To the Graduate Council:

I am submitting herewith a thesis written by William Nicholas Reynolds entitled “Imidacloprid Insecticide Treatments for Hemlock Woolly Adelgid, Adelges tsugae Annand (Hemiptera: Adelgidae), Affect a Non-target Soil Arthropod Community Surrounding Eastern Hemlock, Tsuga canadensis (L.) Carriere.” I have examined the final copy of the thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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A Thesis
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William Nicholas Reynolds
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Dedication

For the loving support from…

Courtney Marie Hendricks
Jennifer Allison Weedman
William Stephen Reynolds
Timothy James Reynolds
Pansy Page Allison
Jean Cunningham Reynolds
Brent Weedman

And in loving memory of…

Norman Lee Allison
William Clarence Reynolds
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Abstract

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an invasive pest that is causing declines in populations of eastern hemlock, *Tsuga canadensis* Carriere, in eastern North American forests. The threat of losing the hemlock as a foundation species in eastern forests prompted reserve managers to devise and implement HWA management strategies integrating cultural, biological, and chemical control tactics. Chemical control methods, systemic imidacloprid applications and horticultural oil foliar sprays, provide the most immediate and effective control of HWA in accessible hemlocks. Non-target impacts of HWA chemical control methods on soil arthropod communities are undocumented.

Empirical studies in the field and in the laboratory were performed to determine the extent of effects of the common HWA chemical control treatments to non-target soil arthropods. Treatments were the horticultural oil foliar spray (no imidacloprid), imidacloprid trunk injection, imidacloprid soil injection, imidacloprid soil drench, and untreated controls. Microarthropods in soil drench plots displayed marginally non-significant decreases in abundance and richness. Microarthropod species composition was distinct in all of the imidacloprid treatments when compared to controls. Acari, the mites, consisted of approximately 50% of the observed abundance, and showed no responses to imidacloprid or horticultural oil treatments. Abundance and richness of Collembola, in contrast, were markedly decreased by the soil drench treatments.
High performance liquid chromatography (HPLC) was used to quantify concentrations of imidacloprid from soils following imidacloprid treatments. Concentrations of imidacloprid observed in soils from imidacloprid treatment plots exceeded the LD$_{50}$ and ED$_{50}$ concentrations for *Folsomia candida* Willem (Collembola: Isotomidae) observed in the laboratory, especially in the soil drench plots, less frequently so in the soil injection plots and in a few of the trunk injection plots.

The springtail *Folsomia candida* were reared in the laboratory on standard soil substrates containing a series of known imidacloprid concentrations to observe impacts to reproduction and survival. The imidacloprid concentration at which *Folsomia candida* adults displayed 50% mortality in the laboratory, as inferred from regression analysis of observed dose responses (LD$_{50}$), was 1.38 mg imidacloprid / kg dry soil. The concentration at which *F. candida* produced half the number of juveniles observed in control microcosms (ED$_{50}$) was 0.598 mg imidacloprid / kg dry soil.
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Chapter 1

Review: Eastern hemlocks, invasive hemlock woolly adelgid, management strategies, and potential for non-target effects on beneficial insects
Abstract

The importance of eastern hemlock forests, invasion by hemlock woolly adelgid (HWA), hemlock decline, and management practices for HWA are reviewed in this chapter. Scientific literature concerning the insecticide imidacloprid and non-target effects on beneficial insects is provided. Potential for effects of HWA chemical treatment methods on non-target soil arthropods is examined. Objectives of empirical studies to determine the extent of non-target effects on soil arthropods caused by HWA chemical control methods are outlined.

Eastern hemlock, *Tsuga canadensis* (L.) Carriere (Pinaceae), is a unique coniferous tree species in eastern North American forests, ranging from Nova Scotia southward along the spine of the Appalachian Mountains to its terminus in northern Alabama and Georgia. The Carolina hemlock, *Tsuga caroliniana* (Pinaceae), occurs in a small, endemic range in western Carolina and southwestern Virginia. Hemlocks are considered foundation species in eastern North American forests, due to their influence on both aboveground and belowground ecosystem processes and community assembly with its uniquely shallow root system, dense canopy and shade, lower quality and quantity of litter inputs into streams and soils, and influence on air, water, and soil temperatures.

Hemlock populations are declining in eastern North America due to an invasive insect, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). Hemlock woolly adelgid is native to Asia and
northwestern North America where populations of the other seven extant hemlock species occur. The two eastern North American hemlock species cannot tolerate HWA herbivory, leading to decline of hemlock forests. Hardwood forest species are colonizing former hemlock stands and changing floral and faunal community assembly and ecosystem processes.

To preserve declining hemlock populations due to HWA, forest managers have implemented integrated pest management strategies that incorporate cultural, biological, and chemical controls. Effects for non-target soil arthropods of the HWA chemical treatments, most of which contain the active ingredient imidacloprid, are undocumented. Evidence from scientific literature indicates that imidacloprid can affect non-target beneficial insects.

**Importance of hemlocks**

*Tsuga spp. in Appalachian forests*

Hemlocks (*Tsuga spp.*) are long-lived, late-successional conifers that occur in Asia and North America. Of the nine extant species world-wide, two occur in the forests of the Appalachian Mountains in eastern North America: the eastern hemlock and Carolina hemlock.

Eastern hemlock, *Tsuga canadensis* (L.) Carriere, occurs in eastern North America from Nova Scotia southward along the spine of the Appalachian Mountains to Georgia and Alabama. In the southern Appalachians, eastern hemlocks grow in moist, cool ravines and valleys, rocky streambeds and are
common on mid-elevation slopes (Swanson 1994). The hemlock’s unique shade
tolerance, foliar chemistry, dense canopies, and shallow root systems offer
characteristic habitats to which many taxa have adapted.

The Carolina hemlock, Tsuga caroliniana, is ecologically similar, but
morphologically distinct, to the Eastern hemlock. The Carolina hemlock occurs
most frequently on south-facing slopes (Swanson 1994) in a small range in the
mountains of the western Carolinas and southwestern Virginia. Due to the small
size and endemic nature of its native range, the Carolina hemlock is more
vulnerable to species extinction than its more common and broadly ranging
cousin, the eastern hemlock.

Hemlocks in Appalachian forests are economically, aesthetically, and
ecologically important (Quimby 1996). The hemlocks of the Southern
Appalachians have a long history of economic importance. Hemlock was
important in the tanning industry until other sources of tannins were discovered
(Quimby 1996). More recently, hemlock has become economically important in
the lumber and pulpwood industry. Hemlocks also occur in a large number of
yards and on private property, and were a popular tree in nursery trade before
the invasion of HWA. Hemlocks can moderate temperatures of homes by
providing dense shade and make excellent privacy hedges. Many thousands of
dollars are spent every year on planting, maintaining, and preserving hemlocks
on private lands. Decreases in land and property values have been negatively
correlated to HWA presence and hemlock decline (Holmes et al. 2006). Hemlock
trees are a signature member of forests in the Great Smoky Mountains National
Park. Tourists spent more than $1.5 billion in Blount and Sevier Counties in 2005 according to the Travel Industry Association Of America in 2006. These Tennessee counties benefit from the tourism that the Great Smoky Mountains attracts. Public affection for the hemlock tree is represented by the successful fundraising that public non-profit organizations such as the Friends of the Smokies have done on behalf of HWA biological control programs in Tennessee.

The hemlocks in eastern North American forests are considered an irreplaceable foundation species that has far-reaching influences on the associated biota and microclimate of these forests (Ellison et al. 2005). These influences extend to both aboveground and belowground communities and ecosystem processes.

*Hemlock influence on aboveground environments and biota*

Hemlock forests are characterized by cool, damp microclimate, low light levels, depauperate understory vegetation cover, and relatively stable forest composition (Orwig and Foster 1998). Hemlock seedlings and saplings grow slowly in the shade underneath shorter lived hardwood trees in early successional forests. The hemlock photosynthesizes in the cold winter months, when the hardwoods have long since dropped their leaves. Eventually, the hardwoods succumb to age and the longer-lived hemlock assumes its role as the dominant, late successional climax species. The microclimate, soil, floral and faunal assemblages, and forest ecosystems are influenced heavily by hemlock stands.
The forest floor in hemlock-dominated stands is resistant to colonization by herbaceous and hardwood plant species because of the acidic, nutrient-poor soils, low light levels, and cool, damp microenvironments that the dominant hemlock species creates. Monospecific hemlock stands also exhibit slow rates of nitrogen cycling and nutrient poor soils which make the hemlock-dominated environment uninhabitable to plants that require high nutrient availability (Jenkins et al. 1999). Hemlocks have a higher leaf area index than surrounding hardwood forests (Catovsky and Bazzaz 2000). High leaf area index results in hemlock’s shade tolerance, and a dark, cool microclimate which few other species of plant can tolerate. However, some species of plants that do not occur in other forest types thrive in hemlock forests (Yorks et al. 2003).

The dense canopies of hemlock stands provide unique habitats for a number of vertebrate fauna. At least 4 species of birds, including the Acadian flycatcher, *Empidonax virescens*; blue-headed vireo, *Vireo solitaries*; black-throated green warbler, *Dendroica virens*; and Blackburnian warbler, *Dendroica fusca* live primarily in hemlock canopies (Ross et al. 2004). Hemlocks also moderate forest floor temperatures in winter, and serve as refuge for many vertebrates during the coldest winter months (Lishawa et al. 2007).

**Hemlock influences on aquatic systems**

Hemlock forests influence not only terrestrial environments and biota, but also influence aquatic abiotic characteristics and community structure. The influence of hemlocks on streams is especially important in the southern ranges
where hemlocks occur along stream banks in great numbers. Water temperatures tend to be cooler in streams that lie underneath a hemlock overstory than in similar streams underneath hardwood forests (Snyder et al. 2002, Yorks et al. 2003). Stream flows have been shown to be more consistent in hemlock forests, due to the shallow root systems of hemlock trees that do not encroach upon groundwater that maintain stream flows. hardwoods tend to root deeper into the ground, and tend to cause lower ground water levels in the hyporeic zone of streams, which decreases stream levels in dry periods. Due to the loss of ecohydrologic roles that hemlocks play as the dominant riparian tree species, increases in discharge and decreases in the daily amplitude of streamflow are predicted (Ford and Vose 2007). Water chemistry in hemlock-covered steams differs from that of streams dominated by litter inputs from hardwood trees (Lewis and Likens 2007). These differences in aquatic environments lead to dissimilar compositions of fishes and aquatic invertebrates in hemlock and hardwood-shaded streams (Snyder et al. 2002, Ross et al. 2003).

Hemlock influences on belowground communities and ecosystem processes

Hemlocks influence soil community assembly of flora and fauna by inputting litter of unique chemistry, structure, and quantity into the soil and the decomposer food web. The classic mull and mor theories that stress the importance of plant litterfall types dictating the characteristics of soils and soil biota. Wardle (2002) summarized the following ways that plant species can
affect soil communities. Plant species produce different amounts of net primary productivity by fixing varying amounts of carbon into aboveground and belowground biomass. The quality of the resources produced by different plant species also affects soil community assembly and ecosystem processes. Some plants deplete different amounts of particular nutrients in the soil. The chemical composition of leaf and root litter that enters the decomposer food web differs among plant species.

Litter from hemlock trees is typical for a conifer species. Evergreen leaves have long life spans and provide less litter quantity than deciduous trees. High levels of defensive phenolic compounds and low nutrient content of conifer leaves tend to decompose slowly due to the relative unpalatability (Cornelissen et al. 1996, Cornelissen et al. 1997). These characteristics lead to lower rates of litter decomposition and characteristically nutrient poor soils in hemlock dominated stands.

Due to these attributes of conifer species’ litter, soils in hemlock-dominated forests are typical mor-type soils. The primary decomposer guild in these soils tends to be dominated by fungal as opposed to bacterial communities (Wardle 2002). The abundance of fungi leads to the domination of soil fauna by microarthropods. Soils that are dominated by coniferous litter inputs tend to have higher numbers of microarthropods than do hardwood forests (Petersen and Luxton 1982).
Invasion of hemlock woolly adelgid

Hemlock woolly adelgid in eastern North America

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an invasive insect that causes declines in populations of hemlock trees in eastern North America. Individual hemlock trees are weakened and killed by the feeding of HWA on the starch stored in the tree’s ray parenchyma cells (Young et al. 1995).

Severely decreased prominence of the hemlock as a mid-elevation, late successional foundation species in Appalachian forests is likely due to this invasive pest. Loss of hemlocks as the dominant, foundation species in mid-elevation forests affects forest tree composition and environmental characteristics in eastern North American forests, which will change above and belowground microclimates, biota, and ecosystem processes.

The hemlock woolly adelgid, or HWA, was inadvertently introduced into the native range of the eastern hemlock in North America in the 1950s through nursery trade with Asia. The HWA population in eastern North America originated from Japan (Havill et al. 2006). Following an establishment lag time from the 1950s until the early 1990s, HWA has rapidly expanded into most of the range of Eastern and Carolina hemlocks and impacted the forest ecosystems by decimating populations of hemlocks. The adelgid reproduces parthenogenetically, has no natural enemies in eastern North America that exert noticeable control of populations, and indiscriminately kills hemlocks of all age
classes. Eggs hatch twice per year in great numbers and crawlers disperse phoretically on birds and other transient species.

Eastern and Carolina hemlocks have shown no signs of resistance or tolerance of heavy populations of HWA. Adelgid infestations lead to thinning of hemlock canopies, needle drop, and ultimately stand mortality (McClure 1991a). Hemlocks can only tolerate HWA infestation for three to four years before displaying 80-90% mortality in the northern ranges. In the southern portion of the hemlocks range, increased temperatures have led to a reduction in winter mortality of HWA and accelerated the hemlock decline rate (Deal 2007).

**Aboveground impacts of hemlock woolly adelgid**

Forest tree composition is changing dramatically due to the HWA-induced hemlock decline. The microenvironment that occurs in the presence of hemlocks is unique in mid-elevation Appalachian forests. There is no tree species that is predicted to replace the hemlock and create a similar forest type in the hemlock’s stead. With increased sunlight due to thinning *Tsuga* canopies, hardwood species, particularly *Betula*, *Quercus*, and *Acer* in the northern range (Orwig and Foster 1998), and possibly rhododendron in the southern ranges (J. Vose et al., unpublished), are likely to invade sites previously dominated by hemlock.

Colonization of former hemlock stands by hardwoods is leading to increased homogeneity in eastern forests (Orwig and Foster 1998). It has been shown that black birch, *Betula lenta*, and other hardwoods replaced hemlock following declines. Seed bank analysis and observations of seedling and sapling
occurrence gave no indication that hemlocks would regain a presence in the forests in the near future (Kizlinski et al. 2002). As the forests of eastern North America transition from a mixture of hemlock and hardwood trees to forests comprised almost entirely of hardwoods, the unique assemblages associated with hemlocks will be lost. Thus, gamma, or overall, diversity of eastern forests is expected to decrease.

Herbaceous species that are not found in monospecific hemlock stands are able to colonize HWA-damaged sites as well. Increased light at the forest floor in HWA damaged hemlock stands leads to an increase in herbaceous cover. (Kizlinski et al. 2002) showed that HWA-infested sites had higher incidences of saplings, seedlings, shrubs, and herbs, resulting most likely from increasing light levels associated with hemlock mortality. Similar colonization of former hemlock stands by herbaceous shrubs and invasive vines has been exhibited in Connecticut forests (Small et al. 2005).

The decline of hemlock has been predicted to be a future catastrophe of a magnitude similar to that of the decline of American chestnut, *Castanea dentate*, due to the chestnut blight. Hemlocks are a unique mid-elevation conifer in Appalachian Mountain forests that are unlikely to be replaced by a similar species following their removal due to HWA. When chestnut blight decimated the American chestnut as the dominant eastern hardwood species, oak and hickory species filled the mast niche of the chestnut. In contrast, no similar tree species that can influence the habitat in the way hemlock does, and the effects of their loss will be far-reaching. The only species that provides litter of similar
quality and provides similar shade is *Rhododendron maximus*, a shrub that commonly co-occurs with hemlock in its southern ranges (J. Vose *et al*. unpublished).

Large vertebrate species are expected to be stressed by extreme winter temperatures with the loss of hemlock as a refuge from cold temperatures (Lishawa *et al*. 2007). There are at least 4 species of birds, including the Acadian flycatcher, *Empidonax virescens*; blue-headed vireo, *Vireo solitaries*; black-throated green warbler, *Dendroica virens*; and Blackburnian warbler, *Dendroica fusca* live primarily in hemlock canopies (Ross *et al*. 2004) and are threatened by HWA-induced hemlock decline.

**Belowground impacts of hemlock woolly adelgid**

As eastern hemlock forests give way to colonizing species, belowground environments and biota are expected to change because hardwood and herbaceous colonizers’ litterfall into the decomposer food web is much different from that provided in a hemlock environment. These differences are expected to induce changes in soil communities and ecosystem processes.

HWA-induced forest composition shifts are predicted to alter belowground microbial and faunal communities. Litter that falls from hemlocks and other conifers is generally of less quality and quantity than litter from deciduous species. The temporal distribution of litterfall is dissimilar as well. In response to litter differences and microbial community responses, microarthropods in the secondary decomposer and predatory functional groups are expected to change.
Microbes, consisting of fungi and bacteria, play important roles in forest soils as primary decomposers of decaying plant material. The composition and functionality of these groups are expected to change with the loss of hemlocks, and the colonization of hardwoods. Changes in hemlock-associated ant community structure and composition have been shown in HWA-damaged forests, because the few ant species that are specialist in New England hemlock forests were unable to survive in the forest without hemlock (Ellison et al. 2005).

Hemlock decline due to HWA has been shown to affect belowground ecosystem processes such as nutrient cycling and litter decomposition. Nitrification rates in HWA-infested sites were more than 40x greater than the near-zero rates in uninfested sites (Kizlinski et al. 2002). Adelgid-induced changes in needle development, production, and chemistry are predicted to alter throughfall chemistry and litter quality (Stadler et al. 2005). During the summer, increases of dissolved organic carbon (DOC) in throughfall water chemistry were attributed to large amounts of HWA wax wool decomposing and being washed from the canopy leading to 24.6% higher DOC fluxes in throughfall (Stadler et al. 2005). Also, inorganic N decreased 40%, and organic N increased 29% in during the summer leading up to aestivation. Once HWA enter aestivation, throughfall chemistry was similar in uninfested and infested sites (Stadler et al. 2006). These HWA-induced changes in canopy leaf chemistry and water throughfall chemistry alter the quality of litter and nutrients being put into the decomposer food web.
The adelgid has been shown to alter decomposition rates in infested hemlock forests. Slower rates of standard substrate (cellulose filter paper) mass loss were observed in adelgid-damaged hemlock plots due to decreased moisture in the forest floor (Cobb et al. 2006). Decomposition of litter is important in forest ecosystems. The complex nutrients and structural proteins associated with newly desiccated woody material are unavailable for uptake by plants. The dead material must be metabolized by microbes and microfauna to simpler compounds to be available for recirculation into nutrient cycles. HWA threatens the balance of these important ecosystem processes.

**Management of Hemlock Woolly Adelgid**

*Invasive species costs and management*

The Millennium Ecosystem Assessment report ranks invasive species as the second most important threat to global biological diversity, endangered species conservation, and ecosystem services, just behind anthropogenic habitat destruction (Reid et al. 2005). The estimated annual cost of invasive species in the United States is over $120 billion, due to management expenses and environmental losses (Pimentel et al. 2005).

Integrated pest management strategies have been developed to protect eastern hemlock populations from extirpation in all but its most northern range in eastern North America. Control measures include cultural, biological, and chemical approaches.
Cultural and Biological Control

Successful cultural control methods against HWA are few. No silvicultural practices are successful in containing the spread of HWA. The harvesting of declining trees, while economical, has been shown to affect forest communities and ecosystem processes more dramatically than hemlock decline alone (Orwig and Abrams 1999, Foster and Orwig 2006). The adelgid benefits from trees with high nitrogen content (McClure 1991b). Therefore, fertilization of infested or threatened hemlocks is not recommended. Plantations of *T. canadensis* and *T. caroliniana* are being established in South America to forestall the loss of hemlock genetic diversity in the event of near extirpation in the eastern United States.

No native predators, parasites, or infectious organisms are known to exert noticeable effect on HWA populations in eastern North America. Several importations of predaceous beetles have been made in attempts to slow the spread of HWA and subsequent decline of hemlock. The first of these beetles to be studied, reared, and released was *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), native to Japan (formerly *Pseudoscymnus tsugae*). *Sasajiscymnus* exhibits HWA-specific feeding habits and a life cycle synchronous with HWA, and is amendable to laboratory rearing (Sasaji and McClure 1997). Since its acceptance as a suitable biological control agent, more than 3 million *S. tsugae* have been released in eastern forests.

*Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is another biological control agent that has been released in large numbers from rearing
Laricobius nigrinus feeds exclusively on HWA in hemlocks and is native to the Pacific Northwest region of North America. Laricobius nigrinus has proven to be less amendable to lab rearing than Sasajiscymnus tsugae, but rearing methods continue to improve leading to increased numbers released each year from labs at the University of Tennessee, Clemson, and Virginia Tech. Laricobius nigrinus has displayed more evidence of establishment in the field compared to Sasajiscymnus tsugae (Zilahi-Balogh et al. 2003, Lamb et al. 2005, Zilahi-Balogh et al. 2005, Flowers et al. 2006, Lamb et al. 2006, Zilahi-Balogh et al. 2006, Flowers et al. 2007, Zilahi-Balogh et al. 2007). A new species of Laricobius collected from China is being studied by Salom and Lamb (personal communication) in quarantine at Virginia Tech, and is expected to be approved for release.

Several other biological control agents are being evaluated and implemented for HWA control. A number of small Coccinellids, primarily Scymnus spp., are currently being reared and released from laboratories at the University of Georgia for biological control of HWA. In addition, a group at Oregon State is evaluating a Dipteran parasitoid, native to the Pacific Northwest, for its potential use in HWA management. Fungal pathogens may be applicable to adelgid control, according to recent unpublished work by Scott Costa’s lab at the University of Vermont.

The goal of these biological control efforts is to establish a complex of self-sustaining adelgid predator and pathogen populations capable of lowering pest populations to non-damaging levels. The cost of rearing the biological control
agents is very high, and the efficacy of control has yet to be determined. Predator populations are difficult to monitor and require time to establish. In the meantime, forest managers rely on chemical insecticides to preserve valued hemlocks in physically accessible sites.

**Chemical Control**

Use of chemical insecticides provides the most effective and immediate control of HWA. Most common insecticides that are used against HWA contain the active ingredient imidacloprid. Horticultural oils are also utilized and do not contain imidacloprid. Forest managers in the Great Smoky Mountains National Park have chemically treated over 56,000 hemlocks within the park boundaries (T. Remaley, personal communication).

Foliar sprays of horticultural oil may be used for the control of hemlock woolly adelgid. Large amounts of water mixed with horticultural oil are required along with a high pressure sprayer capable of giving full coverage of the canopy. The horticultural oil and water solution must contact the pest insect in order to effectively control the pest.

Systemic applications of insecticides containing the active ingredient imidacloprid are most commonly used against HWA. Imidacloprid, 1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine, is a synthetic derivative of nicotine. It is the most widely used compound in a new class of pesticides, the neonicotinoids. The many formulations of insecticides containing imidacloprid are available to the public and are among the most widely used insecticides due
to its novel mode of action, low application rate, longevity, efficacy, selectivity, low mammalian toxicity, and relatively low environmental impact (Cox et al. 1997, Cox et al. 1998a, Cowles et al. 2006).

The mode of action is unique in that it blocks the activity of nicotinic acetylcholine receptors (nAChR) (Abbink 1991, Bai et al. 1991, Tomizawa and Yamamoto 1993, Tomizawa et al. 2007) in insects. Imidacloprid can work as a contact insecticide, but is most often applied to soils surrounding the infested plant for uptake by the roots, followed by translocation of the active ingredient by the plant’s vascular system. Insecticidal activity is observed at low application rates because piercing-sucking pests feed directly on plant metabolites of the active ingredient. This insecticide exhibits extended efficacy and low leaching potential due to its strong binding to organic matter in the root zone of the plant that is to be protected (Oi 1999, Cox et al. 2001, Cox et al. 2004, Papiernik et al. 2006). Imidacloprid formulations are systemic, thus they have been touted as selective insecticides with low non-target effects. In addition, the insecticide has low leaching potential due to strong chemical binding with organic soils (Tomizawa et al. 2007).

Impacts of HWA Chemical Control

While insecticides are effective at controlling adelgid populations, it is important to consider non-target effects of their use in conservation reserves and in forestry and agriculture. Imidacloprid has been tested on a small number of arthropod groups in aboveground systems. The non-target effects of
Imidacloprid on soil arthropod and belowground ecosystems are largely unstudied. Imidacloprid is commonly applied directly to soil habitat in which diverse flora and fauna contribute to the decomposer food web, responsible for litter turnover and nutrient cycling. The importance of these ecosystem processes and the value of the biological diversity harbored in the soil warrant a closer look at the potential for non-target impacts of HWA chemical treatments in soil ecosystems.

**Impacts of imidacloprid to non-target arthropods**

Imidacloprid has been shown to be injurious to Carabidae. Ground beetles exposed to imidacloprid displayed paralysis, impaired mobility, and excessive grooming that led to increased vulnerability to ant predation (Kunkel et al. 1999). The US EPA dissuades imidacloprid application during the flowering season due to imidacloprid’s toxicity to honeybees (Nauen et al. 1998). Imidacloprid was acutely toxic to mirid bugs, lady beetles, and lacewings (Mizell and Sconyers 1992). Imidacloprid has even been shown to be toxic to the HWA biological control agents, *Laricobius nigrinus* and *Sasajiscymnus tsugae* (B. Eisenback, in press). Imidacloprid soil drench applications were shown to negatively impact non-target arthropod abundance, richness, and composition inhabiting hemlock canopies (Dilling 2007).
Objectives of current research

The non-target impacts of imidacloprid on soil communities are unknown, in spite of the common practice of applying it directly to soil habitat. The soil arthropod community contributes to the forest ecosystem by facilitating litter turnover and nutrient cycling. Thus, it is important to document non-target effects of HWA chemical treatments on soil arthropods.

The goal of this study is to determine if non-target ground and soil arthropod diversity and numbers were affected by several common HWA chemical control practices. Empirical studies were established with the objectives to: 1) observe the effects of HWA insecticide treatments on soil and ground arthropod community structure in replicated field experiments; 2) determine imidacloprid concentrations in soil following different treatment strategies; and 3) observe survival and reproductive dose responses of *Folsomia candida* Willem (Collembola: Isotomidae) to imidacloprid in laboratory microcosms. In the final chapter, continued HWA chemical management will be considered in the light of evidence of non-target effects on soil arthropods.
Chapter 2

Non-target effects of hemlock woolly adelgid chemical controls on soil arthropod communities in field experiments
Abstract

Systemic imidacloprid insecticide treatments and foliar applications of horticultural oil are used to control the invasive pest, hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, which is responsible for declines of the eastern hemlock, *Tsuga canadensis* (L.) Carriere. Non-target effects of these insecticide treatments on soil arthropods are undocumented in the hemlock management system.

To determine the extent of non-target effects on soil arthropods following HWA insecticidal treatments, a two-year manipulative field experiment was established in November 2005. Treatments consisted of imidacloprid soil drench, imidacloprid soil injection, imidacloprid tree injection, horticultural oil (not containing imidacloprid) foliar spray, and untreated controls. Species abundance, richness, evenness, and composition of microarthropods extracted from soil cores were compared across the five treatments and two application times (fall and spring).

Total microarthropod abundance was non-significantly decreased in both fall and spring soil drench applications, along with total microarthropod richness for fall application time. Soil drench treatments in the spring application had decreased microarthropod species richness (Tukey-Kramer, P < 0.05). Total microarthropod species composition in control and foliar horticultural oil plots were dissimilar from all imidacloprid treatment plots (ANOSIM, P < 0.10). Compositional shifts in fall and spring application times were due to marked
decreases in abundance and richness of Collembola, which comprised approximately 40% of the microarthropod community in control plots. Mites and other arthropods that comprised the remaining 60% of the community, and were not affected by any treatments.

**Introduction**

The Millennium Ecosystem Assessment report ranks invasive species as the second most important threat to endangered species, global biological diversity, and ecosystem services (Reid et al. 2005). The estimated cost of invasive species in environmental losses and management expenditures was calculated to be more than $120 billion in the United States, annually (Pimentel et al. 2005).

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an invasive pest that has caused populations of *Tsuga canadensis* and *Tsuga caroliniana* to decline in eastern North America. Following its introduction and establishment in the northeastern United States, HWA spread along the spine of the Appalachian mountain range from Maine to Georgia, encompassing most of the range of the hemlock in eastern North America. The HWA threatens the prominence of hemlock as an economically, ecologically, and aesthetically important (Quimby 1996) foundation species (Ellison et al. 2005) in eastern North American forests.

To mitigate the losses imparted by the invasion of HWA-induced loss of hemlock, forest managers have developed an integrated pest management
strategy involving cultural, biological, and chemical control. Of these control measures, chemical controls provide the most immediate and effective management of hemlock woolly adelgid, although it is limited to accessible roadside and trailside trees. Infested hemlocks treated with imidacloprid or horticultural oil foliar sprays resume the production of new growth, which is essential for photosynthesis and survival.

The insecticides most commonly used to control HWA contain the active ingredient imidacloprid. Imidacloprid formulations are used widely in HWA and other pest management strategies. The Bayer Tree and Shrub formulation of imidacloprid is labeled for HWA, and is available to consumers for use on private land. In Great Smoky Mountains National Park, more than 56,000 hemlocks have been chemically treated for HWA (T. Remaley, personal communication).

In a crisis situation, reserve managers are sometimes forced to enact management practices before non-target risks can be documented. Conservation biology is a mission-oriented (Wilcox 1980) and crisis-driven discipline (Soule 1985, Meine et al. 2006). Reserve managers often must take immediate action in order to conserve natural populations that are endangered, threatened, or stressed by small population size, habitat destruction, or invasive species.

To conserve declining hemlock populations, forest managers took swift action to implement integrated management strategies to control HWA. Large scale efforts of biological and chemical controls have been enacted to date.
However, no empirical studies have been performed to test for non-target impacts of HWA chemical treatments on soil arthropod communities. Disturbance in soil arthropod communities caused by chemical control for hemlock woolly adelgid is undocumented. Insecticides are applied directly to the soil habitat in some of the most commonly utilized chemical control tactics for HWA. Hemlock forest soils contain important microarthropods that influence flora and fauna community assembly and forest ecosystem processes such as litter turnover and nutrient cycling. The goal of this study was to monitor how soil arthropod communities are affected by the most common HWA insecticide treatment methods, applied at two different application times.

Materials and Methods

Field Sites

A replicated field experiment was established in 2005 to determine the extent of non-target disturbance in a hemlock-associated soil arthropod community caused by the most common chemical control methods of HWA. Experimental sites were chosen near the invasion front of HWA surrounding Indian Boundary Campground in the Cherokee National Forest, Monroe County, TN. All plots were located between 545m (1789’) and 550m (1804’) in elevation and within a 0.549km (0.34 mile) radius of N35 23.858, W84 06.525. Thirty hemlocks with little to no adelgid infestation and good qualitative health ratings were selected as experimental plots. In addition, each hemlock canopy was
adequately isolated from canopies of other hemlocks to avoid overlap in soil
treatment zones in the current study and in canopy arthropod communities that
were monitored by collaborators for non-target effects (Dilling 2007).

Experimental Design

Thirty hemlocks were organized into fifteen pairs of trees. Each pair
member was proximal to its counterpart, and similar in stem diameter, height,
HWA infestation levels, and qualitative health. Pairs were randomly classified
into the five treatment groups to give the experiment a split plot 3 replicates of 2
treatment times and 5 treatment blocks. Treatments were administered to a
random member of each pair on November 29-30, 2005, and served as the fall
application time. The other member of each pair was treated with the same
application method on April 16, 2006, and served as the spring application time.
This design allowed testing for differences in soil arthropod community species
abundance, richness, evenness, and composition between four chemical
treatment plots against untreated controls, and timing of the application.

Treatments

Chemical pesticide treatments were the most common application
methods used by forest managers in the fight against the hemlock woolly
adelgid. The five treatments were foliar horticultural oil application, imidacloprid
soil drench, imidacloprid soil injection, imidacloprid trunk injection, and untreated
control plots.
For foliar spray treatments a solution of water and SunSpray® horticultural oil was administered with a FMC® high pressure hydraulic sprayer. In accordance with label instructions, 7.57L (2 gallons) of SunSpray® oil were added to 378L (100 gallons) of water. The solution was applied to the entire canopy of hemlocks in the foliar treatment group until runoff occurred from tips of the branches. Each foliar treatment plot/tree was treated with approximately 125L (33 gallons) of the horticultural oil solution.

Trunk injections of the imidacloprid formulation Imicide® were performed with the Mauget® system. A hole, 1.75cm (11/16") in diameter, was drilled to a depth of 1.27cm (1/2") at a slight downward angle in the trunk of the tree 20.3cm (8") above the soil, per the label instructions. Each Imicide capsule contained 3ml of 10% imidacloprid solution. One of the pressurized capsules was inserted into the corresponding hole for every 15cm of stem diameter at breast height (dbh), to give an application rate of 0.15ml of imidacloprid per 2.54cm dbh. Capsules remained inside the hole in the trunk until the contents of each were emptied. The capsules were then removed and discarded.

Soil injections consisted of a small volume of a highly concentrated solution of imidacloprid in water that was applied with a Kioritz® soil injector 6-8cm beneath the soil surface near the base of the hemlock trunk at a rate of 1.0g of imidaclorid per 2.54cm dbh. Merit® 75 WP was mixed in 60ml of water inside of the injector. The volume of solution injected into the soil at each plot varied with the dbh of the hemlock tree being treated.
Soil drench treatment was administered by soaking the soil underneath the drip-line of each soil drench hemlock plot with a high volume of a relatively lower concentration solution of Merit® 75 WP. Each soil drench was applied at a rate of 1.5g of active ingredient per 2.54cm dbh. A large volume, approximately 125L (33 gallons), of imidacloprid and water was applied directly to the soil surface with an FMC® high pressure sprayer.

Arthropod Collection

Quarterly, six soil cores (15cm deep, 3cm diameter) were randomly collected from underneath the drip line of the hemlock in each plot, from November 2005 to August 2007. Euedaphic arthropods were extracted from soil core samples for one week at 15ºC inside high-gradient Tullgren funnels (Crossley and Blair, 1991). Specimens were stored in 95% ethanol. Species and morphospecies abundance data were tabulated for each sample.

Statistical Analysis

Abundance data for each microarthropod species or morphospecies from the six soil cores in each plot were summed for each triplicate treatment plot. Species abundance and observed, rarefied, and estimated species richness means for each plot were calculated for each season and for the total observed throughout the two-year study. In addition, observed and estimated richness means were standardized for differences in abundance across samples by rarefaction to test if richness, per se, was affected by treatments (Sanders 1968,
Foote 1992, Colwell and Coddington 1994, Gotelli and Colwell 2001). Total species richness was estimated with the Chao2 estimator (Chao and Bunge 2002, Shen et al. 2003). Relative abundances and evenness were compared across treatments in Whittaker’s ranked abundance plot to test for community abundance distributions (Whittaker 1952, Whittake.Rh 1966). Evenness was also compared with Shannon’s Diversity index. Species composition was tested by organizing the species abundance into a Bray-Curtis similarity matrix (Bray and Curtis 1957, Gauch 1973, Beals 1984), ordinated into non-metric multidimensional scaling figures (NMDS), and statistical differences were determined using analysis of similarity (ANOSIM) in Primer® (Primer-E, United Kingdom).

Means of each treatment plot’s aforementioned community parameters were analyzed with ANOVA and a Tukey-Kramer mean separation test with an alpha level of $P = 0.05$. Data collected from plots treated either in the fall or in the spring were not summed together, but were kept separate to test for differences in impacts due to treatment times.

**Results**

*Group proportions and Seasonal Variation*

The soil arthropod community total abundance in the untreated control plots and in the foliar spray plots consisted of 48.1% and 45.8% mites (Acari), 39.3% and 39.5% springtails (Collembola), and 7% and 5.8% Protura,
respectively. The rest of the arthropod community consisted of less than 1% proportions of Symphyla, Hymenoptera, Thysanoptera, Coleoptera, Pauropoda, Chilopoda, Diplura, Isoptera, Diplopoda, Hemiptera, Pseudoscorpionida, and Diptera. The proportion of Collembola in the imidacloprid tree injection, soil injection, and soil drench treatments decreased to 30.2, 23.3, and 12.8%, respectively (Fig. 1).

Soil arthropods from all of the treatments displayed seasonal variation in richness and abundance. Some of the insecticide treatment plots had lower means of richness or abundance in particular seasons (Fig. 2 and Fig. 3). Some of these means were outside the ranges of standard error, but no significant differences in all taxa species richness or abundance were shown in any particular treatment for any season. Decreases in Collembolan abundance occurred in collections from soil drench plots in November 2006 and January 2007 (Fig. 4). Decreases in Collembolan richness occurred in soil drench plots in the collections of April 2006, November 2006, and January 2007, and in the soil injection plots from April 2007 (Fig. 5).

**Whole Community**

Mean observed abundances of all taxa collected from soil cores were non-significantly different in the fall or spring applications (Table 2; $df = 2$, Fall: $F=0.77$, $P=0.57$; Spring: $F=0.31$, $P=0.87$). The abundance data were highly variable within and among treatment groups (Fig. 8).
There was no difference observed across treatments in relative abundance of species as demonstrated by Whittaker plots (Fig. 6). No differences in evenness were demonstrated by comparing mean Shannon diversity index across insecticide treatments and times. (Table 4 and Fig. 7; df = 2, Fall: $F = 1.29, P = 0.34$; Spring: $F = 0.46, P = 0.76$). The foliar treatment’s standard error did not overlap that of the control plots in the spring application, indicating a trend towards lower richness and evenness in foliar plots in the spring application time. Slight differences in Shannon’s index were most likely due to drops in richness, which appeared more variable than evenness as indicated in rank abundance plots (Figs. 6 and 7).

Mean observed richness for all taxa collected from soil cores in fall treatment plots showed no significant differences across treatments (Table 1; df = 2, $F = 1.3, P = 0.32$). However, standard error about the means of the control and the drench treatments did not overlap, indicating a trend towards lower observed richness in drench plots. For spring application, mean species richness in the drenched plots was lower than species richness in control plots (Table 1; df =2, $F=6.09, P=0.001$). The standard error range about the mean of the foliar treatment did not overlap with that of the control plot, indicating a weak trend of lower observed richness in foliar treatment plots (Fig. 9).

To correct for abundance differences across treatments, species richness was rarefied to the lowest observed abundance in any one plot, which was 322 individuals. No significant differences were observed in rarefied species richness in the fall treatment (Table 3; df = 2, $F = 0.19, P = 0.93$). The rarefied all taxa
species richness in the spring treatment was marginally insignificant (Table 3; \(df = 2, F = 2.65, P = 0.0961\)), whereas, the drench and foliar treatment plots had lower means with standard error that did not overlap standard error about the means in control and soil injection plots (Fig. 10).

Species composition of cumulative taxa was dissimilar across some treatments in both fall and spring application times. The cumulative, all-taxa abundance data were standardized to a percentage of the total observed abundance in each sample and organized into a Bray-Curtis similarity matrix. From the matrix, analysis of similarity (ANOSIM) was performed to test for statistically significant pair-wise differences in species composition across treatments. For fall treatment plots, Global R-statistic calculated for the null community composition created by permutation of actual data was \(R = 0.19\) with a significance level of \(P = 0.07\). The observed R-statistics of pair-wise treatments were then compared to the null community to test for deviations from the Global R-statistic. Species composition was shown to be significantly different across the control and soil injection plots (R-statistic = 0.519, \(P = 0.10\)), the control and drench plots (R-statistic = 0.556, \(P = 0.10\)), and across the drench and trunk injection plots (R-statistic = 0.519, \(P = 0.10\)). Species composition was plotted in NMDS to help visualize the similarity between treatment groups (Fig 11). The Global R-statistic in the spring application plots was calculated to be \(R = 0.23\) with a significance level of \(P = 0.073\). Significant dissimilarity in species composition was observed between control and drench treatments (R-statistic = 0.926, \(P = 0.10\)), trunk injection and drench treatments.
(R-statistic = 0.889, \( P = 0.10 \)), and between soil injection and drench treatments (R-statistic = 0.630, \( P = 0.10 \)). Species composition of each plot was represented in NMDS plots to help visualize compositional similarity between treatment groups (Fig. 12).

**Acari**

Mites comprised nearly 50% of the microarthropods collected from soil cores, and did not respond to HWA insecticide treatments. No significant differences in cumulative species richness, abundance, or composition across treatments or treatment times were found for mites (Tables 1-4). The mean of the observed richness in the foliar plots treated in the spring was lower than the control, drench, and the soil injection treatment plots. The standard errors of these means did not overlap indicating slight impacts of horticultural oil foliar sprays on total mite richness (Fig. 13; \( df = 2, F = 1.98, P = 0.17 \)).

The abundance data for Acari were further divided into the sub-Orders Mesostigmata and Oribatida. Analyses indicated no significant differences or trends in species richness, abundance, evenness, or composition between treatments or seasons for Mesostigmata or Oribatida. The Oribatida group displayed a marginally insignificant trend, well outside the range of standard error, toward lower species richness in the foliar treatment plots in the fall treatment (Fig. 14; \( df = 2, F = 2.33, P = 0.126 \)).
Collembola

Collembola comprised over 40% of the microarthropods collected from soil cores, and exhibited the most marked responses to imidacloprid treatments. Decreases in species richness, abundance, and evenness were observed, along with changes in species composition.

Cumulative Collembola abundances in each plot were averaged, and significant differences were observed in the fall and spring treatment plots across different treatments (Fig. 15). In the fall treatment plots, Collembola were the most abundant in the control plots, followed by foliar, trunk injection, soil injection, and drench plots. Springtail abundance in drench plots was only one quarter of the abundance observed in the control plots (Table 2; $df = 2$, $F = 10.3$, $P = 0.001$). Abundance in the spring drench treatment plots were also significantly lower than abundances observed in control plots (Table 2, $df = 2$, $F = 4.03$, $P = 0.03$).

Drench treatment plots displayed dissimilar relative abundance distributions of Collembola when compared to all other treatments in the spring and fall treatment times. The slopes of the drench treatment distribution of abundance representation in the Whittaker plots for fall and spring was steeper than the other treatments (Fig. 16). Shannon’s Index of richness and evenness did not display any differences in either treatment time or across any of the treatments (Table 4; $df = 2$, Fall: $F = 0.167$, $P = 0.95$; Spring: $F = 0.75$, $P = 0.58$).

Collembola species richness was decreased by HWA chemical treatments that contained the active ingredient imidacloprid. In fall treatments, springtail
richness of control and foliar plots were higher than the species richness observed in the drench plots (Table 1; $df = 2$, $F = 5.75$, $P = 0.01$). In spring treatments, drench treatment plots also displayed the lowest springtail richness. However, the soil injection treatment had the highest springtail richness, but the control plot mean richness was not statistically different from any of the other treatments (Table 1; Fig. 17; $df = 2$, $P = 0.006$). When species richness was standardized by rarefaction for differences in abundance of samples, there were non-significant differences (Table 3; $df = 2$; Fall: $F = 0.28$, $P = 0.88$; Spring: $F = 1.30$, $P = 0.33$), indicating that richness, per se, was not affected by treatment.

Springtail species composition was different across some treatments in both application times. Global R-statistic of 0.29 and $P = 0.012$ was calculated for the null community in fall treatment plots. Significant dissimilarity of springtail species compositions were observed between control and all other treatments, except the foliar treatment. (R-statistics of 0.444, 0.556, and 0.889 between control and trunk injection, soil injection, and trunk injection, respectively, with $P = 0.10$). Dissimilarity of drench treatments to both soil injection and trunk injection was also shown (Fig. 18; $R = 0.519$ and $R = 0.333$, respectively with $P = 0.10$). Spring treatment had a Global R-statistic of 0.238 with a significance level of $P = 0.032$. Drench treatment composition was distinct from the control, trunk injection, and soil injection treatments (Fig. 19; $R = 1.0$, $R = 0.815$, and $R = 0.889$ respectively, $P = 0.10$).
Collembola family-level responses

Collembola abundance data were analyzed at the family-level to test for unique responses by groups. Entomobryidae and the Sminthuridae/Dicyrtomidae, the most abundant family groups, were analyzed for differences in abundance, richness, evenness, and composition. The other families collected were Isotomidae, Onychuridae, Tomoceridae, and Neelidae, and were analyzed only for differences in observed abundance.

Differences in Entomobryidae abundances in control and drench plots were marginally insignificant in fall or spring treatments (Fig. 20; df = 2 Fall: F = 2.87, P = 0.080; Spring: F = 2.41, P = 0.12). Entomobryidae richness was not affected in neither fall nor spring treatments (df = 2, Fall: F = 1.22, P = 0.35; Spring: F = 2.86, P = 0.081); however, species richness in both fall and spring drench treatments were lower than control plot richness and the standard errors did not overlap, indicating a weak trend towards lower richness of Entomobryidae in drench plots (Fig. 21). Neither rarefied richness, Shannon’s diversity indices, nor species composition were different across any treatments or application times for Entomobryidae.

The Sminthuridae and Dicyrtomidae abundance data were analyzed as one group, due to taxonomic similarity. The observed abundance was significantly lower in the spring drench plots, but no so in the fall (Fig. 22; df = 2, Fall: F = 1.22, P = 0.36; Spring: F = 3.67, P = 0.044). Sminthuridae/Dicyrtomidae mean richness was lower in fall and spring drench treatments (Fig. 23; df = 2, Fall: F = 4.11, P = 0.032; Spring: F = 5.93, P = 0.01). Evenness and
richness as indicated by Shannon’s index was significantly lower in drench plots in fall and spring treatments (\(df = 2\), Fall: \(F = 4.99, P = 0.018\); Spring: \(F = 7.54, P = 0.005\)). Sminthuridae and Dicyrtomidae group in drench plots were less even as indicated in rank abundance plots (Fig. 24).

Isotomidae and Onychuridae were more abundant than either Neelidae or Tomoceridae. Isotomidae were significantly less abundant in drench treatments than in controls in both fall and spring treatments (Fig. 25; \(df = 2\), Fall: \(F = 3.66, P = 0.04\); Spring: \(F = 3.48, P = 0.049\)). Onychuridae were significantly less abundant in drench than control plots in fall treatment (Fig. 26; \(df = 2\), \(F = 3.70, P = 0.042\)). Mean abundance of the drench plots in spring treatment was nine-fold lower than the mean of the control plots. However, variance was high, and the difference was not statistically significant (\(df = 2\), \(F = 1.27, P = 0.34\)). The standard error ranges did not overlap, so a weak decrease in abundance was shown in spring treatments (Fig. 26).

The frequencies of occurrence of the families Neelidae and Tomoceridae were very low in the soil cores. No significant differences were observed. However, there was not a single member of either family collected from drench plots from either treatment time over the course of the two-year study (Figs. 27 and 28).
**Other Groups**

Other groups collected from soil cores that were analyzed individually for richness and abundance were the Thysanoptera, Coleoptera, and Hymenoptera. Protura and Symphyla were analyzed for differences in observed abundance.

Thysanoptera and Coleoptera from soil cores showed no significant differences in abundance and richness across treatments or application times. The Hymenoptera, consisting entirely of Formicidae, showed no significant differences in abundance or richness. Abundance of Protura and Symphyla did not differ among the treatments or application times.

**Discussion**

**Summary**

Strongest non-target effects on soil arthropods were observed in soil drench treatments in this study. Overall community species richness was decreased in the spring drench plots. Species composition analyses indicated that overall decreases in species richness were driven by decreases in Collembola. Approximately 50% of the microarthropod community was comprised of mites, which were not affected by any of the common HWA treatment methods. Both tolerance and susceptibility of Acari to imidacloprid have been observed by others (Sclar et al. 1998, Badejo and Tian 1999, Ako et al. 2004, Anhalt et al. 2007, Laurin and
In contrast, Collembola species richness, abundance, evenness, and composition were decreased by soil drench treatments, indicating that springtail populations decline in the presence of imidacloprid in soil. Changes in Collembola abundance, richness, and evenness were negatively correlated with increased concentrations of imidacloprid observed in HWA insecticide drenched sites (Chapter 5, Reynolds 2007). Microcosm studies of springtail Folsomia candida Willem (Collembola: Isotomidae) survival and reproduction in the presence of imidacloprid in standard soil substrate indicated that the springtail was sensitive to concentrations of imidacloprid in soil (Chapter 3, Reynolds 2008). Similar results were demonstrated for the springtails Folsomia candida (Collembola: Isotomidae) and Heteromurus nitidus (Collembola : Entomobryidae) (Idinger 2002, 2003). Concentrations of imidacloprid in soils collected from drench plots were demonstrated to be higher than the LC$_{50}$ and EC$_{50}$ values for Folsomia candida observed in the laboratory (Chapter 4, Reynolds 2007). Implications to biological diversity and ecosystem function

This study indicates that the non-target Collembola in the soil arthropod community are disturbed by these chemical control tactics. These results are important to conservation of biological diversity and ecosystem function. The Collembola, which decreased the most in this study, are a diverse group of
ancient arthropods, and warrant consideration in a conservation context. The soil arthropods influence their environment by facilitating the turnover of decaying organic matter in the soil and metabolizing complex nutrients in dead plant material to simpler forms that are again available for plant uptake. Stability of these important ecosystem functions may be at risk with reductions in arthropod abundance, richness, and changes in composition.

Even with decreases in the soil arthropod community (especially Collembola), the benefits from the insecticide treatments are obvious. Trees that are treated with imidacloprid in the soil drench and soil injection treatments have the highest probability of successful translocation the active ingredient to plant tissues upon which HWA feeds, thus saving the tree from almost certain death. An imidacloprid-treated hemlock maintains its role as a foundation species in eastern North American forests.

*Improving HWA chemical tactics*

Greatest non-target effects to soil arthropods observed in this study occurred in the soil drench plots. Soil injection and trunk injection plots had less effect on non-target soil arthropods. Foliar spray of horticultural oil to the canopy of infested hemlocks had no measured non-target effects. Decreases in HWA follow the same patterns. Soil applications of imidacloprid provide the highest efficacy of reducing HWA infestations (Cowles et al. 2006, Dilling 2007). Of the imidacloprid treatments, the trunk injection had the least effect on soil arthropod communities. However, the trunk injection treatment does not provide control of
HWA as well as the soil injection or soil drench treatment (Cowles et al. 2006, Dilling 2007)

Trees in experimental plots were observed to have low to no adelgid populations at the beginning of the study. Control plots were very heavily infested after two years. Impacts to soil arthropod communities from the loss of the hemlock may prove to be worse than those incurred from the application of insecticide. Insecticide applicators should consider these non-target impacts before choosing an insecticide treatment method in especially sensitive conservation areas where endemic populations of soil arthropods are known to occur.

Exact locations of these especially sensitive, Collembolan conservation areas are undocumented, and no Collembola are listed as threatened or endangered. In contrast, soil arthropods are known for their cosmopolitanism and ecological functional redundancy. Cosmopolitanism, or wide distribution of common species, is in fact common in many soil arthropods. Impacting a population at a particular locality would not threaten a cosmopolitan species with extinction. However, cosmopolitanism is not universal throughout the whole soil community, and is prone to false assumptions because soil arthropods are inconspicuous. New species of soil arthropods are discovered every year by organizations like Discover Life in America. Functional redundancy occurs when a species of soil arthropod is reduced by a disturbance, another species flourishes in its stead that performs the same ecosystem function (i.e. litter
turnover, or fungal feeder), causing no net change to the decomposer food web process.

This experiment was a two year study, thus the long term effects on soil arthropods are unknown. If, in fact, soil arthropods such as Collembola are cosmopolitan, one would expect for recolonization to occur following degradation of imidacloprid into innocuous metabolites. Yet, to successfully protect a hemlock tree from HWA, retreatment with imidacloprid is recommended every two to three years, which would disallow recolonization. Mites and other microarthropods that are more tolerant to imidacloprid applications will hopefully fill ecological niches left empty by Collembolan decline.
Appendix: Tables

Table 1 Effects of treatments and application times on species richness of All Taxa, Mites, and Collembola collected from soil cores. Means in a fall or spring column followed by a common letter are not significantly different according to the Tukey-Kramer HSD test with \( P<0.05 \).

<table>
<thead>
<tr>
<th>Cumulative Species Richness</th>
<th>Fall Treatment</th>
<th>All Taxa ±SE</th>
<th>Mites ±SE</th>
<th>Collembola ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drench</td>
<td>61 3.6 a</td>
<td>33 1 a</td>
<td>11 0.6 b</td>
<td></td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>66.3 2 a</td>
<td>30.3 0.3 a</td>
<td>17.7 0.9 a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>69.3 2.3 a</td>
<td>34.7 0.7 a</td>
<td>18 1.5 a</td>
<td></td>
</tr>
<tr>
<td>Soil Injection</td>
<td>67 1.5 a</td>
<td>34.3 1.2 a</td>
<td>16.3 1.9 ab</td>
<td></td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>64.7 3.3 a</td>
<td>33 2.1 a</td>
<td>16 0 ab</td>
<td></td>
</tr>
</tbody>
</table>

\[ F=1.3; P=0.32 \] \[ F=1.98; P=0.17 \] \[ F=5.75; P=0.01 \]

<table>
<thead>
<tr>
<th>Spring Treatment</th>
<th>All Taxa ±SE</th>
<th>Mites ±SE</th>
<th>Collembola ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drench</td>
<td>51.7 1.8 b</td>
<td>30 0 a</td>
<td>10.7 0.88 c</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>59.3 1.2 ab</td>
<td>30.3 1.7 a</td>
<td>12.3 1.76 bc</td>
</tr>
<tr>
<td>Control</td>
<td>67.7 2.8 a</td>
<td>33 1.5 a</td>
<td>15.7 0.33 abc</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>67.7 3.8 a</td>
<td>34 1.2 a</td>
<td>18 1.15 a</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>61 3 ab</td>
<td>30 2.6 a</td>
<td>16 1 ab</td>
</tr>
</tbody>
</table>

\[ F=6.09; P=0.001 \] \[ F=1.33; P=0.32 \] \[ F=6.96; P=0.006 \]
Table 2 Effect of treatment and application timing on species abundance for All Taxa, Mites, and Collembola collected from soil cores. Means in a fall or spring column followed by a common letter are not significantly different according to the Tukey-Kramer HSD test with $P < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Cumulative Species Abundance</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall Treatment</td>
<td>All Taxa</td>
<td>Mites</td>
<td>Collembola</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±SE</td>
<td>±SE</td>
<td>±SE</td>
</tr>
<tr>
<td>Drench</td>
<td>778.7</td>
<td>183 a</td>
<td>595.7</td>
<td>147.5 a</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>820</td>
<td>109 a</td>
<td>378 77.9 a</td>
<td>321.7</td>
</tr>
<tr>
<td>Control</td>
<td>1013.3</td>
<td>51.6 a</td>
<td>491 68.5 a</td>
<td>395.7</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>901.3</td>
<td>128 a</td>
<td>599 108.1 a</td>
<td>207</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>759.3</td>
<td>81 a</td>
<td>438.3 51.1 a</td>
<td>227.7</td>
</tr>
<tr>
<td></td>
<td>(F=0.77; $P=0.57$)</td>
<td>(F=1.01; $P=0.45$)</td>
<td>(F=10.3; $P=0.001$)</td>
<td></td>
</tr>
<tr>
<td>Spring Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drench</td>
<td>586.3</td>
<td>175 a</td>
<td>495 169.2 a</td>
<td>40.3</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>701.7</td>
<td>12.8 a</td>
<td>438.3 160.5 a</td>
<td>185.7</td>
</tr>
<tr>
<td>Control</td>
<td>693</td>
<td>27.1 a</td>
<td>313.7 7.5 a</td>
<td>282</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>648.7</td>
<td>74.2 a</td>
<td>365 20.7 a</td>
<td>205</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>570</td>
<td>86.2 a</td>
<td>319 71.5 a</td>
<td>193.7</td>
</tr>
<tr>
<td></td>
<td>(F=0.31; $P=0.87$)</td>
<td>(F=0.51; $P=0.73$)</td>
<td>(F=4.03; $P=0.03$)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Effect of treatments and application timing on rarefied species richness of All Taxa, Acari, and Collembola from soil cores. Means in a fall or spring column followed by a common letter are not significantly different according to the Tukey-Kramer HSD test with \( P<0.05 \). Numbers in parentheses just below group names indicate the abundance to which richness was standardized.

<table>
<thead>
<tr>
<th>Cumulative Rarefied Species Richness</th>
<th>All Taxa</th>
<th>±SE</th>
<th>Mites</th>
<th>±SE</th>
<th>Collembola</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fall Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drench</td>
<td>49.2</td>
<td>3.3 a</td>
<td>27.8</td>
<td>1.3 a</td>
<td>7.54</td>
<td>0.54 a</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>49.8</td>
<td>1.3 a</td>
<td>26.1</td>
<td>1.2 a</td>
<td>7.92</td>
<td>0.68 a</td>
</tr>
<tr>
<td>Control</td>
<td>50.7</td>
<td>1.9 a</td>
<td>29</td>
<td>1 a</td>
<td>7.37</td>
<td>1.19 a</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>52.1</td>
<td>2.7 a</td>
<td>28.1</td>
<td>0.4 a</td>
<td>8.51</td>
<td>1.18 a</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>51.7</td>
<td>3.8 a</td>
<td>28.7</td>
<td>2.3 a</td>
<td>7.62</td>
<td>0.19 a</td>
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<tr>
<td>( F=0.19; P=0.93 )</td>
<td></td>
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</tr>
<tr>
<td><strong>Spring Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drench</td>
<td>44.7</td>
<td>3.8 a</td>
<td>25.6</td>
<td>1.9 a</td>
<td>9.08</td>
<td>0.09 a</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>47.4</td>
<td>3.4 a</td>
<td>26.9</td>
<td>2.6 a</td>
<td>7.21</td>
<td>0.5 a</td>
</tr>
<tr>
<td>Control</td>
<td>54.7</td>
<td>1.6 a</td>
<td>30</td>
<td>1.3 a</td>
<td>7.6</td>
<td>0.41 a</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>54.3</td>
<td>1.8 a</td>
<td>29.3</td>
<td>1.1 a</td>
<td>8.96</td>
<td>1.02 a</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>51.4</td>
<td>2.1 a</td>
<td>26.9</td>
<td>1.1 a</td>
<td>8.35</td>
<td>1.06 a</td>
</tr>
<tr>
<td>( F=2.65; P=0.10 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\( F=1.18; P=0.38 \) \( F=1.30; P=0.33 \)
Table 4 Effects of treatments and application timing on Shannon's diversity index for richness and evenness for All Taxa, Acari, and Collembola from soil cores. Means in a fall or spring column followed by a common letter are not significantly different according to the Tukey-Kramer HSD test with $P<0.05$.

<table>
<thead>
<tr>
<th>Cumulative Shannon's Index</th>
<th>Fall Treatment</th>
<th>All Taxa ±SE</th>
<th>Mites ±SE</th>
<th>Collembola ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drench</td>
<td>3.3 ±0.11 a</td>
<td>2.86 ±0.11 a</td>
<td>1.86 ±0.09 a</td>
<td></td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>3.24 ±0.08 a</td>
<td>2.68 ±0.12 a</td>
<td>1.84 ±0.18 a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3 ±0.13 a</td>
<td>3 ±0.01 a</td>
<td>1.77 ±0.12 a</td>
<td></td>
</tr>
<tr>
<td>Soil Injection</td>
<td>3.4 ±0.08 a</td>
<td>2.81 ±0.05 a</td>
<td>1.93 ±0.18 a</td>
<td></td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>3.4 ±0.07 a</td>
<td>3 ±0.1 a</td>
<td>1.78 ±0.05 a</td>
<td></td>
</tr>
</tbody>
</table>

$(F=0.46; P=0.76)$  $(F=2.30; P=0.13)$  $(F=0.16; P=0.95)$

<table>
<thead>
<tr>
<th>Spring Treatment</th>
<th>All Taxa ±SE</th>
<th>Mites ±SE</th>
<th>Collembola ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drench</td>
<td>3.1 ±0.2 a</td>
<td>2.71 ±0.16 a</td>
<td>1.98 ±0.15 a</td>
</tr>
<tr>
<td>Foliar (Hort. Oil)</td>
<td>3.1 ±0.15 a</td>
<td>2.65 ±0.21 a</td>
<td>1.71 ±0.11 a</td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ±0.08 a</td>
<td>2.97 ±0.05 a</td>
<td>1.87 ±0.1 a</td>
</tr>
<tr>
<td>Soil Injection</td>
<td>3.4 ±0.13 a</td>
<td>2.83 ±0.11 a</td>
<td>2.06 ±0.19 a</td>
</tr>
<tr>
<td>Trunk Injection</td>
<td>3.4 ±0.1 a</td>
<td>2.88 ±0.08 a</td>
<td>1.94 ±0.23 a</td>
</tr>
</tbody>
</table>

$(F=1.29; P=0.33)$  $(F=0.99; P=0.46)$  $(F=0.76; P=0.58)$
Appendix: Figures

Control (N = 3017)

Trunk Injection (N = 2263)

Soil Injection (N = 2663)

Soil Drench (N = 2325)

Foliar Oil Spray (N = 2446)

Fig. 1. Effect of treatments on proportional abundance of arthropods from soil cores in the fall treatment time over a two-year period.
Fig. 2. Effects of treatments on microarthropod abundance. A) Control vs. trunk injection; B) Control vs. foliar oil spray; C) Control vs. soil injection; and D) Control vs. soil drench. Error bars indicate Standard error about the mean.
Fig. 3. Effects of treatments on microarthropod richness. A) Control vs. trunk injection; B) Control vs. foliar oil spray; C) Control vs. soil injection; and D) Control vs. soil drench. Error bars indicate Standard error about the mean.
Fig. 4. Effects of treatments on Collembola abundance through the course of the two-year study. Error bars indicate standard error about the mean. Stars above collection dates indicate statistically significant differences as inferred from a Tukey-Kramer HSD (P < 0.05).
Fig. 5. Effects of treatments on Collembola richness through the course of the two-year study. Error bars indicate standard error about the mean. Stars above collection dates indicate statistically significant differences as inferred from a Tukey-Kramer HSD (P < 0.05)
Fig. 6. Effects of treatments and application times on distribution of abundance of all-taxa.
Fig. 7. Effects of treatments and application times on Shannon's Diversity index for the cumulative microarthropods. Bars with a common letter are not significantly different according to the Tukey-Kramer HSD test ($P<0.05$). Error bars indicate standard error about the mean.
Fig. 8. Effect of treatments and application times on microarthropod abundance. Bars with a common letter are not significantly different according to the Tukey-Kramer HSD test ($P < 0.05$). Error bars indicate standard error about the mean.
Fig. 9. Effects of treatments and application times on microarthropod species richness. Bars with a common letter are not significantly different according to the Tukey-Kramer HSD test ($P < 0.05$). Error bars indicate standard error about the mean.
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Bars with a common letter are not significantly different according to the Tukey-Kramer HSD test ($P < 0.05$). Error bars indicate standard error about the mean.

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Chapter 3

Imidacloprid concentrations in hemlock soils following hemlock woolly adelgid chemical treatment
Abstract

Objectives in this study were to determine active ingredient, imidacloprid (IMI), concentrations in soils following insecticide treatments for the invasive insect, hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, in an eastern hemlock forest, *Tsuga canadensis* (L.) Carriere. Imidacloprid was extracted from soil cores and quantified with high-performance liquid chromatography (HPLC). Insecticide application methods were imidacloprid soil drench, imidacloprid soil injection, imidacloprid stem injection, and untreated controls. Soil drench had the highest concentrations of imidacloprid (8.88, 7.54, and 5.94 mg IMI / kg dry soil in November 2005, January 2006, and April 2006). Soil injection treatment displayed infrequently high concentrations of imidacloprid, due to the localized soil injection procedure (1.45, 42.1, and 1.56 mg IMI / kg dry soil in November 2005, January 2006, and April 2006). Tree injections had detectable amounts of imidacloprid, as well, indicating that active ingredient is fed into soil by either litterfall or root exudates (0.49mg, 0.14mg, and 0.49mg of imidacloprid / kg dry soil in November 2005, January 2006, and April 2006) Untreated controls did not have any imidacloprid detected in soils. An understanding of concentrations of imidacloprid in soil following treatment for HWA can be used to assess non-target risk of HWA chemical control methods.
Introduction

Background: Imidacloprid

Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a systemic, neonicotinoid insecticide used in a wide range of forest, landscape, and crop systems to control piercing-sucking insect pests. Imidacloprid is currently one of the most popular insecticides in the world (Cox et al. 1998a), because of the novel mode of action, low applications rate, duration of effect, and favorable toxicological and environmental profiles.

Imidacloprid has a novel mode of action that is useful in avoiding development of insecticide resistance in systems that have historically used chemicals with other modes of action. Imidacloprid’s insecticidal activity is attributed to its interference with nicotinic acetylcholine receptors in insects (nAChR) (Abbink 1991, Bai et al. 1991, Tomizawa and Yamamoto 1993, Tomizawa and Casida 2005, Tomizawa et al. 2007). This action is highly specific to insect nACh receptors, thus imidacloprid displays low mammalian toxicity (Tomizawa and Casida 2005). Neonicotinoid systemic insecticides are increasingly replacing organophosphates and methylcarbamates for management of piercing-sucking insect pests (Tomizawa and Casida 2005).

Imidacloprid is systemic, and therefore, is commonly applied directly to the soil in the root zone of plants. Imidacloprid is absorbed by the roots and
translocated to the rest of the plant, leading to effective control of plant-feeding insect pests.

Half-life of imidacloprid was determined, ranging from 40-129 days in soils associated with imidacloprid-coated sugar beet seeds (Rouchaud et al. 1994). Imidacloprid has a long lasting insecticidal activity because it binds to soil organic matter and, thus, is available for plant uptake and presentation to the pest for an extended period of time.

Imidacloprid is soluble in water, leading one to believe that it would be leached through soils to pollute groundwater. However, the chemical binds very strongly to organic matter, and so has low leaching potential in organic soils. Sorption of imidacloprid is positively correlated with organic matter amendments in soil (Cox et al. 2004). The strength of sorption to organic matter, and thus persistence, in soil was shown to increase with time (Oi 1999). The binding of imidacloprid to the soil is strong and it is not released readily. Thus, the compound remains in the upper root zone and does not leach into groundwater (Krohn and Hellpointner 2002).

Ecosystem processes and biological diversity in the soil at risk

The invasion of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, into eastern North American forests elicited a response from forest managers. Insecticide treatments containing the active ingredient, imidacloprid, have been used to protect the eastern hemlock, *Tsuga canadensis*, as a foundation species in eastern North American forests. In the Great Smoky
Mountains, alone, more than 56,000 hemlocks have been treated with imidacloprid and horticultural oil sprays (T. Remaley, personal communication). Imidacloprid is the active ingredient in most of the applications used to control HWA. Most of these insecticide applications involve applying insecticides to the soil in the root zone of the hemlock trees. Concerns have been raised about the potential for non-target declines in soil arthropods following soil applications of imidacloprid. The objectives of this study were to determine if imidacloprid was present in soils following HWA chemical treatments in eastern hemlock forests, rates of disappearance from soil over time, and differences in imidacloprid concentrations among the four treatments (imidacloprid soil drench, as part of a larger study to determine non-target effects of these treatments on non-target soil arthropods.

**Materials and Methods**

*Field Sites*

Experimental site was established near the invasion front of HWA surrounding the Indian Boundary Campground in the Cherokee National Forest, Monroe County, TN. All of the plots were located between 545m (1789’) and 550m (1804’) in elevation and within a 0.549km (0.34 mile) radius of N35 23.858, W84 06.525. Thirty hemlocks with little to no adelgid infestation and good qualitative health ratings were selected as experimental plots. In addition, each hemlock canopy was adequately isolated from canopies of other hemlocks to avoid overlap in treatment zones.
Experimental Design

A replicated field experiment was established in November 2005 to test non-target effects in hemlock associated soil arthropods caused by the most common chemical control methods of hemlock woolly adelgid. Soil cores were collected in November 2005, January 2006, and April 2006 to quantify the average concentrations of the active ingredient, imidacloprid, in soils underneath hemlock trees treated following common chemical control methods used against the adelgid. The thirty hemlocks were organized into fifteen pairs of trees. Trees were randomly classified into the five treatment groups to give the experiment 3 blocks of 5 treatments. Treatments were administered on November 29-30, 2005. This design allowed the testing of differences in persistence of imidacloprid concentrations in the three chemical treatment plots against untreated control plots.

Treatments

Chemical pesticide treatments mimicked the four most common application methods used by forest managers to control HWA. The five insecticide treatments were the foliar horticultural oil application, imidacloprid soil drench, imidacloprid soil injection, imidacloprid trunk injection, and untreated control plots.

Foliar spray treatments were the only insecticide treatments included in the study that did not contain imidacloprid. The foliar spray treatments consisted
of horticultural oil. Thus, these plots were not included in the chemical concentrations analysis.

Trunk injections of the imidacloprid formulation Imicide® were performed with the Mauget® system. A hole, 1.75cm (11/16") in diameter, was drilled to a depth of 1.27cm (1/2") at a slight downward angle in the trunk of the tree 20.3cm (8") above the soil, per the label instructions. Each Imicide capsule contained 3ml of 10% imidacloprid solution. One of the pressurized capsules was inserted into the corresponding hole for every 15cm of stem diameter at breast height (dbh), to give an application rate of 0.15ml of imidacloprid per 2.54cm dbh. Capsules remained inside the hole in the trunk until the contents of each were emptied. The capsules were then removed and discarded.

Soil injections consisted of a small volume of a highly concentrated solution of imidacloprid in water that was applied with a Kioritz® soil injector 6-8cm beneath the soil surface near the base of the hemlock trunk at a rate of 1.0g of imidaclorid per 2.54cm dbh. Merit® 75 WP was mixed in 60ml of water inside of the injector. The volume of solution injected into the soil at each plot varied with the dbh of the hemlock tree being treated.

The soil drench treatment was administered by soaking the soil underneath the drip-line of each soil drench hemlock plot with a high volume of a relatively lower concentration solution of Merit® 75 WP. Each soil drench was applied at a rate of 1.5g of active ingredient per 2.54cm dbh. A large volume, approximately 125L (33 gallons), of imidacloprid and water is applied directly to the soil surface with an FMC® high pressure sprayer.
**Soil core collection**

Four soil cores (15cm deep, 3cm diameter) were collected from each plot in November 2005, January 2006, and April 2006. Soil cores were dried and kept out of the light and below freezing to disallow degradation of imidacloprid until the extraction procedure could be performed. The EPA states that soil cores can be stored in this manner for up to 24 months without changing the results.

**Extraction of imidacloprid from soil samples**

From each soil core, a 20g dry weight subsample (Bonmatin et al. 2003) was placed into 70ml of water and placed on a shaking table for one hour. Water was an excellent solvent for quantifying the concentration of imidacloprid biologically available for plant uptake and leaching (Felsot et al. 1998). The pH of the soil and water mixture was then lowered with acetic acid and then raised again with sodium bicarbonate to induce the release of imidacloprid from its strong bonds with organic matter. The solution was vacuum filtered from the soil and mixed with two 30 ml methylene chloride elutions in a 125ml separatory funnel (Felsot et al. 1998). The methylene chloride was collected into a 75ml round bottomed flask then dried in a 50ºC water bath under vacuum in a Rotovap. The residue was then dissolved into 1ml of 1:1 Acetonitrile:water solution. The samples were syringe filtered and placed into chromatography vials (Baskaran et al. 1997).
Samples were analyzed with high performance liquid chromatography. The mobile phase consisted of a 0.1% solution formic acid in water and acetonitrile (20:80) (Proenca et al. 2005). The isocratic flow rate of the mobile phase was 1mL per minute. Samples were analyzed with a UV sensor set at 270nm wavelength (Proenca et al. 2005). Standard solutions of known concentrations were run in addition to samples to establish a standard curve. Imidacloprid standards were used to determine that the compound had a retention time of 8-10 minutes.

**Statistical Analysis**

The area inside the imidacloprid peaks were calculated in the Breeze software results analysis interface. Peak areas observed from internal standards of known concentrations and were used to establish standard curves. Observed peak areas correlated to peak area of known concentrations of imidacloprid through regression analyses to find the observed imidacloprid in mg per 20g dry soil. These values were standardized to mg imidacloprid / kg dry soil. Mean values for each season were calculated and statistical significance was inferred from ANOVA and Tukey-Kramer Honestly Significant Difference test with $P < 0.05$.

**Results:**

The most consistently, high concentrations of imidacloprid were observed in soil cores from the drench treatment. Soil drench plots in November 2005,
immediately following insecticide treatments had concentrations of 8.88 mg IMI / kg dry soil. This concentration decreased in the January 2006 collection to 7.54 mg IMI / kg dry soil, and further decreased in the April 2006 collection to 5.94 mg / kg dry soil. Means for the soil injection treatment plots in the same collection times were 1.45, 42.1, and 1.56 mg IMI / kg dry soil. Concentrations of imidacloprid were lower in the soil injection plots on average, but much more sporadic, than in drench plots. Collection of soil cores were less likely to be taken from one of the points of injection, than from the widespread drench treatment. When imidacloprid was observed in soils from the soil injection plots, the concentration was very high. Soil from tree injection plots were observed to contain low levels (0.49mg, 0.14mg, and 0.49mg of imidacloprid / kg dry soil in November 2005, January 2006, and April 2006, respectively) even though insecticide was not applied directly to the soil. Concentrations of imidacloprid were sporadically observed in the soil injection plots. Low, but consistent, imidacloprid concentrations were observed in the trunk injected sites, and no imidacloprid was found in the control plots (Fig. 29, 30, and 31).

Discussion

Observable concentrations of imidacloprid were present in each HWA imidacloprid insecticide treatment over the six months of observations. Soil drench had the highest concentrations of imidacloprid following treatment. Spraying a large volume of insecticide solution to the soil beneath the drip line of
the hemlock distributed the active ingredient in the highest concentrations more or less evenly.

Soil cores from soil injection plots were observed to have sporadically, high concentrations of imidacloprid. Soil cores were randomly sampled from underneath the drip line of the tree, and not necessarily from the points of insecticide injection that were very close to the stem. The active ingredient was in exceedingly high concentrations near the point of injection and did not spread to the rest of the soil in the drip line of the tree.

Imidacloprid was surprisingly collected in soils from tree injection plots. It has been thought that applying imidacloprid to the stem of the tree was a way to avoid non-target effects in soil communities, because the active ingredient reached neither the soil nor the decomposer food web. However, imidacloprid did occur in the soil from tree injection plots, possibly due to direct leakage during injection, presence in senesced, plant tissues from litterfall, or from root exudates. Empirical studies to elucidate these mechanisms were not performed in this study.

Imidacloprid concentrations observed in this study were conducted as part of a larger study to determine the extent of non-target effects of imidacloprid insecticide treatments used to control the invasive pest, hemlock woolly adelgid, *Adelges tsugae* Annand. Laboratory microcosms predicted that 50% mortality of *Folsomia candida* Willem (Collembola: Isotomidae) adults will occur at an
imidacloprid concentration of 1.38 mg imidacloprid / kg dry soil, and that reproduction will be reduced by 50% at a concentration of 0.598 mg imidacloprid / kg dry soil (Reynolds 2008, Chapter 3). Drench treatments observed in treatment plot soils in this study readily exceeded these concentrations even six months after treatment, which leads one to believe that imidacloprid treatments in the field may lead to non-target impacts to springtails. Non-target effects of treatments on soil arthropod species composition were caused by decreases in Collembola abundance and richness in this same manipulative field experiment following treatment with imidacloprid used for HWA control (Reynolds 2008, Chapter 2).

Imidacloprid concentrations in the soil have implications leading to the optimization of chemical control tactics of HWA and the reduction of non-target impacts on soil arthropod communities. These arthropods are important members of the decomposer food web responsible for litter turnover and nutrient cycling. In addition, the information provided in this study may be used to speculate on non-target impacts to soil communities in the numerous other systems in which imidacloprid is utilized.
Appendix: Figures

Fig. 29. Effects of treatments on imidacloprid concentrations from soils immediately following treatments in November 2005. Bars with common letters are not considered significantly different according to a Tukey-Kramer HSD test with $P < 0.05$. Error bars indicate 95% confidence intervals about the mean.
Fig. 30. Effects of treatments on imidacloprid concentrations of three months following treatments in January 2006. Bars with common letters are not considered significantly different according to a Tukey-Kramer HSD test with $P < 0.05$. Error bars indicate 95% confidence intervals about the mean.
Fig. 31. Effects of treatments on imidacloprid concentrations in soils six months following treatments in April 2006. Bars with common letters are not considered significantly different according to a Tukey-Kramer HSD test with $P < 0.05$. Error bars indicate 95% confidence interval about the mean.
Chapter 4

_Folsomia candida_ tolerance to imidacloprid concentrations in laboratory microcosm soils
Abstract

Imidacloprid (IMI), 1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine, is the active ingredient of most insecticides labeled for control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, which is an invasive pest causing declines in the eastern North American forest species, eastern hemlock, *Tsuga canadensis* (L.) Carriere. The use of imidacloprid formulations against HWA is widespread in potentially sensitive conservation areas, such as the Great Smoky Mountains National Park. Understanding Collembolan tolerance of imidacloprid in soils is fundamental to minimizing non-target effects in the soil faunal community caused by prevailing HWA-insecticide control tactics. A four-week, replicated microcosm experiment was conducted in which *Folsomia candida* (Collembola: Isotomidae) were reared on standard soil substrates containing a range of concentrations of imidacloprid. *Folsomia candida* reproduction was reduced by imidacloprid in treatments equal to or greater than 0.24 mg IMI/kg dry substrate. The mean adult survival of *F. candida* was reduced in treatments equal to or greater than 2.1 mg IMI/kg dry substrate. Regression indicated that the predicted concentration at which 50% adult mortality occurred was 1.38 mg IMI/kg dry soil, and that a 50% reduction in reproduction rate occurred at 0.598 mg IMI/kg dry soil.
Introduction

Imidacloprid (IMI), 1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine, is the active ingredient of most insecticides labeled for control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, which is an invasive pest causing declines in the eastern North American forest species, eastern hemlock, *Tsuga canadensis* (L.) Carriere. A unique attribute of hemlock forests is the high microarthropod diversity, which is comprised primarily by mites (Acari) and springtails (Collembola). Understanding Collembolan tolerance of imidacloprid in soils is fundamental to minimizing non-target effects in the soil faunal community caused by prevailing HWA-insecticide control tactics.

Imidacloprid is often used in agriculture, forestry, and industry. Imidacloprid is a synthetic derivative of nicotine. It is the most popular of the new class of pesticides termed neonicotinoids. Insecticides containing imidacloprid are available to the public and are among the most widely used insecticides in the world due to their novel mode of action, low application rate, longevity, efficacy, selectivity, and relatively low environmental impact (Cox et al. 1998a, Cox et al. 1998b). Mode of action is unique in that it blocks the activity of nicotinic acetylcholine receptors (nAChR) in insect nervous systems (Abbink 1991, Bai et al 1991, Tomizawa et al 1992; 2007).

Imidacloprid is applied to soil as a plant systemic insecticide for uptake by roots and translocation of active ingredient to the rest of the plant. Insecticidal activity is observed at low application rates because piercing-sucking pests feed
directly plant fluids containing active ingredient. Insecticidal action displays endurance of efficacy and low leaching potential due to its strong binding to organic matter in soil (Cox et al. 1998a, Cox et al. 1998b, Oi 1999, Papiernik et al. 2006). In addition, the insecticide has low leaching potential due to binding with organic soils and low levels of mammalian toxicity (Abbink 1991, Bai et al. 1991, Tomizawa and Yamamoto 1993, Tomizawa and Casida 2005, Tomizawa et al. 2007). Evidence indicates that non-target declines occur in Collembola abundance and richness following imidacloprid soil drench applications for HWA control (Reynolds 2008, Chapters 2 and 5). Understanding tolerance of Collembola to residual concentrations of imidacloprid is fundamental to strengthening HWA chemical management techniques by reducing non-target impacts. Determination of *Folsomia candida* Willem (Collembola: Isotomidae) tolerances to soil pollutants in the laboratory can be compared to predicted or observed concentrations of pollutants in field situations to determine the potential for environmental risk (Reynolds 2008, Chapter 3).

*Folsomia candida* is commonly used in laboratory toxicology studies to estimate tolerances of soil Collembola to a wide variety of soil pollutants. In a review of *Folsomia candida* biology, history, and utility to ecotoxicology, Fountain and Hopkin (2005) described *Folsomia candida* as an excellent candidate for toxicology studies due to its ease of rearing in the laboratory and short generation times at room temperature (Fountain and Hopkin 2005).
A four-week, replicated microcosm experiment was conducted in which *Folsomia candida* (Collembola: Isotomidae) were reared on standard soil substrates containing a range of concentrations of imidacloprid. Objectives of the study were to determine concentrations of imidacloprid in standard soil substrate at which *Folsomia candida* colonies displayed 50% mortality (LC$_{50}$) and 50% reduction in reproduction of juveniles (EC$_{50}$) in laboratory microcosms.

**Materials and Methods**

Experiments were designed following the protocols provided by the International Organization of Standardization (ISO 1999) for toxicology studies on inhibition of reproduction and survival of *Folsomia candida* by soil pollutants. A large culture of *Folsomia candida* was established on activated charcoal and plaster of Paris substrate from laboratory stock. Springtails were fed Fleischmann Active Dry® yeast and water was added to substrate twice per week. Large cultures of *F. candida* were divided into fresh containers to induce egg production. Eggs were collected over a two-day period and isolated in new containers to isolate a large number of 10-12 day old juveniles.

Ten juvenile springtails of the same age (10-12 days) were portioned into each replicate microcosm. Replicate microcosms were consisted of a 100mL screw-top jar that contained 25.8g dry mass standard soil substrate (10% Sphagnum peat, 20% kaolinite clay, and 70% industrial quartz sand), 2mg of Active Dry yeast, and a 4.2ml portion water containing the appropriate imidacloprid concentration. Reagents were prepared from solid imidacloprid from...
the Bayer Corporation® in a liquid-liquid dilution series, and included 2.1 and 1.05 mg IMI / kg dry substrate concentrations, which were prepared separately.

Test containers were spatially randomized to standardize for potential differences in light or temperature in the laboratory. Containers were momentarily opened twice a week to allow for aeration. After two weeks, an additional 2mg of Fleischmann Active Dry® yeast was added to each container.

At the end of four weeks, the test was concluded. From each test container, substrates and springtails were washed into a 1-liter flask with approximately 300mL of water. The water and substrate mixture was stirred lightly with a spatula to induce springtails to float to the surface of the water. Springtails on the surface of the water in each sample were photographed in order to facilitate quantification of the abundance of adult and juvenile specimens. Because juvenile numbers were so great in some of the treatments, they were estimated by counting a linear transect in the photographs.

Results

All validity requirements stated by the ISO (1999) were satisfied. The ISO guideline 11267 (1999) states that in the control containers: 1.) adult mortality cannot exceed 20%, 2.) there should be at least 100 juveniles on average, and 3.) the coefficient of variation should not exceed 30% to consider the test valid. The control replicates in this study fit well within these parameters. Also, subsamples of the substrate were tested for pH and water-holding capacity, and matched test requirements.
*Folsomia candida* reproduction was reduced by imidacloprid in treatments equal to or greater than 0.24 mg IMI/kg dry substrate (Fig. 32). The mean adult survival of *F. candida* was reduced in treatments equal to or greater than 2.1 mg IMI / kg dry substrate (Fig. 33).

Regression analyses were performed of adult survival and juvenile production. The concentration at which 50% of the adults were predicted to survive (LD$_{50}$) was calculated to be 1.38 mg IMI / kg of dry substrate (Fig. 34). The concentration at which reproduction was predicted to be 50% of the control mean (ED$_{50}$) was calculated to be 0.598 mg IMI / kg of dry substrate (Fig. 35).

**Discussion**

This study shows that survival and reproduction of a standard soil arthropod, *Folsomia candida*, are affected by the presence of the insecticide, imidacloprid. Concentrations of insecticide that caused decreases in survival and reproduction are concentrations that have been observed in soils following imidacloprid application for HWA control in the field. It has been shown that average imidacloprid concentrations in soils from HWA chemical management plots can be as high as 3.5 mg IMI / kg dry soil (Reynolds et al Chapter 3). Average concentrations of imidacloprid in soil drenched plots were higher than the concentrations at which no *F. candida* adults survived this four-week laboratory study.
The number of adults observed in control containers and in containers at very low concentrations was higher than the number of adults initially added to containers. Increases in the final numbers of adults from the initial number, most likely stemmed from the maturation of first juveniles hatched during the study. *Folsomia candida* reached sexual maturity at the beginning of the 6th instar at an average age of 16.4 days (range of 13-29 days). This early instar typically only lays around 20 eggs (Snider 1973). The eggs of *F. candida* hatch on average in 7-10 days. Some of the F2 generation may have developed quickly in the conditions provided in the laboratory, and these instars may have been mistaken for the parental adults.

Although these tests were performed in the laboratory microcosms that may or may not mimic natural systems, the results from this study suggest that *Folsomia candida* is sensitive to imidacloprid. It is not reasonable to assume that all springtails are similarly sensitive to imidacloprid, because the biology of *Folsomia candida* is not universal throughout the class. Yet, these findings correspond with evidence of Collembola declines following imidacloprid applications for HWA control in replicated field experiments (Reynolds 2008; Chapter 2 and 5). *Folsomia candida* and *Heteromurus nitidus* (Collembola: Entomobryidae) were survival were similarly reduced in laboratory microcosms due to residues of Confidor®, an imidacloprid containing insecticide (Idinger 2003).

Sensitivity and resilience of Collembola and other soil arthropods should be considered during the planning and implementation of insecticide treatment
protocols. Ideally, these results will help hemlock conservation, in the future, by providing information that will aid in identifying chemical treatment methods with high efficacy of HWA control and low non-target effects in soil fauna.
Appendix: Figures

Fig. 32. Effect of increasing concentrations of imidacloprid on adult survival of *Folsomia candida*. Bars with common letters are not considered significantly different by a Tukey-Kramer HSD test with $P < 0.05$. Error bars indicate standard error about the mean.
Fig. 33. Effect of increasing concentrations of imidacloprid on juvenile production of *Folsomia candida*. Bars with common letters are not considered significantly different by a Tukey-Kramer HSD test with $P < 0.05$. Error bars indicate standard error about the mean.
Fig. 34. Regression analysis of *Folsomia candida* adult survival by imidacloprid concentration in soil. The red arrow indicates the predicted concentration at which 50% adult mortality would have occurred.
Fig. 35. Regression analysis of juvenile production of *Folsomia candida* by imidacloprid concentration in soil. The red arrow indicates the predicted concentration at which a 50% reduction in juvenile production would have occurred.

\[ y = 60.73x^2 - 244.3x + 252.9 \]

\[ R^2 = 0.815 \]

\[ F = 114.5 \]

\[ P < 0.0001 \]

EC\textsubscript{50} = 0.598 mg IMI / kg soil
Chapter 5

Synthesis: Conserving the hemlock community: Hemlock woolly adelgid chemical management vs. non-target soil arthropod effects
Abstract

In Chapter 1, a review of biology of eastern hemlock, invasion by hemlock woolly adelgid (HWA, *Adelges tsugae* Annand), hemlock decline, and HWA management practices provided a justification for assessment of non-target effects of HWA insecticide treatments on soil arthropods. In Chapter 2, empirical evidence from a manipulated field experiment indicated that overall microarthropod species composition was altered by the three imidacloprid treatments, when compared to control plots and foliar horticultural oil treatments. Microarthropod community composition changes were a consequence of decreases in abundance and richness of Collembola, which comprised approximately 35% of microarthropods in control plots. Mites comprised approximately 50% of the microarthropod community and other arthropods comprised the remaining 15%, neither of which responded to any insecticide treatments. In Chapter 3, imidacloprid concentrations in soil were quantified with high-performance liquid chromatography (HPLC). Soil drench treatments had the highest concentrations of imidacloprid, followed by soil injection, and tree injection. No active ingredient was found in control plots. In Chapter 4, results from laboratory microcosms were presented that indicated that the reproduction and adult survival of the springtail *Folsomia candida* Willem (Collembo: Isotomidae) are decreased by the presence of imidacloprid in standard soil substrates.
This final chapter’s intention is to synthesize these results into the context of the future of HWA chemical management. Do decreases in Collembola abundance and richness warrant discontinuation of insecticide treatments? Are the non-target impacts to soil arthropods an affordable or temporary loss necessary to conserve irreplaceable hemlock stands? Can these results be used to strengthen our ability to conserve hemlock ecosystems in eastern North America by reducing declines in non-target soil arthropods?

**Introduction**

Eastern hemlock, *Tsuga canadensis*, is rapidly declining in eastern North American forests due to the invasive insect hemlock woolly adelgid (HWA), *Adelges tsugae*. Insecticidal treatments containing the active ingredient imidacloprid (IMI) provide the most effective and immediate control of HWA. These pesticides are often applied to soils surrounding infested trees, which raised concerns about non-target effects on soil arthropods. Evidence in previous chapters indicated that springtail (Collembola) abundance and richness were decreased in soils following insecticide treatment for control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, in field trials (Reynolds 2008, Chapter 1) and survival and reproduction were decreased in the presence of residual imidacloprid in laboratory microcosms (Reynolds 2008, Chapter 4). Collembola are members of the soil decomposer food web. Collembola, microbes and other soil arthropods are members of the decomposer food web,
responsible for facilitation of forest ecosystem processes including litter turnover and nutrient cycling.

Hemlocks are considered a foundation species in eastern North American forests. Insecticide treatments are among the only management practices that readily protect hemlock trees from nearly certain death following infestation of HWA. Ecosystem-level consequences of hemlock decline are far-reaching into aboveground and belowground terrestrial systems and aquatic systems. Conserving hemlock stands using insecticides may be more important in the long-term than Collembola declines in the soil arthropod community following HWA insecticide treatments observed over the course of this two-year study.

It is important to evaluate the costs and benefits of insecticide treatments in hemlock forests in conservation reserves like the Great Smoky Mountains National Park. Do decreases in Collembola abundance and richness warrant discontinuation of insecticide treatments? Are the non-target impacts to soil arthropods an affordable or temporary loss necessary to conserve irreplaceable hemlock stands? Can these results be used to strengthen our ability to conserve hemlock ecosystems in eastern North America by reducing declines in non-target soil arthropods?
Cost of non-target Collembola declines vs. Benefits of hemlock protection with imidacloprid

Evidence for non-target effects in soil arthropods

This two-year study provided evidence from the field and laboratory that Collembolan survival and reproduction were decreased by imidacloprid presence in soil, which leads to decreases in springtail abundance and richness. Concentrations of imidacloprid in soils collected from the field following HWA chemical treatments exceeded concentrations (LC50 and EC50) which were not tolerated by *Folsomia candida* in laboratory microcosms (Fig. 36). Concentrations of imidacloprid in soils from treatment plots were negatively correlated with Collembolan richness (Fig. 37, $R^2 = 0.23; P = 0.0011$) and abundance (Fig. 38, $R^2 = 0.18; P = 0.0047$). In contrast, mite species richness and abundance were not affected by different imidacloprid concentrations. Mite species richness (Fig. 39, $R^2 = 0.02; P = 0.3574$) and abundance (Fig. 40, $R^2 = 0.02; P = 0.3910$) were non-significantly correlated with imidacloprid concentrations of imidacloprid in field trials. Decreases in Collembolan richness and abundance along with increases in imidacloprid concentrations in soils provide further evidence that springtails are affected by HWA imidacloprid applications.

If Collembola of special conservation interest occur in a proposed treatment area, imidacloprid application would not be recommended. To date, no
springtail is listed as threatened or endangered. Yet, soil arthropod species are be
being discovered every year by taxonomists and groups such as Discover Life in Ameri
America. Levels of endemism of Collembola are unknown. Mutualisms between Collembola
and important microbial, arthropod, or plant associates may exist, and decreases in Collembola may lead to unforeseen indirect effects.

Abundance and richness decreases along with compositional shifts in soil arthropods may alter important processes that free nutrients from litter that collects on the forest floor for cycling back into forest biomass. Alterations of ecosystem processes due to reductions in Collembola were not tested in this study, and warrant further examination.

Protecting hemlocks in spite of non-target effects

In Chapter 1, a review of the importance of eastern hemlock in eastern North American forests was provided. Eastern hemlock is a foundation species upon which a unique ecosystem relies in mid-elevation Appalachian Mountains. Hemlock decline due to HWA threatens faunal and floral assemblages and ecosystems in aquatic and aboveground and belowground terrestrial environments. Standing dead hemlocks are a safety hazard and have prompted closing of hiking trails in Shenandoah National Forest. Hemlock decline has also caused a decrease in hemlocks' prominence in nursery trade and landscaping on private property. All of these negative effects of hemlock decline may sum to a greater loss than that incurred by soil arthropod declines following HWA insecticide treatment.
No decreases were observed in mites and other microarthropods (excluding Collembola). This tolerance and functional redundancy in soil arthropod communities may decrease ecosystem-level consequences of Collembola decline. Mites and arthropods other than springtails comprised more than 60% of the total microarthropod abundance. Prominence and stability of these groups in treated and untreated plots is encouraging, because many ecosystem functions, like litter turnover and nutrient cycling, may be facilitated by mites and other arthropods in the stead of springtail decline.

No Collembola are listed as threatened or endangered. Soil arthropods, such as Collembola, are commonly considered to be cosmopolitan in distribution, indicating that local declines in springtails are not of conservation interest because other populations exist elsewhere.

This study was only a two-year study. Long-term effects of imidacloprid treatments on soil arthropod communities will be monitored in the future. As concentrations of active ingredient decrease due to natural degradation, one could expect that recolonization by arthropods to occur.

Towards hemlock ecosystem management

Implications for HWA management

Soil drench treatment was shown to have the highest concentrations of imidacloprid and the greatest declines in Collembola abundance and richness. Soil injection and trunk injection treatments had lower levels of imidacloprid in the
soil and more moderate declines of Collembola. Foliar sprays of horticultural oil elicited no response from any soil arthropod group.

Soil applications of imidacloprid (soil drench and soil injection) are the most effective and long term means of controlling HWA. Trunk injection of imidacloprid into hemlock trees has a poor record of successful translocation of active ingredient to the entire canopy, and thus does not provide equally effective control of HWA when compared to soil applications. Imidacloprid was detected in soils from tree injection plots, indicating that active ingredient still enters soil decomposer food webs through either litterfall or root exudates. Negative consequences incurred by the soil arthropod community due to HWA insecticide treatments, must be compared to the positive outcome of saving the trees upon which the arthropods rely.
Appendix: Figures

Fig. 36. Effects of treatments on concentrations of imidacloprid in soil compared to LC50 and EC50 of *Folsomia candida* observed in laboratory microcosms. LC50 represents the predicted concentration at which 50% adult mortality would occur. EC50 represents the predicted concentration at which a 50% reduction in juvenile production would occur.
Fig. 37. Relationship between Collembola species richness and imidacloprid concentrations from soil cores.

\[ y = -0.42x + 5.912 \]

- \( R^2 = 0.232 \)
- \( F = 12.41 \)
- \( P = 0.0011 \)
Fig. 38. Relationship between Collembola species abundance and imidacloprid concentrations in soil.

\[ y = -2.05x + 22.76 \]
\[ R^2 = 0.178 \]
\[ F = 8.93 \]
\[ P = 0.0047 \]
Fig. 39. Relationship between mite species richness and imidacloprid concentrations in soil.

The linear relationship can be described by the equation:

\[ y = -0.362x + 18.08 \]

- \( R^2 = 0.020 \)
- \( F = 0.87 \)
- \( P = 0.3574 \)
Fig. 40. Relationship between mite species abundance and imidacloprid concentrations in soil.
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Vita

Wm. Nicholas Reynolds was born in Chattanooga, TN, in November of 1977. His family moved to Hendersonville, TN, where Nick graduated from high school with honors. Interest in ecology and conservation led Nick to studies at the University of Tennessee where he received his BA, BS, and MS in Environmental Studies, Ecology and Evolutionary Biology, and Entomology and Plant Pathology, respectively.

His interests in arthropod ecology are broad. He has utilized a multidisciplinary approach (microcosms, manipulative field trials, chemical analyses, and natural experiments) to answer questions about how biological diversity and community assembly are affected by human and natural mechanisms. In addition, he is interested how ecosystem processes are affected by biological diversity and composition.

After completing his undergraduate thesis, reviewing the effects of global climate change on aquatic systems, he labored in the hemlock woolly adelgid biological control effort. During his M.S. research in Entomology and Plant Pathology at the University of Tennessee, he documented non-target impacts of pesticide treatments that are used against hemlock woolly adelgid. During his master’s work, he collaborated on a number of ecological studies to test metacommunity dynamics, productivity, elevation, competition, and disturbance effects on insect, spider, ant, springtail, and mite communities. Nick continually seeks clever methods of statistical and empirical analyses.
Nick hopes to strengthen his abilities as a soil ecologist by testing ecological theory pertaining to the maintenance of biological diversity and the assembly of communities, and how biological diversity and composition affect ecosystem processes. He has the unique ability to identify soil arthropod fauna, and is always interested in collaboration with new colleagues. Nick is a positive, self-motivated, and enthusiastic worker, with an insatiable thirst for ecological knowledge.