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Impact of Imidacloprid and Horticultural Oil on Non–target Phytophagous and Transient Canopy Insects Associated with Eastern Hemlock, *Tsuga canadensis* (L.) Carrieré, in the Southern Appalachians

Carla Irene Dilling
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Carla Irene Dilling entitled "Impact of Imidacloprid and Horticultural Oil on Non–target Phytophagous and Transient Canopy Insects Associated with Eastern Hemlock, *Tsuga canadensis* (L.) Carrieré, in the Southern Appalachians." I have examined the final electronic copy of this 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.

Paris L. Lambdin, Major Professor

We have read this thesis and recommend its acceptance:

Jerome Grant, Nathan Sanders, James Rhea, Nicole Labbé

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Carla Irene Dilling
August 2007

DEDICATION

To
Richard Allen Dilling Jr.
my husband

for his loving support, his constant humor in life, and his love of all things science

and

Grace Irene Snelbaker
and
Robert Levi Snelbaker
my parents

who have always given me undying support
and love in all my endeavors, for teaching me the value of nature, and for all the sacrifices they have made to ensure my education

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ABSTRACT

Hemlock woolly adelgid, *Adelges tsugae* Annand, is an exotic insect species dramatically reducing populations of eastern hemlock, *Tsuga canadensis* (L.) Carrieré, throughout the eastern United States. Systemic imidacloprid and horticultural oil are the two primary chemicals used to control infestations of the hemlock woolly adelgid. However, the effect of application timing (fall versus spring) and method on the translocation of imidacloprid throughout the canopy in addition to the quantity of imidacloprid translocated is unknown. Also, the potential effect of both imidacloprid and horticultural oil on non-target canopy insects is unknown. A study was initiated to determine the effect of application timing (fall versus spring) for three imidacloprid application methods (soil drench, soil injection, and tree injection) on the translocation of imidacloprid and concentration levels accumulated in eastern hemlock sap and twig and needle samples, assess the effect of these treatments and horticultural oil on the overall species richness and abundance, guild species richness and abundance, and specific species of non-target phytophagous and transient canopy insects.

Eastern hemlocks (n = 30) were selected at Indian Boundary in Cherokee National Forest located in southeast Tennessee on 5 November 2005. This test was arranged in a split plot 2 x 5 factorial complete randomized block design with three replications. Three blocks were established. Each block contained ten trees, arranged in five tree pairs, with one tree in the pair treated in the fall (29-30 November 2005) and the other during the spring (16 April 2006). Five treatments were made; horticultural oil, imidacloprid soil drench, imidacloprid soil injection, imidacloprid tree injection, and the control (no

treatment). Enzyme-linked immunosorbant assays were used to determine imidacloprid concentration in sap and combined twig and needle concentrations collected from hemlock branches at three strata (bottom, middle, and top) of the hemlock canopy collected every three months post-treatment. To determine effect on phytophagous and transient canopy insects, monthly sampling consisting of malaise traps, beat-sheets, direct observation/trunk vacuuming/handpicking, and branch pruning was conducted from 16 March 2006 - 18 April 2007.

Concentration levels progressively decline from the bottom strata to the top strata of the canopy. This trend was consistent in all chemically treated trees. Tree injections provided the lowest concentration and the most non-uniform distribution of imidacloprid throughout the canopy. Soil drench consistently provided the highest insecticide concentration within the tree across all strata.

Species richness and abundance were significantly effected by one or more application methods when compared to the control trees; however, the timing of the applications (fall versus spring) had no significant effect on the insect species. The detritivore and phytophaga guilds were effected by one or more chemical applications. Species richness was significantly lower across all guilds and differed significantly from those species on the control trees. Some 35 insect species were found to be directly effected by these chemical treatments. Of the 35 species, 27 feed directly on eastern hemlock, and as such, ingest the chemical. Eight of the species were psocopterans that feed on decaying organic material (detritivore). The soil drench had the greatest effect on species richness and abundance and guild richness and abundance among non-target phytophagous and transient canopy insects, followed by soil injection, while horticultural oil and tree injections had minimal effect. This data provides more flexibility in the timing and method of application used to have a minimal effect on non-target phytophagous and transient canopy insects.

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I. Literature Review

Eastern Hemlock

Distribution And Biology Of Eastern Hemlock

Two species of hemlocks are found in the eastern United States, eastern hemlock, *Tsuga canadensis* (L.) Carrieré, and Carolina hemlock, *Tsuga caroliniana* Engelmann. Eastern hemlock is a shade tolerant, slow growing conifer (Ward et al. 2004) found on nearly eight million hectares of forest in the eastern United States and is the dominant tree on about one million of those hectares (Schmidt and McWilliams 1996). Its geographic range extends from Nova Scotia south to northern Georgia and west to Minnesota (Figure 1). Throughout its range, eastern hemlock occurs at elevations between 300 m (984.25 ft) and 1,520 m (5,000 ft). Carolina hemlock is considered a rare relic species limited in range to the Blue Ridge Mountains in the Southern Appalachians.

Eastern hemlocks are monoecious trees that begin to produce male strobili developing from flower clusters in the axis of the needles after about 15 years. The bud scales develop around the strobili forming the male conelet. Female conelets are formed from the short, more ovate flowers found on the terminals of the previous year's branchlets. Female cones contain multiple bracts from which two ovules develop on each of the bracts. Female cones begin to open and leaf buds burst open releasing pollen in the spring that is dispersed by the wind for two weeks. After pollination receptivity, the female cones close and fertilization is completed within six weeks. Cones grow to their full size (13–19 mm in length) between late August and early September (Nienstaedt and Kriebel 1955). The female cones reopen in October with a color change from a

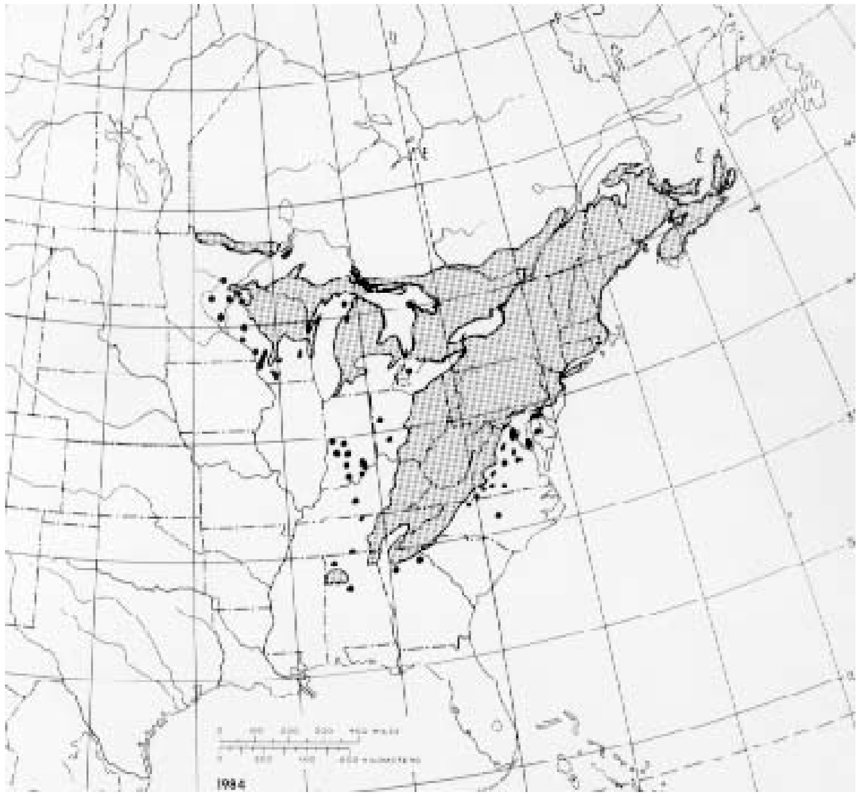

Figure 1. Native range of eastern hemlock in North America (Godman and Lancaster 2003).

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yellowish-green to a dark brown indicating a reduction in cone moisture (136 mm (5.35 in) in length). Seeds are dispersed throughout the winter months (Nienstaedt and Kriebel 1955).

Seedling development is limited by the germinative capacity which in most cases is less than 25% (USDA 1974). Ten weeks at or below freezing temperatures are required to break partial dormancy of the seed. Alternatively, light exposure can aid in breaking partial dormancy. Germination is epigeal leaving the seed susceptible to drying (USDA 1974). The seedling stage is slow in growth with most seedlings reaching an average of 31 mm in height (1.3 m (4.2 ft)) and with relatively shallow roots. Seedlings become fully established when they reach approximately 1.3 m (4.2 ft) tall and develop as saplings (Godman and Lancaster 2003). In addition to being highly intolerant of drought during this period, survival and growth of seedlings have been shown to be greatly reduced by deer browsing (Ward 2002). In some forest preserves with large herds of deer, seedlings are almost absent (Frelich and Lorimer 1985).

After completion of the sapling stage, the tree enters a pole stage consisting of trees with a dbh less than 20 cm (8 in) but greater than 2.5 cm (1 in) dbh (Godman and Lancaster 2003). Trees in this stage tend to retain good health despite suppression by overstory crowding (Tubbs 1977; Godman and Lancaster 2003). Once the tree reaches a dbh greater than 20 cm (8 in), it is considered to be mature. Eastern hemlocks generally reach maturity between 250-300 years.

Eastern hemlocks are a long lived species with some trees having life spans over 800 years (Godman and Lancaster 1990). The largest documented eastern hemlock has a height of 50 m (165 ft) with a circumference measuring 513 cm (202 in) (Blozan et al.

1995), and is located in the Great Smoky Mountains National Park ca. 1.6 km west of Brushy Mountain along Surry Fork. Two main characteristics of eastern hemlock allow its high survival rate as an understory tree. One is the high degree of shade tolerance exhibited by eastern hemlocks that contributes to the tree's survival in the understory with as little as 5 % of full sunlight (Godman and Lancaster 1990). As a result, eastern hemlocks often produce dense canopies extending almost to the forest floor (Ward et al. 2004). The deep, dense canopies form cool, moist microclimates contrasted to other hardwood stands of similar age in the same area (Daubenmire 1931; Friesner and Potzger 1932, 1934, 1936, 1944; Hough 1945; Moore et al. 1924; Oosting and Hess 1956; Shreve 1927; Ward et al. 2004). The second characteristic is the ability of eastern hemlocks to maximize rates of photosynthate storage during the winter when surrounding hardwoods are bare, enabling development under a variety of deciduous trees (Hadley and Schedlbauer 2002; Ward et al. 2004). As a late successional climax species capable of colonizing established forest stands, they can become a dominant species within the stand, if left undisturbed (Graham 1941; Hough 1936; Martin 1959; Quimby 1996).

Eastern hemlocks have a shallow root system, and as such, are drought and flood intolerant (Graham 1943; McIntyre and Schnur 1936; Secrest et al. 1941; Stickel 1933). Shallow root systems also make them vulnerable to wind throw (Willis and Coffman 1975). The healthiest eastern hemlock stands are found on north and east facing slopes and in gorges characterized by high humidity and cool temperatures during all seasons (Benzinger 1994a, 1994b, 1994c; Thornthwaite 1948).

Associated Forest Cover

Eastern hemlock is associated with 29 forest cover types (Eyre 1980). It is dominant in four forest cover types: in the north, it is associated with white pine-hemlock (Type 22), eastern hemlock (Type 23), and hemlock-yellow birch (Type 24); in the mid-west, yellow-poplar-eastern hemlock (Type 58). It is commonly found in association with the seven forest cover types: white pine-northern red oak-red maple (Type 20), eastern white pine (Type 21), red spruce-yellow birch (Type 30), red spruce-sugar maple-beech (Type 31), red spruce (Type 32), red spruce-balsam fir (Type 33), and red spruce-Fraser fir (Type 34). In addition it is a minor component of 18 forest cover types (Table 1).

The deep dense evergreen canopy produced in mature eastern hemlock stands reduces the amount of light that reaches the forest floor and reduces diversity in ground cover (Simpson et al. 1990). Dominant plants in the understory are well adapted to developing in minimal sunlight and include: great rhododendron, *Rhododendron maximum* (L.), doghobble, *Leucothoe fontanesiana* (Steud.), common witchazel, *Hamamelis virginiana* (L.), mountain silverbell, *Halesia tetraptera* var. *monticola* (L.), mountain pepperbush, *Clethra acuminate* Michx., sourwood, *Oxydendrum arboreum* (L.), woodfern, *Dryopteris* spp., goldthread, *Coptis groenlandica* Salisbury, seges, *Carex* spp., moss, *Polytrichum* spp., starflower, *Trientalis borealis* (Hook), and clubmoss, *Lycopodium* spp. (Rogers 1980; Eyre 1980; Willis and Coffman 1975; Alverson et al. 1988).

Table 1. Forest types in which eastern hemlocks are a minor component (Eyre 1980).

Type number*	Forest Type
15	Balsam Fir
17	Pin Cherry
18	Paper Birch
25	Sugar Maple-Beech-Yellow Birch
26	Sugar Maple-Basswood
27	Sugar Maple
28	Black Cherry-Maple
35	Paper Birch-Red Spruce-Balsam Fir
37	Northern White-Cedar
39	Black Ash-American Elm-Red Maple
44	Chestnut Oak
52	White Oak-Black Oak-Northern Red Oak
53	White Oak
57	Yellow-Poplar
59	Yellow-Poplar-White Oak-Northern Red Oak
60	Beech-Sugar Maple
97	Atlantic White-Cedar
108	Red Maple

***Society of American Foresters (SAF) recognized forest types.**

Insects Associated With Eastern Hemlock

Several studies have focused on insect communities and their association with a specific tree, all varying relative to species richness and abundance (e.g., Hijii 1986; Moran and Southwood 1982; Nielsen 1975; Schowalter 1989; Schowalter et al. 1981; Southwood 1961; Winchester 1997). Trees in general are structurally complex; thus, provide numerous niches for arthropods to occupy resulting in a diversity of insects that are associated with specific host trees (Moran and Southwood 1982; Lawton 1978; Strong and Levin 1979). Studies in Tennessee have focused on dogwood, yellow poplar, southern magnolia, northern red oak, and eastern hemlock, with varying species richness and abundance as well (Neitch 1995; LaForest 1999; Werle 2002; Stanton 1993; Trieff 2002; Buck et al. 2005). However, differences in species richness and abundance may be attributed to differences in sampling methodology, making comparisons across different tree species difficult. Few studies have been designed to compare arthropod communities among different tree species (Moran and Southwood 1982; Stork 1987; Schowalter 1994, 1995; Didham 1997).

In the southern Appalachians, 281 species of insects were found in associated with eastern hemlock (Buck et al. 2005) representing 86 families and nine orders, and species richness was estimated at between 420 and 550 species. This study determined insect species diversity associated with eastern hemlock prior to disturbances by hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), and the use of insecticides to control this pest (Buck et al. 2005). Ellison et al. (2005a) examined the differences in ant community associated with undisturbed eastern hemlock and those in varying degrees of decline as a result of hemlock woolly adelgid infestation. Fifteen ant species were found in undisturbed eastern hemlock stands in southern New England, four southern species were found occurring at their northern boundary, (the formicids, *Prenolepis imparis* (Say), *Acanthomyops interjectus* (Mayr), and *Camponotus chromaiodes* Bolton, and the mymicinid, *Stenamma schmitti* Wheeler)(Ellison et al. 2005a). However, these species were not recorded by Buck et al. (2005).

The most abundant species found by Buck et al. (2005) was the carabid *Sphaeroderus stenostomus* Weber, which feeds exclusively on snails found on the forest floor (Arnett and Thomas 2002a; Buck et al. 2005). Two other coleopteran species were found in high abundance: *Geotrupes horni* Blanchard (Scarabaeidae), a scavenger found throughout the United States, and *Glischrochilus sanguinolentis* (Olivier), a nitidulid that feeds primarily on sap but will also feed on fungus. The second most abundant species found was *Monoclona elegantula* Johannsen (Diptera: Mycetophilidae). Mycetophilids are also known as fungus gnats most often found in damp habitats near decaying material. A few mycetophilid larvae are predaceous but most are fungivores. The most abundant hymenopteran collected was the formicid, *Aphaenogaster picea* Emery, a species indigenous to the southern Appalachian highlands, New England, and Nova Scotia (Creighton 1950). Two rare species were collected in this study, *Dryomyza simplex* Loew (Diptera: Dryomyzidae) and *Necrophilus pettiti* Horn (Coleoptera: Agyrtidae). The species *N. pettiti* is associated with cool climates near mountainous streams (Peck 2001), a microhabitat provided by eastern hemlock. In addition to those lepidopteran species reported by Buck et al. (2005), other species that were not found belonging to the families Gelechiidae, Geometridae, Lymantriidae, Noctuidae, and

Tortricidae are closely associated with eastern hemlock utilizing the tree as a food resource during their larval stage (Table 2).

Of the species associated with eastern hemlock, 24 are known to attack eastern hemlock and are considered pests; however, despite their label as pests, most do not produce extensive damage to the tree (Godman and Lancaster 2003). Known pests of eastern hemlock include: the hemlock borer (*Melanophila fulvoguttata* (Harris)), which only attacks weakened trees, three Lepidopteran defoliators: fall hemlock looper (*Lambdina fiscellaria fiscellaria* (Guenée)), spring hemlock looper (*Lambdina athasaria athasaria* (Walker)), and the spruce budworm (*Choristoneura fumiferana* (Clemons)), that cause localized mortality, the larvae of two curculionids, strawberry root weevil, (*Otiorhynchus ovatus* L.) and black vine weevil (*Otiorhynchus sulcatus* (F.)) that attack the roots of eastern hemlock, two scale insect species, hemlock scale (*Abgrallaspis ithacae* (Ferris)) and the invasive elongate hemlock scale (*Fiorinia externa* Ferris), and the invasive hemlock woolly adelgid that threatens the survival of this tree throughout the eastern U.S.

The eight guilds (Dilling et al. 2007) determined from the species collected from eastern hemlock by Buck et al. (2005) include: transient, scavenger, predator, detritivore, phytophagous, parasitoid, haematophagous, and fungivore. Also, respective species richness estimates were calculated for the various guilds (Figure 2), with the exception of the fungivore guild which was only represented by 1 species. The community documented by Buck et al. (2005) is dominated by insects belonging to transient and scavenger guilds (Dilling et al. 2007). The dominance of transient species within tree communities and the low abundance of specialist phytophagous insects have been well

Table 2. List of Lepidoptera, generation(s) per year, and time of presence for caterpillars that feed on eastern hemlock (Maier et al. 2004).

Common Name	Family	Genus	Species	Author	Generation(s) per year	Caterpillars Present
Brown Hemlock Needleminer	Gelechiidae	*Coleotechnites*	*macleodi*	Freeman	1	May- June
Fringed Looper	Geometridae	*Campaea*	*perlata*	Guenée	2	April-September
Saddleback Looper	Geometridae	*Ectropis*	*crepuscularia*	Denis and Schiffermüller	3	July-August
Dashed -lined Looper	Geometridae	*Protoboarmia*	*porcelaria*	Guenée	1	May-August
White Slant	Geometridae	*Tetracis*	*cachexiata*	Guenée	1	July-September
Pine Looper	Geometridae	*Hypagyrtis*			1	May-July
Gray Spruce Looper	Geometridae	*Caripeta*	*divisata*	Walker	1	August-October
Morrison's Pero	Geometridae	*Pero*	*morrisonaria*	Edwards	1	July-August
Spring Hemlock Looper	Geometridae	*Lambdina*	*athasaria*	Walker	1	August-October
False Hemlock Looper	Geometridae	*Nepytia*	*canosaria*	Walker	1	July-September
Yellow-lined Conifer Looper	Geometridae	*Cladara*	*limitaria*	Walker	1	May-June
Hemlock Angle	Geometridae	*Macaria*	*fissinotata*	Walker	2	July-November
Spruce Fir Looper	Geometridae	*Macaria*	*signaria dispuncta*	Hübner	2	July- November
Small Pine Looper	Geometridae	*Eupithecia*	*palpata*	Packard	1	June-October
Fir Needle Inchworm	Geometridae	*Eupithecia*	*lariciata*	Freyer	1	June-October
Transverse-banded Looper	Geometridae	*Hydriomena*	*divisaria*	Walker	1	August-November
White-fringed Emerald	Geometridae	*Nemoria*	*mimosaria*	Guenée	1	August-October
Larch Tolype	Geometridae	*Tolype*	*laricis*	(Fitch)	1	July-August
Northern Conifer Tussock Moth	Lymantriidae	*Dasychira*	*plagiata*	Walker	1	May-June
White-marked Tussock Moth	Lymantriidae	*Orgyia*	*leucostigma intermdia*	Smith	2	May-September
Rusty Tussock Moth	Lymantriidae	*Orgyia*	*antiqua nova*	L.	1	June-August
Gypsy Moth	Lymantriidae	*Lymantria*	*dispar*	L.	1	June-August
Abstruse False Looper	Noctuidae	*Syngrapha*	*abstruse*	Eichlin and Cunningham	1	May-June
Angulated Cutworm	Noctuidae	*Syngrapha*	*rectangular*	Kirby	1	May-June
Red-Marked Caterpillar	Noctuidae	*Feralia*	*jocose*	Guenée	1	May-July
Nameless Pinion	Noctuidae	*Lithophane*	*innominata*	Smith	1	June-July
Woodgrain	Noctuidae	*Morrisonia*	*latex*	Guenée	1	June-August
Confused Wooodgrain	Noctuidae	*Morrisonia*	*confusa*	Hübner	1	June-November
White Pine Cutworm	Noctuidae	*Xestia*	*badicollis*	Grote	1	May-July
Fir Harlequin	Noctuidae	*Elaphria*	*versicolor*	(Grote)	2	June-October
Tufted Spruce Caterpillar	Noctuidae	*Panthea*	*acronyctoides*	Walker	1	July-September
Early Polypogon	Noctuidae	*Polypogon*	*cruralis*	Guenée	1	September-October
White-lined Leafrollar	Tortricidae	*Amorbia*	*humerosana*	Clemens	1	July-September
Eastern Blackheaded Budworm	Tortricidae	*Acleris*	*variana*	Fernald	1	May-July
Fall Spruce Needle Moth	Tortricidae	*Argyrotaenia*	*occultana*	Freeman	2	June-July, September-October
Green Needleworm	Tortricidae	*Clepsis*	*persicana*	Fitch	1	May-June

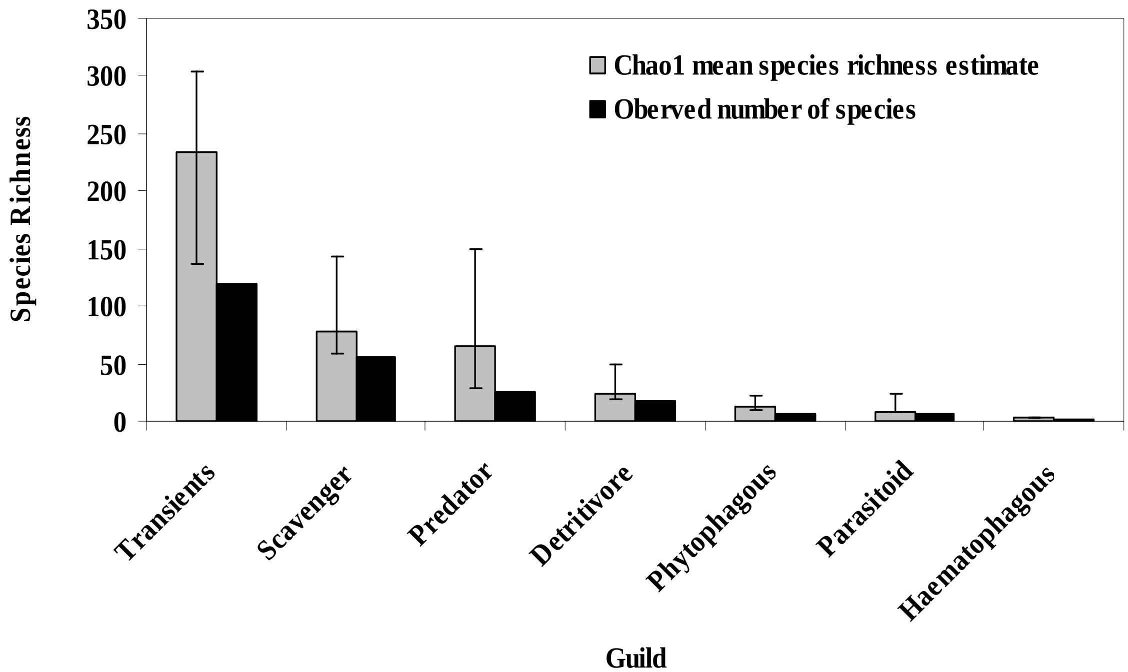

Figure 2. Chao1 mean (± 95 % confidence limits) species richness estimate and the observed number of species per guild. Means whose intervals do not overlap are significantly different.

documented in studies on tropical trees (Stork 1987, 1991; Basset, 1992, 1999; Chey et al. 1997; Basset and Novotny 1999; Novotny and Basset 2000; Ødegaard 2000). Studies are limited for coniferous trees and most do not include the transient guild. Two independent studies of predators associated with eastern hemlock produced similar results. Dilling et al. (2007) found the predatory guild determined from the Buck et al. (2005) study included 26 predatory species in the orders Coleoptera, Diptera, Neuroptera, and Hymenoptera with an estimated species richness for predators of 56. An earlier study by Wallace and Hain (1999) reported 22 predatory species associated with eastern hemlock in the orders Coleoptera, Diptera, and Neuroptera.

Ninety-two percent of the insects found were canopy dwelling species. The deep dense canopy produces an inimitable habitat with gradients in light, temperature, moisture and foliage quality (Erwin 1995; Winchester 1997); thus, resulting in a unique community of insects associated with trees. Canopy insects provide a variety of functions and their responses to disturbances can alter forest productivity and nutrient cycling (Schowalter et al. 1981, 1986; Erwin 1995; Stork et al. 1997; Winchester 1997). Insect herbivores control nutrient turnover and leaf area (Janzen 1981; Wiegert and Evans 1967) and function as the primary herbivores in forest ecosystems removing between 3–20% of photosynthetic biomass in temperate deciduous and tropical evergreen forests (Coley and Aide 1991; Landsberg and Ohmart 1989; Odum and Ruiz–Reyes 1970; Schowalter and Ganio 1999; Schowalter et al. 1986; Van Bael et al. 2004). Insect parasitoids and predators function in regulating insect populations within the community (Schowalter and Ganio 1999). Insect scavengers and detritivores aid ecosystem function by breaking down organic material and recycling nutrients back into their surrounding environments.

Importance of Eastern Hemlock

Eastern hemlocks are a vital component of biological diversity, environmental stability, and economic stability within their geographic range (Beatty 1984; Buck et al. 2005; Kelty 1989; DeGraaf et al. 1992; Snyder et al. 2004). They are considered a foundational species (Ellison et al. 2005b), which is defined as “a single species that defines much of the structure of a community by creating locally stable conditions for other species, and modulating and stabilizing fundamental ecosystem processes” (Dayton 1972).

Eastern hemlock provides imperative cover species for turkey (*Meleagris* spp.), ruffed grouse (*Bonasa umbellus* (L.)), snowshoe rabbit (*Lepus americanus* Erxleben), rabbit (*Oryctolagus* spp.), and porcupine (*Erethizon dorsatum* (L.)) (Jordan and Sharp 1967; Quimby 1996; Wydeven and Hay 1996). In addition it is a vital foliage resource for deer in the winter (Lapin 1994; Reay et al. 1990), eastern hemlock is correlated to avian community composition (Tingley et al. 2002; Ward et al. 2004), is associated with over 281 species of insects (Buck et al. 2005), and its canopy is a preferred habitat for a variety of mammals (Ward et al. 2004; Wydeven and Hay 1996). Eastern hemlocks also serve as a key component of riparian habitats lowering stream temperature, stabilizing diel variation in stream temperature, regulating streamflow, and producing an aquatic environment favorable to fish and aquatic macroinvertebrates (Evans 2002; Snyder et al. 2004).

Eastern hemlock also fulfills unique ecological roles that contribute to environmental stability. The coverage produced by deep dense canopies in hemlock dominant stands moderate cold temperatures and snow depths in extreme northern climates (Lishawa et al. 2007). Deep shade and slowly decomposing acidic litter result in a microclimate characterized by temperature reduction, moisture retention, lowered rates of nitrogen cycling, and nutrient poor soils (Jenkins et al. 1999). Hydrologically, this tree fills the two roles of maintaining transpiration rates year-round with higher transpiration rates in the spring and constituting a dominant tree along riparian corridors (Ford and Vose 2007).

Economically, eastern hemlock forests provide revenue in the form of tourism in eastern Tennessee (Travel Industry Association 2006), supports production of over four million cubic feet of timber annually in the northeastern United States, are components of ornamental nurserystock worth millions of dollar (Brisbin 1970; Rhea 1996; Woodsen 2001), makes up 22 % of the softwood growing stock in the northeast (Powell et al. 1993). The wood harvested from eastern hemlock was used for making a variety of low-value containers like boxes and crates (Brisbin 1970).

Hemlock Woolly Adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae)

Origin, Distribution, and Life History

Throughout its expansion into North America, eastern hemlock populations have gone through two major declines. The first decline coincided with an increase in human forest resource use about 200 year ago (McMartin 1992). The second and most rapid decline is the direct result of the introduced hemlock woolly adelgid. Hemlock woolly adelgid has proven to be detrimental to both eastern hemlock, and Carolina hemlock in eastern North America, since its introduction in Richmond, Virginia in the 1951 (McClure 1990, 1991a; Souto et al. 1996; Royle and Lanthrop 1997; Danoff–Burg and Bird 2002). It now has a range as far north as Massachusetts, south to North Carolina and north Georgia, and west to Tennessee and West Virginia (Figure 3).

This pest of eastern hemlocks was first introduced in the western U.S. around 1924 where it had minimal impact on western hemlock, *Tsuga heterophylla* (Raf.) and mountain hemlock, *Tsuga mertensiana* (Annand) (Havill et al. 2006; McClure and Cheah 1999; Stoetzel 2002). Mitochondrial DNA analysis of the hemlock woolly adelgid introduced in western and eastern United States indicates that they represent different lineages (Havill et al. 2006). The variety of hemlock woolly adelgid found in the eastern United States matches the lineage of hemlock woolly adelgid from Honshu, Japan. The lineage introduced in the western U.S. is from an unknown source (Havill et al. 2006).

The lifecycle of hemlock woolly adelgid is parthenogenetic and bivoltine on eastern hemlock: the winter generation is known as sistens (present in the southern Appalachians from mid July–mid March) and the spring generation known as progrediens (present in the southern Appalachians from mid March–mid June) (Deal 2006). Each female is capable of laying 100-300 eggs within a protective woolly wax coating in late March.

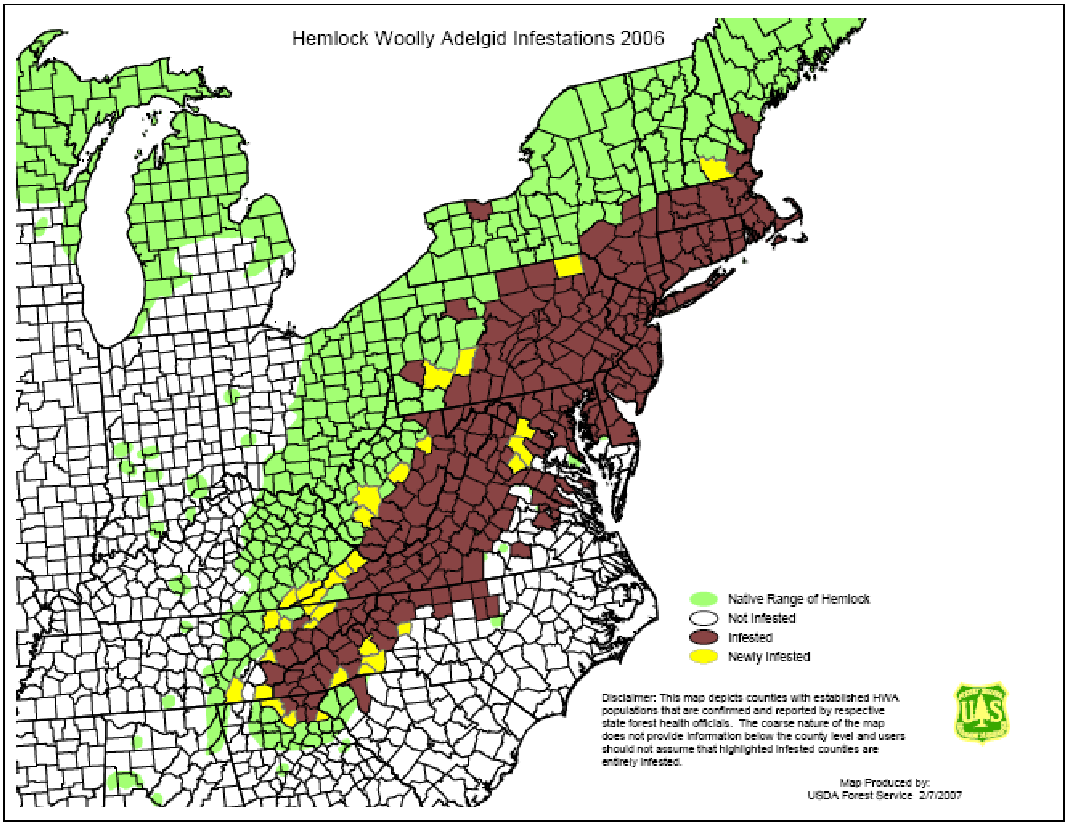

Figure 3. Distribution of hemlock woolly adelgid, *Adelges tsugae* Annand, in the eastern United States in 2006 (USDA 2006).

Eggs begin to hatch into first instars (crawlers) in April and May, and begin searching the branches for an appropriate place to settle and insert their stylets for feeding. The settled crawler inserts its stylets into the plant tissue at the base of the hemlock needles and travels to the xylem ray parenchyma cells in the branch (Young 1995). The settled crawler remains on the branch and progresses through four nymphal instars stages before reaching maturity in June. A portion of the progrediens will develop into winged sexupara, flying away from the tree in search of spruce (*Picea* spp.), which is needed to complete its lifecycle. This species of spruce does not exist in North America, so the adult starves to death before it is able to reproduce. It is suggested that the winged sexupara is density dependant and are produced in greater numbers when the health of the tree is declining (McClure 1991a).

Eggs and crawlers are reported to be transported by wind, birds, humans, and other mammals (McClure 1990), as well as through nursery stock (Gibbs 2002; McClure 1987, 1989; Ouellette 2002). Roads, riparian corridors, and major trails all have a high degree of connectivity, which enables long-distance dispersal of hemlock woolly adelgid (Koch et al. 2006). These factors all aid in the rapid dispersal rate of hemlock woolly adelgid estimated at 20-30 km per year (McClure 2001).

After establishment of hemlock woolly adelgid on eastern hemlock, two primary mortality factors that limit the size of the populations. Cold winter temperatures have been shown to reduce hemlock woolly adelgid populations (McClure 1995; Parker et al. 1998, 1999); however, there may be low abundances of cold tolerant individuals within a population (Parker et al. 1998). Intraspecific competition limits hemlock woolly adelgid populations through negative density dependent feedback (McClure 1991a; McClure et al. 2002). Interspecific competition with other herbivores, such as the elongate hemlock scale, hemlock looper, and hemlock borer is hypothesized to limit hemlock woolly adelgid populations, but competition between such species has not been convincingly documented (McClure 2001). In Japan, native predators, parasitoids, and competition severely limit hemlock woolly adelgid populations, and as such, they never reach pest status (McClure 1995, 1996; McClure and Cheah 1999).

Hemlock mortality is caused by reduced carbohydrate reserves in the tree as a direct result of adelgid feeding (Ward et al. 2004) and effects trees of all size and ages classes (McClure 2001). Carbohydrates are critical for proper growth, maintenance, reproduction, defense, and storage (Shigo 1991), and reduction of carbohydrate reserves retards development (Ward et al. 2004). Mortality generally occurs within 2 to 12 years, depending on the level of infestation (McClure 2001; Mayer 2002; Orwig 2002a, 2002b). Declining tree health is characterized by branch dieback, foliage thinning, and needle drop (McClure 2001).

Impacts of Hemlock Woolly Adelgid

Loss of this foundational tree species results in the opening of the forest floor, replaced by deciduous trees such as maple (*Acer* spp.), birch (*Betula* spp.), beech (*Fagus grandifolia*) Ehrh, and oaks (*Quercus* spp.) (Orwig and Kizlinski 2002; Sullivan and Ellison 2006) and understory vegetation like brambles (*Rubus* spp.) and sedges (*Carex* spp.) (Orwig and Kizlinski 2002; Sullivan and Ellison 2006). The replacement of hemlock with hardwood tree species results in a dramatic shift in the ecosystem processes. The unique microclimate underneath the canopy shifts from cool to warm temperatures in the summer and from warm to cold temperatures in the winter. In addition, it is suggested that such a change in the general make-up of the habitat would result in an increase in the diel thermal variation, which is more stable in eastern hemlock stands (Ellison et al. 2005b; Lishawa et al. 2007). Soil characteristics where eastern hemlocks once dominated, shift from acidic low-quality soil, with moderate C:N:P ratios, moderate metals, low rates of nitrogen mineralization and nitrification, to seasonal inputs of high quality leaf litter produced by the deciduous trees, low C:N:P ratios, low metals, high rates of nitrogen mineralization and nitrification (Evans 2002; Ellison et al. 2005b; Jenkins et al. 1999; Mladenoff 1987; Yorks 2000). The low light penetration of eastern hemlock stands are replaced with high light, shifting species poor understory of hemlock to a species rich understory (Ellison et al. 2005).

In addition to the change in ecological stability, loss of this species has the potential to effect the insect, bird, and other vertebrate species discussed in previous sections of this thesis. Economically, the loss of eastern hemlocks will reduce timber production for lumber and pulpwood (Godman and Lancaster 1990), reduce revenue from loss of tourism to states who have highly visited parks which contain a great number of hemlocks, like Tennessee, and severely impact the nurserystock industry.

Control Methods of Hemlock Woolly Adelgid

Overview

Insect control begins with monitoring for the insect pest. For small scale monitoring, a grove of a few hectares, 10-25 trees, 2-4 branches per tree, should be inspected (Ward et al. 2004). Deciding whether or not to treat is dependant upon cost-benefit analysis relative to locality (Ward et al. 2004). Proportional/percentage infestation estimates (Evans 2002) and hemlock woolly adelgid counts per 100 needles (Mayer et al. 2002) are the standards for determining infestation levels. The decision to treat is usually based on the decline of the tree's health, which has been reported at 45% infestation (Evans 2002) and at ≥ 30 hemlock woolly adelgid per 100 needles (Mayer et al. 2002).

A variety of cultural, biological, and chemical control methods can be used to control hemlock woolly adelgid. As part of a more long-term solution for this pest, a suite of biological control agents are being researched. Unfortunately, there is an immediate need for treatment of these valued trees. Hemlock woolly adelgid has been successfully controlled in both urban and limited forest settings (Cowles et al. 2006; McClure 1991b; Steward and Horner 1994; Cowles and Cheah 2002a, 2002b; Doccola et al. 2003; Webb et al. 2003) using several chemical application methods. The integration of cultural, biological, and chemical controls is considered to be the best long-term solution for controlling hemlock woolly adelgid.

Cultural

Maintaining healthy eastern hemlock trees help increase tolerance of higher densities of hemlock woolly adelgid (McClure 1995). Eastern hemlocks are drought intolerant trees and become easily stressed. Two prophylactic steps are recommended for this: 1) mulching around the tree to aid in water retention and 2) irrigation (Ward et al. 2004). Fertilizers can help improve the overall health of the tree; however, fertilizers containing nitrogen should be avoided as they increase survival and reproduction of hemlock woolly adelgid and elongate hemlock scale (McClure 1991c). Isolated trees that are infested can be cut down and small isolated branch infestations can be hand pruned (Ward et al. 2004). Although wind is the primary means of dispersal of this exotic, birds, deer, and other mammals have been documented as dispersers of eggs and crawlers. As such, discouraging these animals by removal of animal feeders or other food products that would encourage wildlife into the area is recommended.

Human movement between infested and non-infested areas is another mechanism for dispersal. Cleaning vehicles, clothing, camping gear, and recreational equipment reduce the risk of spreading hemlock woolly adelgid (Ward et al. 2004). Reducing the movement of wood products like firewood from areas of known infestations can reduce the spread of hemlock woolly adelgid (Ward et al. 2004).

Silviculturally, stands can be irrigated, reducing drought-induced stress, and large infested trees that may act as a reservoir, removed (McClure 1995). Replanting areas where there has been significant hemlock decline with natives such as white pine and the two western hemlock species, *T. heterophylla* and *T. mertensiana*, which are resistant to the hemlock woolly adelgid, is recommended as these trees act as the closest ecological homologies in North America (McClure 1995).

Biological

A number of non–native biological control agents (i.e., the derodontid *Laricobius nigrinus* Fender, and the coccinellids: *Sasajiscymnus tsugae* (Sasaji and McClure), *Scymnus sinuanodulus* Yu & Yao, *Scymnus ningshanensis* Yu & Yao, and *Scymnus campodromus*) are being reared and evaluated for mass release into infested regions as long-term biological control agents for the hemlock woolly adelgid. *Sasajiscymnus tsugae* is native to Japan and in 1922, was observed feeding on hemlock woolly adelgid in Honshu, Japan. The adelgid does not reach damaging population levels within its native range. Over 90% mortality of hemlock woolly adelgid was observed at sampled sites where *S. tsugae* was present (Sasaji and McClure 1997; Cheah and McClure 2000), making it a favored biological control agent (Cheah and McClure 2000). Currently, these predators are not uniformly established in hemlock forest throughout eastern North America, but research is promising and continues in this area. Native predators such as the multicolored lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), brown lacewings (Neuroptera: Hemerobiidae), and green lacewings (Neuroptera: Chrysopidae), have been reported to feed on hemlock woolly adelgid; however, they are not effective in controlling hemlock woolly adelgid (Wallace and Hain 2000).

Chemical

Imidacloprid and horticultural oil are the primary chemical compounds used to control hemlock woolly adelgid in both urban (McClure 1991b; Steward and Horner 1994; Cowles and Cheah 2002a, 2002b; Doccola et al. 2003; Webb et al. 2003) and limited forest (Cowles et al. 2006) environments. Forest treatment is limited to trees that are of high value as treatment of an entire forest is not practical or economically feasible. High value trees are chosen based on economic (public safety, control vs. removal costs, or salvage harvest), ecological (water quality, protection of endangered or threatened species, impact on species associated with hemlock), or aesthetic criteria (decline in tourism to area due to closed trails, visual impact of dead trees) (Ward et al. 2004). Hemlock woolly adelgid has been effectively controlled using horticultural oil (McClure 1987, 1988) in small scale infestations, but treatment is highly dependant on thorough coverage of the infested tree.

In addition to imidacloprid and horticultural oil and soap, pyrethroids have been shown to be effective against hemlock woolly adelgid. This insecticide is used less often because of its highly negative effect on non-target effects (Cowles and Cheah 2002a). Other chemical such as diazinon, ethion, and malathion have proven effective (Rhea 1996), but such organophosphates also have poor environmental and toxicological profiles. The unique mode of action of imidacloprid, degree of systemic and contact activity, variety of application methods, low application rates, extended residual control, resilient binding to soil organic matter, and good environmental and toxicological profiles result in this being one of the most widely used insecticide globally and one of the most preferred for control of hemlock woolly adelgid (Elbert et al. 1990; Elbert et al. 1991; Kagabu 1997; Cox et al. 1997; Cox et al. 1998; Silcox 2002).

The cost of treatment with horticultural oil, soil drench with imidacloprid, soil injection with imidacloprid, and tree injection with imidacloprid is highly variable. Horticultural oil and soil drenching with imidacloprid are the two least expensive methods, while tree injections are usually the most expensive. Soil injection with imidacloprid is moderate in price.

Imidacloprid

In 1985, Nihon Bayer Agrochem chemists initially synthesized imidacloprid (Elbert et al. 1998). Imidacloprid has two chemical names: one given by the International Union of Pure and Applied chemistry [IUPAC], 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, the other by the Chemical Abstracts Services [CAS], 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2 imidazolidinimine. It is a broad-spectrum chloronicotinyl insecticide, classified in toxicity classes II and II by the Environmental Protection Agency (EPA). Insecticides that contain imidacloprid have a variety of tradenames: Admire®, Bayer Advanced® Condifor®, Gaucho®, Leverage®, Premier®, Premise®, Provado®, Marathon®, Merit®, and Trimax® (Meister 1995). This compound is synthesized from nicotine and works by binding to the post-synaptic nicotinergic acetylcholine receptors, thus, disrupting nerve impulse transmission resulting in death within 24–48 hours after contact or ingestion (Bai et al. 1991; Kid and James 1991; Mullins and Christie 1995).

Imidacloprid is a broad-spectrum insecticide that has an impact on a variety of insects. In turf grass and ornamental settings, imidacloprid has been show to effectively control adelgids, aphids, lace bugs, leafminers, mealybugs, scales, thrips, whiteflies, elm leaf beetles, leafhoppers, and Japanese beetles (Dotson 1994). In forested settings, specifically trees, shrubs, flowers, and groundcover, it is recommended for the control of adelgids, aphids, armored scale, black vine weevil larvae, emerald ash borer, eucalyptus longhorn borer, flathead borers, Japanese beetles, lace bugs, leaf beetles, leafhoppers, leafminers, mealybugs, pine tip moth larvae, psyllids, royal palm bugs, sawfly larvae, soft scales, thirps, white grub larvae, and whiteflies (Bayer 2007).

Imidacloprid is usually applied by soil drench, soil injection, tree injection, foliar spray, and granular application. All of these methods, with the exception of the foliar spray, are considered systemic because the chemical is taken up by the plant and diffused across plant tissue. The foliar application is sprayed directly on the plant and has a direct contact effect. In systemic applications, imidacloprid is transported through the xylem (Steward et al. 1998; Tattar et al. 1998). In eastern hemlock, the chemical diffuses into the xylem ray parenchyma cells located in twigs in trees (Young et al. 1995), where hemlock woolly adelgid feeds. Applications of imidacloprid for hemlock woolly adelgid may be applied either in the fall or the spring.

Foliar applications, soil injections, and soil drenches of imidacloprid have been evaluated and shown to be successful in the control of hemlock woolly adelgid (Steward and Horner 1994; Rhea 1996; Steward et al 1998; Fidgen et al. 2002; and Cowles et al. 2005). The health of tree has been shown to be important in the effectiveness of imidacloprid treatments. Tree injections have been shown to be less effective than foliar application, soil injections, and soil drenches (Cowles et al. 2005), and are preferred less because of tree wounding from the injection. Tree injections not only damage the tree tissue, but the wounds can act as a portal for a variety of diseases (Steward and Horner 1994; Marion and Foster 2000; McClure et al. 2001, Smith et al. 2005). Trees under drought stress and those with needle loss and dieback have difficulty transporting systemic insecticides into the canopy (McClure et al. 2001). Damage to the tree from heavy adelgid infestations reduces the ability of the hemlock to transport imidacloprid throughout the tree (McClure et al. 2001; Webb et al. 2003). Translocation of imidacloprid in trees that have been treated with a soil injection or tree injection have been shown to occur in eastern hemlock; however, concentrations of the insecticide was only monitored for three months (Tattar et al. 1998). Reduction of adelgid populations as the result of imidacloprid treatment has shown to dramatically increase new growth, even trees in poor conditions recovered, although the rate of recovery is highly dependant on the health of the tree at the beginning of therapy (Webb et al. 2003).

Three primary metabolites produced by imidacloprid are one olefin metabolite, imidazoline, and two hydroxy metabolites, 4–hydroxy and 5–hydroxy. The olefin metabolite has been shown to be at least ten times more active than its parent compound (Nauen et al. 1998). The 4–hydroxy metabolite is just as active as the parent imidacloprid, and the 5–hydroxy metabolite is slightly less active than the parent imidacloprid (Nauen et al. 1998). These findings suggest a more long term residual effect that may be catalyzed by the breakdown of imidacloprid, resulting in longer control of pest insects (Nauen et al. 1998).

Horticultural Oil

Paraffinic oil is the active ingredient in most horticultural oils, and is a refined petroleum product. The refining process removes plant injuring aromatic, sulfur, nitrogen, and oxygen containing compounds. Horticultural oils have various tradenames such as: Sunspray®, Scalecide®, and Volck®. Horticultural oils are broad-spectrum insecticides that cover the spiracles on the insect resulting in suffocation. Horticultural oils are recommended for control of the following shade tree, shrub, ornamental, flower and foliage plant, and Christmas tree pests: aphids, adelgids, caterpillars, lacebugs, leaf beetle larvae, leafminers, mealybugs, psyllids (immature), sawfly (larvae), scales (immature), and whiteflies (immature).

Research Objectives

Hemlock woolly adelgid is a non-indigenous insect dramatically reducing eastern hemlock populations throughout the eastern United States. Systemic imidacloprid and horticultural oil are the two primary chemicals used in the control of hemlock woolly adelgid. However, the impact of application timing (fall versus spring) and method on the translocation of imidacloprid throughout the canopy and the quantity of imidacloprid translocated is unknown. Additionally the potential impact of both imidacloprid and horticultural oil on non-target canopy insects is unknown.

The objectives of this study were to:

1. Determine the impact of application timing (fall versus spring) on imidacloprid concentrations in three strata in eastern hemlock.
2. Determine the impact of application method on imidacloprid concentrations in three strata in eastern hemlock.
3. Determine the impact of horticultural oil and imidacloprid treatments on non-target phytophagous and transient canopy insects associated with eastern hemlock.

II. Impact of Application Timing and Method on the Vertical Concentrations of Imidacloprid

Introduction

Hemlock woolly adelgid, *Adelges tsuga* Annand, (Hemiptera: Adelgidae), has proven to be detrimental to both eastern hemlock, *Tsuga canadensis* (L.) Carrieré, and Carolina hemlock, *Tsuga caroliniana* Engelmann, throughout eastern North America (McClure 1990, 1991a; Souto et al. 1996; Royle and Lanthrop 1997; Danoff-Burg and Bird 2002). Imidacloprid, one of the primary insecticides used to control hemlock woolly adelgid, is primarily applied as a soil drench, soil injection, or tree injection, and can be applied in both the fall and spring. However, rates of application in terms of grams of active ingredient per 2.5 cm diameter at breast height (dbh) all vary. The recommended rate as per product label of the soil drench, soil injection, and tree injection are 1.5 g AI/2.5 dbh, 1 g AI/2.5 dbh, and 0.15 ml AI/2.5 dbh, respectively. However, the degree to which imidacloprid is translocated within the canopy with respect to these various application methods and its long-term activity in eastern hemlock is not known.

Translocation of imidacloprid in tree injected and soil injected trees has been shown to occur in eastern hemlock, but concentrations of the insecticide was only monitored for a three month post-treatment period (Tattar et al. 1998). They were not able to determine the length of time the compound remained in high enough concentrations to effectively control the target pest. Soil injections and soil drenches of imidacloprid have been evaluated and shown to be successful in the control of hemlock woolly adelgid (Steward and Horner 1994; Rhea 1996; Steward et al. 1998; Fidgen et al.

2002; and Cowles et al. 2006). Uniform distribution of effective concentrations of imidacloprid throughout the tree is imperative to successful control of this invasive pest. Currently, the effect of application timing and method on translocation of imidacloprid throughout the canopy and the quantity of imidacloprid translocated are unknown.

The objective of this study was to determine the effect of application timing (fall versus spring) and application method (tree injection, soil drench, and soil injection) on imidacloprid concentrations at various strata within the canopy.

Materials and Methods

Study Site and Experimental Design

Eastern hemlocks (n = 24) were selected at Indian Boundary in Cherokee National Forest in southeast Tennessee on 5 November 2005 to evaluate the effect of application timing and method on concentration levels within the canopy. The test site was arranged in a split-split plot 2 x 4 factorial complete randomized block design with three replications. Three test blocks were established (35° 23.787 N, 84° 06. 662 W, elevation: 543 m (1,784 ft); 35° 23.764 N, 84° 06.732 W, elevation: 555 m (1,823 ft); 35° 24.173 N, 84° 06.268 W, elevation: 565 m (1,853 ft), respectively) with each block containing eight trees. These trees were arranged in four pairs with one tree in the pair treated in the fall (29-30 November 2005) and the other during the spring (16 April 2006). To monitor translocation of imidacloprid within the tree, each tree was divided into three strata (bottom, middle, and top) or sections with each strata representing ca. one-third of the tree. Each tree was marked with a numbered identification metal tag. The basic tree characteristics were documented on 25-26 November 2005 and consisted of: tree height, transparency, density, crown class, dbh, foliage color, overall appearance, crown condition, and percent of hemlock woolly adelgid on tree. Tree pairs were selected based on how closely two trees matched morphologically with regard to these characteristics. All three blocks were located in a shortleaf pine-oak forest (type 76).

Insecticide Application

The four imidacloprid treatments evaluated were tree injection (Figure 4a), soil drench (Figure 4b), soil injection (Figure 4c), and the control (no treatment). The tree injection system consisted of the Mauget® 3 ml 10% imicide capsules and feeder tubes (J. J. Mauget Co. Arcadia, CA). The tree injection was applied at a rate of one capsule per 15 cm dbh, which is equal to 0.15 ml AI/ 2.5 cm dbh. A 0.4 cm (11/64 inch) drill bit was used to drill a hole to the depth of 1.2 cm (½ inch) at a downward angle into root flair to penetrate xylem tissue, 20.5 cm (8 in) above the ground. The feeder tubes were placed in the holes and capsules were attached to feeder tubes. Capsules were spread evenly around the circumference of the tree. Capsules were left in the tree until total uptake was completed, ranging from 1 to 5 hours.

Soil injection was made using the Kioritz® soil injector (Kioritz Corp. Tokyo, Japan). Merit ® 75 WP insecticide (Bayer, Kansas City, MO) was diluted to 1 g AI/2.5 dbh in 60 ml of water. Soil injections were made using the basal system in which injections were made within 45 cm of the base of the trunk and were spaced evenly

Figure 4. Imidacloprid applications evaluated: a) tree injection, b) soil drench, and c) soil injection.

around the tree at a depth of 7 cm (2.8 in) below the soil surface with individual injections delivering 30 ml of insecticide. The soil drench was applied using a FMC high pressure hydraulic sprayer (FMC Corporation, Jonesboro, AR). Merit® 75 WP (Bayer, Kansas City, MO) was applied at a rate of 1.5 g AI/2.5 dbh. The recommended dosage of 50 grams (5,000 mg) of Merit® 75 WP was mixed with 379 liters (100 gallons) of water for the fall and spring treatments, respectively. The soil extending from the trunk to the drip line was sprayed with 125 liters (33 gallons) of the designated insecticide.

Branch Sampling

Branch samples were taken at 3, 6, 9, and 12 months post-treatment. One 24 cm branch clipping was taken at each stratum (bottom, middle, top) using a 10 m (32.8 ft) pole pruner or an articulating boom (Genie Z 45/22, Tigard, OR) (Figure 5). Branches were immediately sealed in plastic bags, packed in dry ice, transported to the laboratory, and stored in a freezer at -18° C until sap extraction from branches.

Sap Extraction

Sap was extracted using a PMS pressure chamber (PMS instrument Co. Albany, OR) (Figure 6). Six cm of the cut end of the branch was inserted into a gland gasket and the remaining portion of the branch was placed into the pressurized chamber. The chamber was incrementally pressurized with nitrogen to 575 psi (40 bars). Sample size consisted of 300 - 400 μl of sap micro-pipetted from a collecting chamber located on top of the pressure chamber. Sap was placed back into the freezer at -18° C until quantification. No additional cleanup was needed for sap samples.

Figure 5. Collection of eastern hemlock branches using an articulating boom (Genie Z 45/22, Tigard, OR).

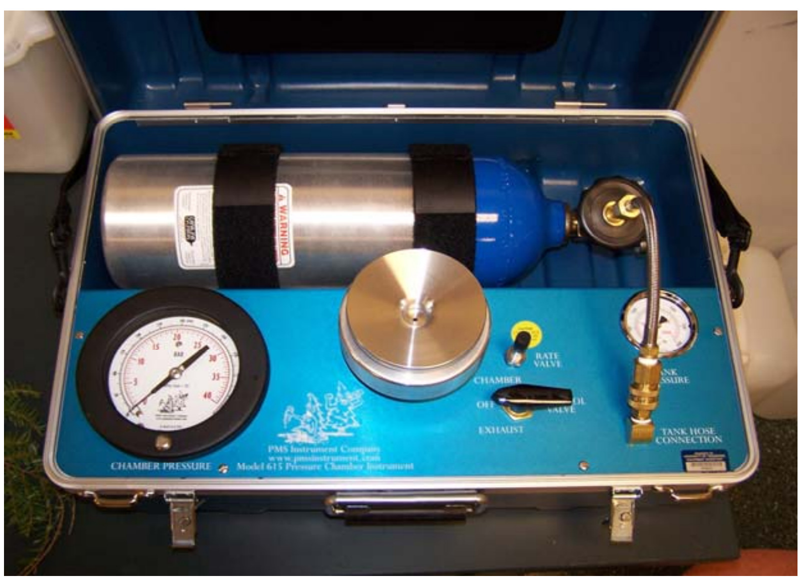

Figure 6. PMS pressure chamber used to extract sap from eastern hemlock samples to test for imidacloprid concentrations.

Needle and Twig Preparation

To determine the amount of imidacloprid in needles and twigs, the same branches used for sap extractions were cut above where they had been pruned, 10 cm samples were pulverized using a coffee grinder (KitchenAid, model BCG1000OB, Shelton CT) and tissue was weighed out to 1 g. The 1 g of tissue was then added to 10.00 ml of histological grade acetone in 10 dram glass vials and shaken horizontally at 2 cycles/s for 24 hours. Samples were removed from the shaker and allowed to sit until particles settled and acetone evaporated. A 1.0 ml aliquot was prepared by vortexing the residue in 1.0 ml of distilled water.

Imidacloprid Quantification

Imidacloprid residues within the sap were measured using a commercially available enzyme linked immunosorbant assay (ELISA) kit (EnviroLogix 2005). In this test, the compound horseradish peroxidase-labeled imidacloprid was used which competes with the imidacloprid residues present in the sample for a limited number of antibody sites on the walls of the test wells. This kit was used to quantify concentrations of imidacloprid between 0.2-6 parts per billion (ppb).

Sample size consisted of 100 μl aliquot per chemical sample, 100 μl aliquot of the negative control and 100 μl aliquot of each calibrator (0.2 ppb, 1 ppb, 5 ppb, and 6 ppb) added to their predetermined wells in this order. Also, 100 μl of imidacloprid–enzyme conjugate was added to each well immediately following the previous step. The solutions were thoroughly mixed by moving the plates in circular motion across countertop for one minute. Plates were then covered in Parafilm® and allowed to incubate at ambient temperature for one hour. After one hour, the plates were rinsed thoroughly making sure all wells were flooded with water. After the plate was rinsed, 100 μl of substrate was added to each well and contents were mixed by moving the plates in a circular motion for one minute. Plates were then covered in Parafilm® and allowed to incubate for 30 minutes. At the end of the 30 minutes, 100 μl of 1.0 N hydrochloric acid was added as a stop solution.

The optical density of each well was read using a 96–well plate reader (Bio-Rad microplate manager model 680, Hercules, CA) measuring absorbance at 450 nanometers (nm). Measured optical densities were used to develop standard curves. All standard curves were graphed using Excel® to provide a linear regression with the log of concentration versus the optical density. The slope and intercept obtained from regression parameters were used to calculate the concentration of imidacloprid in the samples. In the initial analysis, all samples were undiluted; however, if a sample was found to be > 6 ppb, the remaining sample was diluted 1:10, 1:100, and 1:1000 and rerun until the concentration was within a range of 0.2–6 ppb.

Data Analysis

Data were placed into an Excel® file and analyzed using mixed proc ANOVA in SAS (SAS 2005). ANOVA and Least Significant Differences (LSD) procedures were run on chemical concentration data ($P < 0.05$).

Results and Discussion

Fall Versus Spring Applications

A mixed proc ANOVA test for sap concentrations showed no significant interactions between timing of application, strata, application method, and months post-treatment ($F = 1.19$, $df = 12$, $P = 0.29$). Mean separation inferred no significant differences in sap concentrations between fall and spring application times (LSD test; $P < 0.05$). A mixed Proc ANOVA test for twig and needle concentrations showed no significant interactions between timing of application, strata, application method, and months post-treatment ($F = 3.22$, $df = 12$, $P = 0.33$), and mean separations inferred no significant differences in twig and needle concentrations between fall and spring application times (LSD test; $P < 0.05$).

Application Method

A mixed proc ANOVA test for sap concentrations revealed significant interactions ($F = 3.2$, df =12, 96; $P = 0.0007$) between application method, months post-treatment, and strata. Mean separation showed significant differences (LSD test; $P < 0.05$) in sap (Table 3) and combined needle and twig concentrations (Table 4) between the various application methods and months post–treatment at bottom, middle, and top strata. Soil drench and soil injection had significantly higher (LSD test; $P < 0.05$) mean sap concentrations than the tree injection at the bottom strata of the canopy for 3, 9, and 12 months post-treatment. In the sixth month, all application methods had significantly different (LSD test; $P < 0.05$) sap concentration levels with soil drench having the highest sap concentration, followed by soil injection, tree injection, and the control. In the bottom

Table 3. Imidacloprid concentration (ppb) (mean ± SE) in sap for the bottom, middle, and top strata determined by ELISA of eastern hemlocks (n=6 trees per treatment).

Treatment	Months Post-Treatment			
	3	6	9	12
Bottom Strata				
Soil Drench	7.2 ± 1.2a*	8.5 ± 0.4a*	8.9 ± 2.1a*	7.1 ± 1.1a*
Soil Injection	7.2 ± 1.2a*	7.5 ± 0.4b*	7.2 ± 1.9a*	5.2 ± 2.1a*
Tree Injection	5.2 ± 0.5b*	6.6 ± 0.4c**	3.8 ± 0.6b***	1.9 ± 0.5b****
Control	0.0c*	0.0d*	0.0c*	0.0c*
Middle Strata				
Soil Drench	5.6 ± 1.2a*	2.9 ± 0.3a**	2.6 ± 0.3a**	1.8 ± 0.2a***
Soil Injection	5.3 ± 0.9a*	2.4 ± 0.6a**	1.8 ± 0.2b**	1.2 ± 0.4a**
Tree Injection	3.7 ± 0.5b*	0.4 ± 0.1b**	0.8 ± 0.2c***	0.3 ± 0.2b****
Control	0.0c*	0.0c*	0.0d*	0.0c*
Top Strata				
Soil Drench	4.2 ± 1.2a*	2.9 ± 0.4a*	1.7 ± 0.8a**	1.6 ± 1.1a**
Soil Injection	3.7 ± 0.9a*	1.3 ± 1.1b**	0.5 ± 0.2b***	0.2 ± 0.1b***
Tree Injection	1.7 ± 0.5b*	0.1 ± 0.1bc**	0.1 ± 0.1c**	0.1 ± 0.1bc**
Control	0.0c*	0.0c*	0.0c*	0.0c*

Means within the same columns within the same strata category followed by the same letter are not significantly different (LSD test; *P* > 0.05). Means within the same row followed by the same symbol are not significantly different (LSD test; P > 0.05).

Table 4. Imidacloprid concentration (ppb) (mean ± SE) in combined needles and twigs for the bottom, middle, and top strata determined by ELISA of eastern hemlock (n=6 trees per treatment).

Treatment	Months Post-Treatment			
	3	6	9	12
Bottom Strata				
Soil Drench	280.3 ± 22.2a*	250.5 ± 28a*	255 ± 55a*	232 ± 45.1a*
Soil Injection	180.5 ± 32.1b*	177.5 ± 16b*	172.3 ± 12b*	165.2 ± 2.10b*
Tree Injection	120.4 ± 17.2c*	46.6 ± 12c**	65.5 ± 10c**	47.8 ± 18.0c**
Control	0.0d*	0.0d*	0.0d*	0.0d*
Middle Strata				
Soil Drench	255.5 ± 62.1a*	189.4 ± 35a*	192 ± 41a*	179 ± 23a*
Soil Injection	182 ± 24.1a*	179.5 ± 31a*	155.9 ± 16a*	139.2 ± 29a*
Tree Injection	90 ± 8.01b*	49.2 ± 7.1b**	55.2 ± 4.0b**	65.6 ± 12b**
Control	0.0c*	0.0c*	0.0c*	0.0c*
Top Strata				
Soil Drench	192.7 ± 55a*	188.6 ± 41a*	186.7 ± 26a*	155.9 ± 45a*
Soil Injection	150.2 ± 45a*	166.7 ± 36a*	145.2 ± 56a*	138.4 ± 65a*
Tree Injection	40.7 ± 5.0b*	36.4 ± 7.1b*	32.1 ± 12b*	12.6 ± 2.1b**
Control	0.0c*	0.0c*	0.0c*	0.0c*

Means (n = 6) within the same columns within the same strata category followed by the same letter are not significantly different (LSD test; $P > 0.05$). Means within the same row followed by the same symbol are not significantly different (LSD test; P > 0.05).

strata, sap concentrations in soil drenched and soil injected trees did not significantly change (LSD test; $P > 0.05$) over the 12 month period. Trees that were tree injected were significantly lower after month 3, and sap concentrations significantly decreased (LSD test; $P < 0.05$) through month 12 post-treatment. Combined twig and needle concentrations were significantly different (LSD test; $P < 0.05$) across all treatments months 3, 6, 9, and 12 post-treatment, within the bottom strata, with the soil drench having significantly higher (LSD test; $P < 0.05$) concentrations, followed by soil injection, and tree injection. Trees that were tree injected had significantly lower (LSD test; $P < 0.05$) concentrations after month 3 post-treatment. Trees that were soil drenched and soil injected had combined twig and needle concentrations that were not significantly different across months 3-12 post-treatment.

In the middle strata of the tree, soil drench had the highest sap concentrations across months 3, 6, 9, and 12 post-treatment and was significantly different from all other treatments in the middle strata, except in the third month, where it was not significantly different (LSD test; $P > 0.05$) from soil injection. All other application methods differed significantly (LSD test; $P < 0.05$) from one another in the middle strata across 3, 6, 9, and 12 months post-treatment, except for the nine month post-treatment where no significant difference (LSD test; $P > 0.05$) between soil injection and tree injection was noted. Those trees treated with a soil drench, soil injection, and tree injection showed a significant decrease (LSD test; $P < 0.05$) in sap concentration in the middle strata 3 months after treatment. Combined twig and needle concentrations were not significantly different (LSD test; $P > 0.05$) between soil drench and soil injection across months 3, 6, 9, and 12 post-treatment in the middle strata, while tree injection had significantly lower (LSD test; $P < 0.05$) concentrations than soil drench and soil injection. Trees treated with a soil drench and soil injection had combined needle and twig concentrations that were not significantly different (LSD test; $P > 0.05$) across months 3, 6, 9, and 12 post-treatment. Trees treated with a tree injection showed a significant decrease in combined twig and needle concentration after month 3 post-treatment.

In the top strata, soil drench had a significantly higher (LSD test; $P < 0.05$) sap concentration than other treatments across all months post-treatment, with the exception of month 3 post-treatment were it was not significantly different (LSD test; $P > 0.05$) from soil injection. Trees treated with a soil drench and soil injections showed significantly lower (LSD test; $P < 0.05$) concentration levels in the sap after month 6 post-treatment, while those trees treated with a tree injection showed a significant decrease (LSD test; $P < 0.05$) in concentration after month 3 post-treatment. Combined twig and needle concentrations were not significantly different (LSD test; $P > 0.05$) between soil drench and soil injection across months 3, 6, 9, and 12 post-treatment in the top strata, while tree injection was significantly lower (LSD test; $P < 0.05$) than soil drench and soil injection. Trees treated with a soil drench or soil injection showed no significant decrease (LSD test; P > 0.05) in combined twig and needle concentrations through months 3-12 post-treatment.

Two general trends are observed relative to concentration translocation. First, sap and combined twig and needle concentrations progressively decrease from the bottom to the top strata of the canopy, with the highest concentration over time represented in the bottom strata. This trend was consistent in all treated trees. Second, the soil drench consistently provided the highest sap and combined twig and needle concentrations across all strata on the tree; however, the higher concentration translocation may be a result of a higher application rate (1.5 g AI/2.5 dbh) used. The second and third highest sap and combined twig and needle concentration levels were in most cases followed by soil drench and tree injection, respectively. Tree injections were found to be the least uniform in concentration within the tree, especially at the top of the tree. The non-uniform distribution of the concentration may explain why tree injections are often considered to be ineffective (Cowles et al. 2006). Soil drench and soil injections have both been shown to be effective at controlling hemlock woolly adelgid (Steward and Horner 1994; Rhea 1996; Steward et al. 1998; Fidgen et al. 2002; Cowles et al. 2006), and has the most uniform distribution within the canopy. These general trends can be used by land owners and managers to make informed decisions on what types of treatments have the most potential for effectively treating hemlock woolly adelgid over longer periods of time.

Concentrations within the sap and combined twig and needle samples in the bottom strata were similar to those reported by Cowles et al. (2006). They determined that the LC_{50} for hemlock woolly adelgid population in the laboratory was 300 ppb. In forest settings, they found an association with concentrations > 120 ppb maintained a high degree of suppression for over two years (Cowles et al. 2006). An LC_{50} of 150 ppb was reported by Tattar et al. (1998) using the Placke and Weber (1983) total method to determine concentration levels. This method combines imidacloprid and all its metabolites for a total product for analysis, artificially inflating the quantification of the concentration. Thus, some question is noted in the reported amounts needed for control of the target pest. The highest concentration found in this investigation was 280 ppb in soil drenched trees, with all soil drenched and soil injected trees ranging from 138-280 ppb. The highest concentration levels were detected in combined twig and needle samples which may indicate that imidacloprid concentrates in the plant tissue.

Pre- and post-imidacloprid treatment percentage rankings of hemlock woolly adelgid populations showed reductions in percent infested for all trees that were initially infested and treated with a soil drench or soil injection. All trees varied greatly with respect to initial infestations, and some trees were not infested (Table 5). One out of the six trees treated using tree injections showed control in two of the trees there was no infestation prior to treatment, but percentage ranking of <25% after treatment.

Future research is needed to determine if reduced concentrations of imidacloprid will be as effective against the hemlock woolly adelgid and the precise time period the compound persists within the host tree providing protection. A possible reduction in concentration would result in greater financial savings and potentially lessen the effect on non-target species. Eastern hemlock dbh is used to determine rate of application of imidacloprid; however, it has been shown that water uptake in eastern hemlock is related to and varies by tree height and diameter (Ford and Vose 2007). Because water uptake is effected by tree diameter and height, it would seem plausible that translocation of imidacloprid may be effected as well. In addition to determining more optimized control of hemlock woolly adelgid, development of a technique for in field evaluation of imidacloprid concentrations would allow for more customized treatment and monitoring for multiple agencies. Preliminary research shows a high correlation between mid-infrared spectra (r = 0.96) and known concentrations. Field evaluation of the concentration level within a tree has the potential to decrease the cost and time required to obtain information leading to control decisions compared with the use of standard HPLC, GC- MS, and ELISA techniques. Such techniques would provide the user an immediate feedback for analysis. The potential use of near- and mid-infrared spectroscopy will save time and money and would provide an earlier detection time that would be beneficial to a variety of agencies (i.e., U. S. D. A. Forest Service) who could utilize this method in customizing imidacloprid treatment based on the uptake of the insecticide in the tree.

Table 5. Pre-treatment (11/9/2005) and post-treatment (1/3/2007) infestation ratings of hemlock woolly adelgid on eastern hemlock.

Treatment	**Percent infestation ratings per 4–12cm branch samples per tree**	
	Pre-treatment 9 November 2005	**Post–treatment 3 January 2007**
Drench	Absent	Absent
Drench	Absent	Absent
Drench	25-50%	<25%
Drench	<25%	<25%
Drench	Absent	Absent
Drench	<25%	Absent
Horticultural Oil Spray	<25%	Absent
Horticultural Oil Spray	Absent	Absent
Horticultural Oil Spray	Absent	Absent
Horticultural Oil Spray	<25%	Absent
Horticultural Oil Spray	<25%	50 - 75%
Horticultural Oil Spray	25-50%	Absent
No Treatment	Absent	<25%
No Treatment	25-50%	25-50%
No Treatment	<25%	>75%
No Treatment	<25%	>75%
No Treatment	Absent	<25%
No Treatment	<25%	50-75%
Soil Injection	Absent	Absent
Soil Injection	Absent	Absent
Soil Injection	Absent	Absent
Soil Injection	Absent	<25%
Soil Injection	<25%	<25%
Soil Injection	<25%	<25%
Tree Injection	<25%	50-75%
Tree Injection	Absent	50-75%
Tree Injection	<25%	<25%
Tree Injection	<25%	>75%
Tree Injection	<25%	50-75%
Tree Injection	Absent	<25%

III. Impact of Imidacloprid and Horticultural Oil on Non–Target Phytophagous and Transient Canopy Insects Associated with Eastern Hemlock, *Tsuga canadensis* (L.) Carrieré.

Introduction

Imidacloprid and horticultural oil are broad-spectrum insecticides that are the primary insecticides used to control insect pests such as the invasive hemlock woolly adelgid, *Adelges tsuga* Annand, (Hemiptera: Adelgidae). This introduced species has dramatically reduced populations of eastern hemlock, *Tsuga canadensis* (L.) Carrieré, since its introduction into Richmond, Virginia in the 1950's. These insecticides offer effective short-term control until more long-term solutions like biological control agents can be established.

Imidacloprid is a systemic insecticide taken up by xylem (Steward et al. 1998; Tattar et al. 1998) and diffused into the xylem ray parenchyma cells located in the twigs of trees (Young et al. 1995) where the hemlock woolly adelgid feeds causing death with 24-48 hours after ingestion or contact (Bai et al. 1991; Kidd et al. 1991, Mullins and Christie 1995). In forested settings, specifically trees, shrubs, flowers, and groundcover, it is recommended for the control of adelgids, aphids, armored scale, black vine weevil larvae, emerald ash borer, eucalyptus longhorn borer, flathead borers, Japanese beetles, lace bugs, leaf beetles, leafhoppers, leafminers, mealybugs, pine tip moth larvae, psyllids, royal palm bugs, sawfly larvae, soft scales, thirps, white grub larvae, and white flies (Bayer 2007). Paraffinic oil is the active ingredient in most horticultural oils, and is refined petroleum product. In a forest setting, horticultural oils are recommended for control of the aphids, adelgids, caterpillars, lace bugs, leaf beetle larvae, leafminers, mealybugs, psyllids (immature), sawfly (larvae), scales (immature), and whiteflies (immature).

The broad-spectrum nature of both these insecticides have been beneficial in pest management and have been shown to be effective at controlling hemlock woolly adelgid (Cowles et al. 2006a, 2002b; McClure 1991b; Steward and Horner 1994; Cowles and Cheah 2002a; Doccola et al. 2003; Webb et al. 2003). At present the effect of these insecticides on non-target insects associated with eastern hemlock, is unknown. The goal of most pest management strategies is to effectively reduce pest populations, while having a minimal effect on non-target species. However, the effect of horticultural oil and imidacloprid on non-target phytophagous and transient insects associated with eastern hemlock is not known. As such, this study was initiated to determine the effect of imidacloprid and horticultural oil on non-target phytophagous and transient insects.

Materials and Methods

Study Site and Experimental Design

Eastern hemlocks (n = 30) were selected at Indian Boundary in Cherokee National Forest located in southeast Tennessee on 5 November 2005. This test was arranged in a split plot 2 x 5 factorial complete randomized block design with three replications. Three test blocks were established (35° 23.787 N, 84° 06. 662 W, elevation: 543 m (1,784 ft); 35° 23.764 N, 84° 06.732 W, elevation: 555 m (1,823 ft); 35° 24.173 N, 84° 06.268 W, elevation: 565 m (1,853 ft), respectively). Each block contained ten trees, arranged in five tree pairs, with one tree in the pair treated in the fall (29-30 November 2005) and the other during the spring (16 April 2006). Each tree was marked with an identification numbered metal tag. Tree characteristics were documented on 25-26 November 2005: tree height, transparency, density, crown class, dbh, foliage color, overall appearance, crown condition, and percent of hemlock woolly adelgid on tree. Tree pairs were selected based on how close any two trees matched base on these characteristics. All three blocks are located in a shortleaf pine–oak (type 76) forest.

Insecticide Application

Five treatments per block (1 tree per pair) consisting of tree injection, soil injection, soil drench, horticultural oil foliar spray, and control were applied. Tree injection system (J. J. Mauget Co. Arcadia CA) consisted of the Mauget® 3 ml 10% imicide capsules and feeder tubes. The tree injection was applied at a rate of one capsule per 15 cm diameter at breast height (dbh) which is equal to 0.15 ml AI/ 2.5 cm dbh. A 0.4 cm (11/64 in) drill bit was used to drill a hole to the depth of 1.2 cm (½ in) at a downward angle into root flair to penetrate xylem tissue, 20.5 cm (8 inches) above the ground. The feeder tubes were placed in the holes and capsules were attached to feeder tubes. Capsules were spread evenly around the circumference of the tree. Capsules were left in tree until total uptake was completed, ranging from one to five hours.

Soil injection application was made using a Kioritz® soil injector (Kioritz Corp. Tokyo, Japan). Merit ® 75 WP (Bayer, Kansas City, MO) was diluted to 1 g AI/2.5 dbh in 60 ml of water. Soil injections were made using the basal system in which injections were made within 45 cm of the base of the trunk and were spaced evenly around the tree at a depth of 7 cm (2.7 inches) below the soil surface with individual injections delivering 30 ml of insecticide.

The soil drench was applied using a FMC high pressure hydraulic sprayer (FMC corporation, Jonesboro, AR). Merit® 75 WP (Bayer, Kansas City, MO) was applied at a rate of 1.5 g AI/2.5 dbh. The recommended dosage of 50 g (5,000 mg) of Merit® 75 WP was mixed with 379 liters (100 gallons) of water for fall and spring treatments respectively. The soil extending from the trunk to the drip line was sprayed with 125 liters (33 gallons) of insecticide.

SunSpray® horticultural oil (Sun Company, Philadelphia, PA) was applied using a FMC high pressure hydraulic sprayer (FMC corporation, Jonesboro, AR). The mixture consisted of 7.57 liters (2 gallons) AI per 379 liters (100 gallons) of water, in accordance with the product label to treat trees for adelgids. The tree was sprayed to runoff to ensure adequate coverage, as such, the amount of insecticide applied to each tree varied.

Sampling

Sampling methods consisted of malaise traps, beat sheet, direct observation/handpicking/trunk vacuuming, and branch sampling. One modified malaise trap was placed in the mid canopy of each tree (Figure 7a). The modified malaise trap design consists of a 60 cm x 60 cm x 60 cm PVC pipe frame covered in No-Thrips® insect screen. Secured to the traps were two collecting units, a pan (15 cm wide x 65 mm length x 12 deep) containing 900–1000 ml of 50% propylene glycol and water, and a collecting cup (6 cm top diameter x 6 ½ cm deep, 120 ml) which contained 30–60 ml of 50% propylene glycol and water. Pulley systems were set up in each tree to allow for rapid movement of the trap in and out of the canopy for collection. Malaise traps were collected monthly from April 2006 through April 2007, labeled, and taken to the lab for sorting.

Beat-sheet samples were taken monthly (Figure 7b), four per tree representing each cardinal direction, wherein each branch was struck five times with a one-meter stick. Direct sampling (visual observations/handpicking/trunk vacuuming) (Figure 7c) were conducted monthly on each tree for 15 minutes per tree. Samples were placed in pre-labeled (date collected, tree number, and collecting method) 75% alcohol in 6 dram vials and taken to the laboratory for sorting and identification. To assess sedentary insect species, 4-12 cm branch samples, one in each of the cardinal directions, were collected monthly (Figure 7d). Except for larvae that were placed in a Petri dish with a pre–moistened filter paper, an untreated hemlock clipping and a label, specimens on branch samples were sealed in a pre-labeled (date collected, tree number, and collecting method) plastic bag. Caterpillars were taken back to the laboratory and reared to adults.

Preserving and Identification of Specimens

Specimens collected from the malaise traps were placed in a new collecting cup (ca. 60 mm x 65 mm deep; 120 ml vol.) labeled (date collected, tree number and collecting method) with permanent marker. Beat sheet samples were directly placed in pre-labeled 75% alcohol vials with a label (date, tree number, cardinal direction, and collecting method). Specimens collected from direct sampling / handpicking/ and trunk

Figure 7. Sampling methods: a) modified malaise trap, b) beat–sheet, c) visual observations/handpicking/trunk vacuuming, and d) tree pruning.

vacuuming were also placed directly into pre-labeled alcohol vials. Branch samples were examined under the microscope in the laboratory for insect specimens. Insect specimens collected were placed in 75% alcohol and labeled. All specimens were processed in this manner with the exception of caterpillars that were placed in moist Petri® dishes with untreated hemlock branches to complete their development into the adult stage for identification. In the laboratory, the excess propylene glycol was drained from those specimens collected from malaise traps. All specimens collected were sorted to order, family, genus, and species. For each of these categories the specimens were sorted into four dram vials filled with 75% alcohol and labels attached to the side of the vial.

Specimens were identified using standard keys (Arnett and Thomas 2002a; Arnett and Thomas 2002b; Blatchley 1926; Bradley 1930; Creighton 1950; DeLong 1948; Dmitriev 2007; Dillon and Dillon 1961; Ferguson 1978; Fisher 1938; Hall 1948; Johannsen, 1910a, 1910b, 1912; Kissinger 1964; Lafontaine 1987, 1998; LaFontaine and Poole 1991; Linsley and Chemsak 1961, 1962a, 1962b, 1963, 1964, 1972; McAlpine et al.1981, 1987; McPherson 1982; Mitchell 1962; Mockford 1993; Neunzig 1986, 1990, 1997; Otte 1981, 1984; Poole 1995). Specialists (Appendix A) were contacted to identify difficult specimens. Voucher specimens were organized into Cornell drawers and incorporated into the University of Tennessee's insect museum.

Impact was assessed by examining the effect on overall species richness, abundance, and composition, guild species richness and abundance, and specific species. Guilds (a group of organisms that utilized a similar resource in a similar manner) were examined across all treatments to assess any effects on the functional structure of insects associated with eastern hemlock. Species were assigned guilds based on documented feeding habits. Seven guilds were recognized; phytophagous, transient phytophagous, detritivore, scavenger, fungivore, haematophagous and phytophagous/haematophagous. The phytophagous guild consists of insects that feed directly on hemlock. The transient phytophagous guild consists of insects that feed on other living plant material not associated with eastern hemlock. The detritivore consists of insects that feed on decaying organic material, while scavengers consisted of those insects that feed on dead animals or insects and may also exhibit various other feeding habits. Haematophagous insects consisted of insects that feed on blood, and fungivores consists of those insects that feed primarily on fungus. All guild assignments were made based on the life stage at which the insect was collected.

Data Analysis

Data consisting of: collection date, tree number, block number, treatment, fall or spring application, order, family, genus, species, author, number of specimens, and guild were entered into an Excel® spreadsheet. Differences in species abundance and richness and guild species richness and abundance between different treatments were analyzed. using mixed model analysis of variance (ANOVA) in SAS (SAS 2005) and least significant differences (LSD) procedures. ANOVA mixed model type 3 test of fixed effects was used to determine interactions between application timing and treatment. Species richness estimates for different treatments were calculated using Chao1 species richness estimator in EstimateS (Colwell 2005). To determine which species specifically were effected, least squares means (lsmeans) and t-tests were used for each species to determine which treatment means are significantly different from other treatment means. Because t-tests between least squares means involves multiple statistical comparisons, a Bonferroni correct alpha is used to avoid Type I errors.

Results and Discussion

Impact on Species Abundance and Richness

During this study, 2,349,827 insect specimens representing 293 species, 226 genera, 75 families and nine orders were collected and identified (Appendix B). Species composition was most similar among control, horticultural oil, and tree injection; these treatments were most dissimilar with those trees treated with a soil drench or a soil injection. An ANOVA type 3 test of fixed effects revealed a significant difference (F=3.34, df = 4, 18, P < 0.05) in species abundance by treatment method. There was no significant interaction (F = 0.34, df = 4, 18, P > 0.05) between application time and treatment method. The timing of application had no significant effect (F = 0.04, df = 1, 18, P > 0.05) on the total species abundance by treatment. Species abundance was significantly lower (LSD test; P < 0.05) in the soil drench treatment than the control, horticultural oil, soil injection, soil drench, and tree injection (Figure 8). Species abundance was not significantly different (LSD test; P > 0.05) among horticultural oil, soil injection, and tree injection, but these were significantly different (LSD test; P > 0.05) from the control.

An ANOVA type 3 test for fixed effects inferred a significant difference (F = 27.06, df = 4, 18, P < 0.0001) in species richness by treatment method. There was no

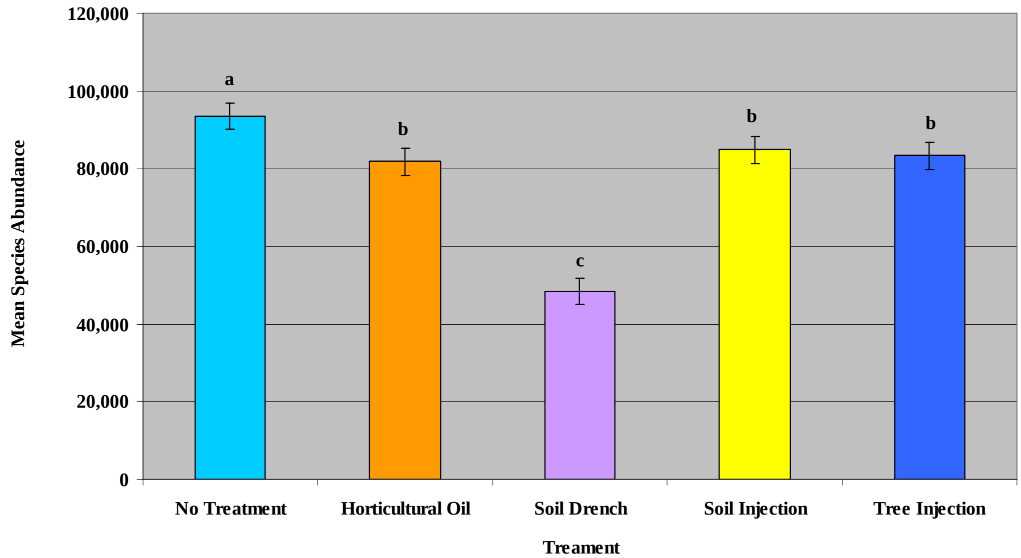

Figure 8. Mean species abundance (± SE) for treatments. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

significant interaction (F = 0.47, df = 4, 18, P > 0.05) between application timing and treatment method. The timing of application showed no significant difference (F = 1.15, df = 1, 18, P > 0.05) in species richness. Observed species richness within soil drench treatments was significantly lower (LSD test; P < 0.05) than horticultural oil, soil injection, and tree injection which did not significantly differ (LSD test; P > 0.05) (Figure 9).

To determine how many insect species were potentially present in each treatment regime, Chao1 species richness estimator was used (Figure 9). The species richness estimate for soil drench was 227 with 183 species observed. The species richness estimates for no treatment, horticultural oil, soil injection, tree injection was 235, 225, 229, 230, respectively, with 230, 221, 227, 224 actual species observed, respectively. The control treatment estimate produced a 95% confidence interval that did not overlap with the other treatments confidence intervals, which means that the estimate for the control is significantly higher from the rest. The small confidence intervals associated with each estimate infers the number of species are reaching an asymptote in the species accumulation curve, however the species richness estimates among treatments also infers that if sampling was taken to completion, there might be an effect seen on the other treatments compared with the control.

Overall mean species richness and abundance were greatly effected by soil drench treatments. Timing of application did not have an effect on mean species richness or abundance. The effect of the soil drench may be due to the higher concentration of imidacloprid translocated throughout the tree. Horticultural oil, soil injection, and tree

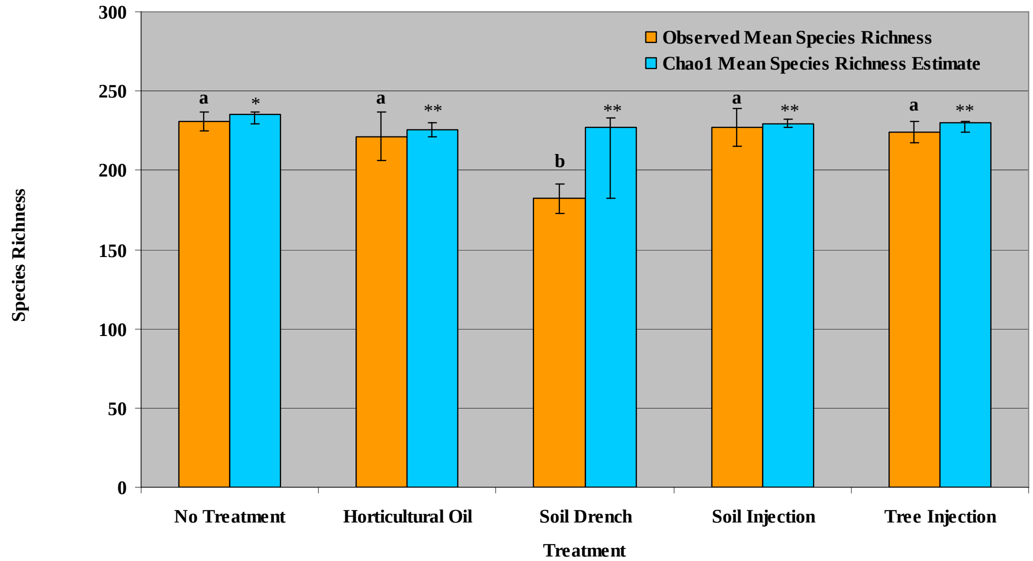

Figure 9. Observed mean species richness and Chao1 mean species richness estimate (± 95% CI) for treatments. Observed means followed by the same letter followed by the same letter are not significantly different (LSD test; $P > 0.05$). Estimated richness means followed by the same symbol are not significantly different.

injection had a moderate effect on mean species abundance and no effect on species richness.

Impact on Guild Structure

An ANOVA type 3 tests of fixed effects showed that there was a significant difference (F = 30057.5, df = 4, 18, P < 0.0001) in detritivore species richness across different treatments. There was no significant difference (F = 0.07, df = 1, 18, P = 0.7886) in species richness across different application timings and there was no significant interaction (F = 0.49, df = 4, 18, P = 0.7426) between application timing and treatment method. Detritivore species richness was significantly lower (LSD test; P > 0.05) in those trees treated with a soil drench than those trees treated with horticultural oil, soil injection, tree injection, and no treatment (Figure 10).

The funigvore guild showed no significant difference (F = 0.94, df = 4, 18, P > 0 .05) in species richness across different treatments (Figure 11). Also, there was no significant difference (F = 0.4854, df = 1, 18, P > 0.05) in species richness in the fungivore guilds across different application timings and there was no significant interactions (F = 0.3590, df = 4, 18, P > 0.05) between application timing and treatment method.

A significant difference (F = 4781. 51, df = 4, 18, P < 0.0001) was noted for species comprising the phytophaga guild in regard to species richness across different treatments. However, no significant differences (F = 4.19, df = 1, 18, P = 0.07) were found in species richness across different application timing and there was no significant interactions (F = 0.86, df = 4, 18, P = 0.5092) between application timing and treatment method. Phytophaga species richness was significantly lower (LSD test; P < 0.05)

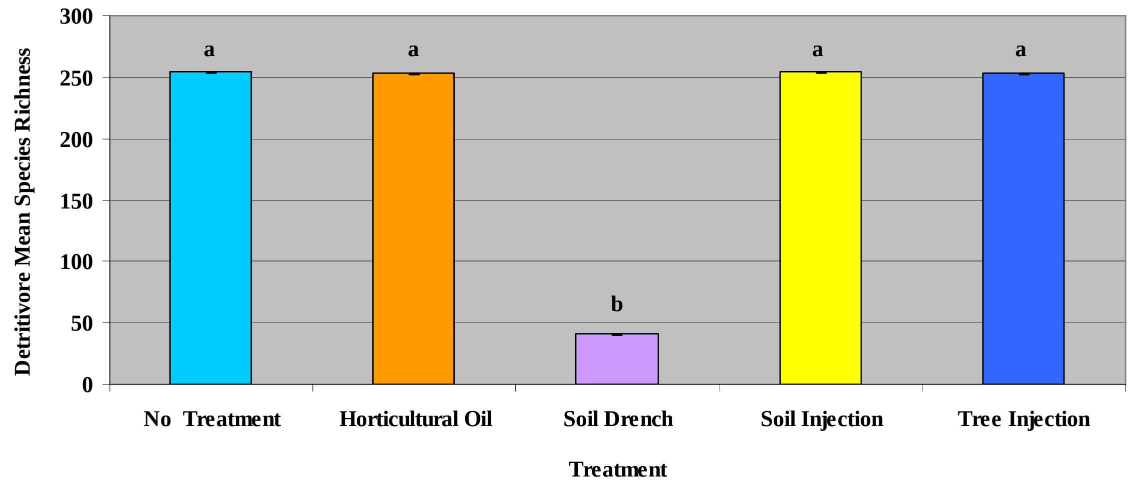

Figure 10. Detritivore guild mean species richness. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

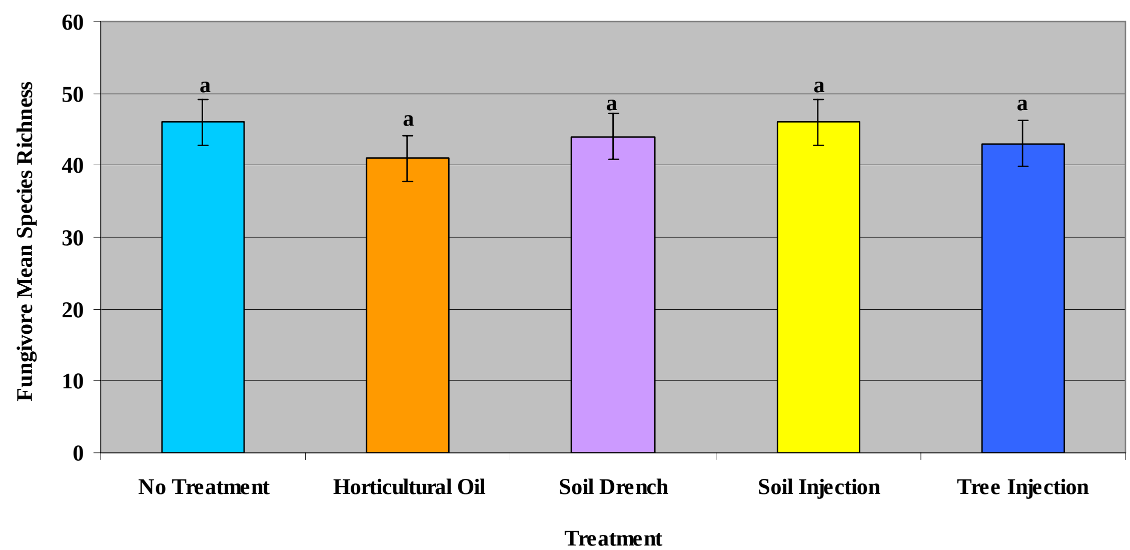

Figure 11. Fungivore guild mean species richness. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

in those trees treated with a soil drench than those trees that received horticultural oil, soil injection, tree injection and the control (Figure 12). Also, no significant differences (LSD test; $P > 0.05$) were found between those trees that received no treatment, horticultural oil, soil injection, and tree injection.

The transient phytophaga guild showed no significant differences in species richness across different treatments (Figure 13), timing of application ($F = 1.14$, $df = 1, 18$, $P = 0.2994$), and in the interaction between application timing and method ($F = 0.58$, $df = 4, 18$, $P = 0.6787$). The scavenger guild species richness showed no significant differences in species richness across different treatments ($F = 0.73$, $df = 4, 18$, $P = 0.5805$), timing of application ($F = 1.94$, $df = 1, 18$, $P = 0.1811$), and in the interaction between application timing and method ($F = 0.57$, $df = 4, 18$, $P = 0.6864$) (Figure 14).

Analysis was not run on the haematophagous and phytophagous/haematophagous guilds, because only one species was represented in the phytophagous/haematophagous guild was *Chrysops geminatus* Wiedeman (Diptera: Tabanidae). The male feeds on plant material and the female feeds on blood. Since the sex of the specimens (n=41) was not identified, this guild category was created. The three species representing the haematophagous guild included: *Culicoides sanguisuga* (Coquillet) (Diptera: Ceratopogonidae), *Prosimilium mixtum* Syme and Davies (Diptera: Simuliidae), and *Atrichopoogon* sp. (Diptera: Ceratopogonidae), and were present on all the trees.

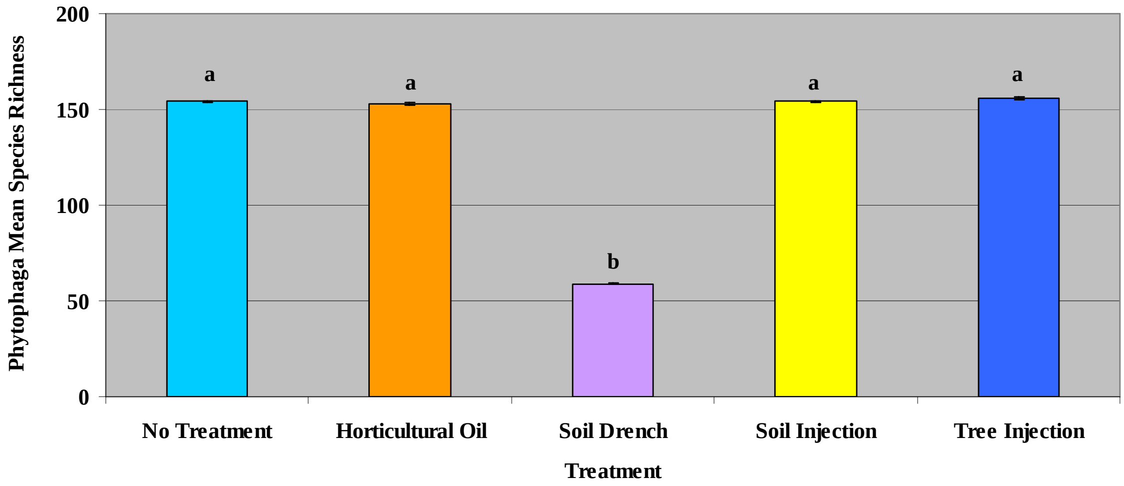

Figure 12. Phytophaga guild mean species richness. Means (n = 6) followed by the same letter are not significantly different (LSD test; *P* > 0.05).

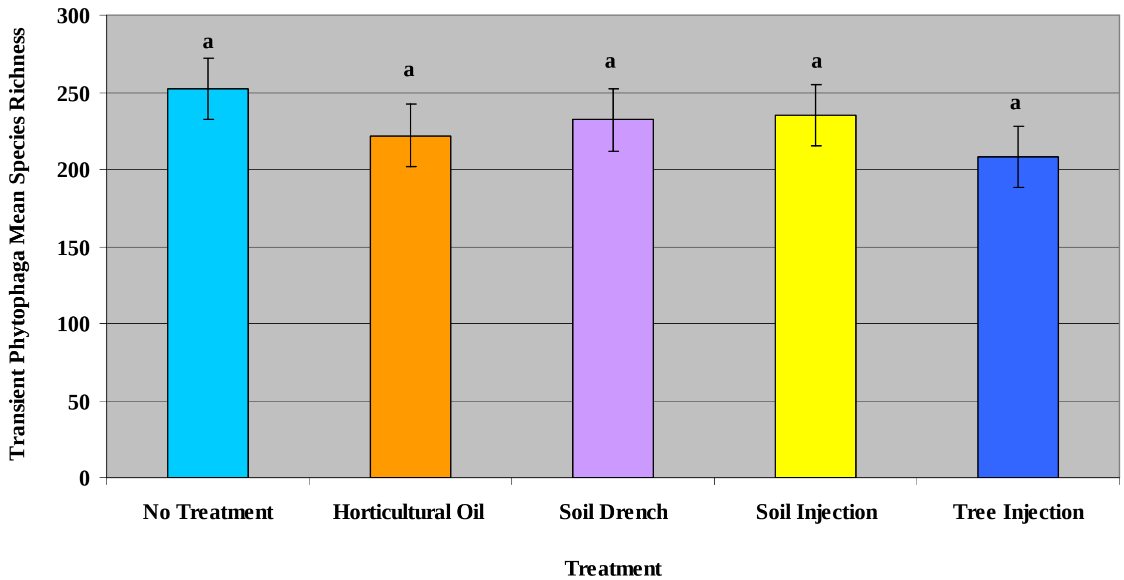

Figure 13. Transient phytophaga guild mean species richness. Means (n = 6) followed by the same letter are not significantly different (LSD test; *P* > 0.05).

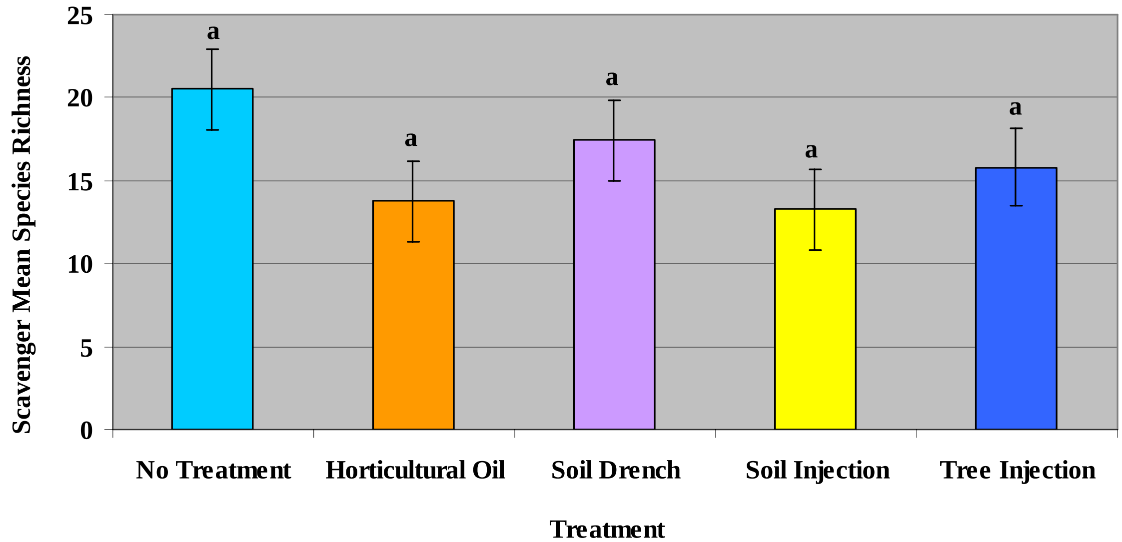

Figure 14. Scavenger guild mean species richness. Means (n = 6) followed by the same letter are not significantly different (LSD test; *P* > 0.05).

An ANOVA type 3 test for fixed effects showed that there was a significant difference (F = 4.43, df = 4, 18, P < 0.05) in detritivore guild species abundance across different treatments. No significant difference was found for timing of application (F = 0.07, df = 1, 18, P > 0.05) and in the interaction between timing of application and method (F = 0.55, df = 4, 18, P > 0.05). The detritivore guild was significantly effected (LSD test; P < 0.05) by the soil drench application (Figure 15) and was not significantly different (LSD test; P > 0.05) among other treatment (no treatment, horticultural oil, soil injection, and tree injection).

An ANOVA type 3 test for fixed effects did infer a significant difference (F = 1.43, df = 4, 18, P < 0.05) in fungivore guild species abundance across different treatments. No significant differences were found for timing of application (F = 0.54, df = 1, 18, P > 0.05) or in the interaction between timing of application and treatment method (F = 0.33, df = 4, 18, P > 0.05). Those trees treated with a soil drench had a significantly lower species abundance than those treated with horticultural oil, soil injection, tree injection, and the control (Figure 16).

An ANOVA type 3 test for fixed effects showed that there was no significant (F = .15, df = 4, 18, P > 0.05) differences in the haematophagous guild species abundance across different treatments, different application times (F = .09, df = 1, 18, P > 0.05), and in the interaction between application timing and method (F = 0.22, df = 4, 18, P > 0.05) (Figure 17).

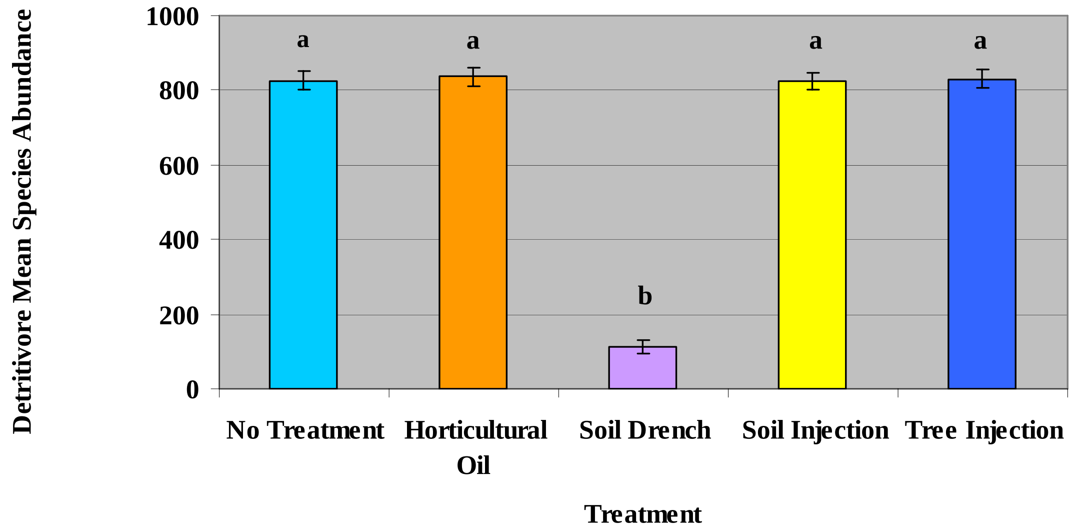

Figure 15. Detritivore mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

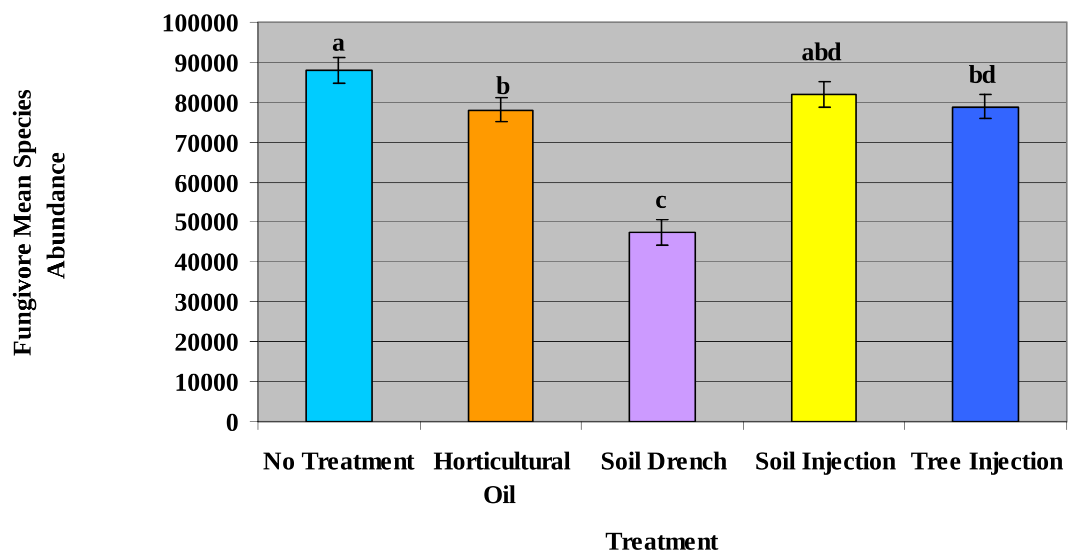

Figure 16. Fungivore mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

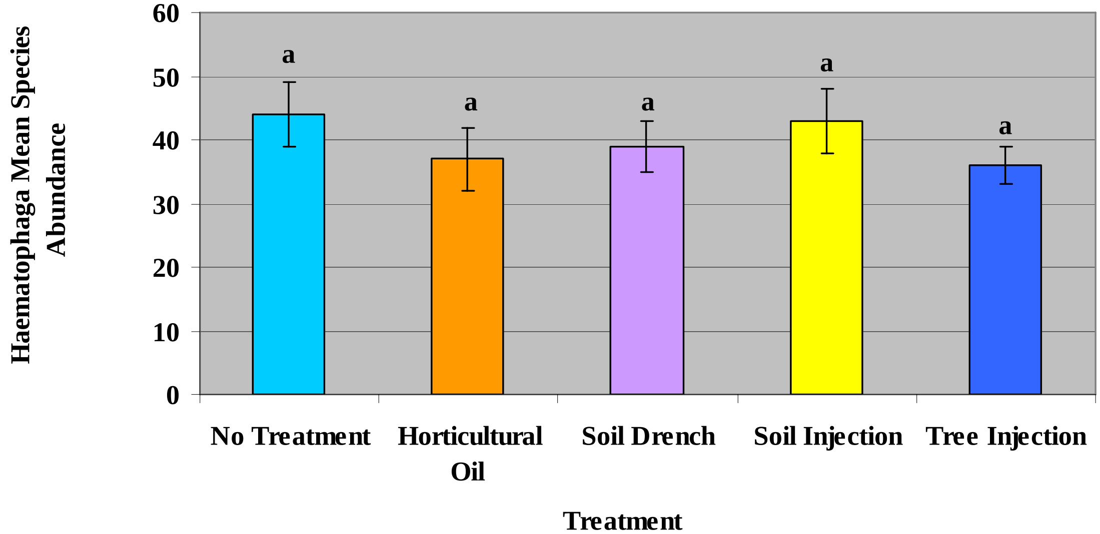

Figure 17. Haematophaga mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

The phytophaga guild was significantly impacted (LSD test; $P < 0.05$) by the soil drench and soil injection (Figure 18). An ANOVA type 3 test for fixed effects revealed significant differences ($F = 2.22$, $df = 4, 18$, $P < 0.05$) in phytophaga species richness across treatments. Following the general trend for other guilds, no significant differences were found for the timing of application ($F = 0.25$, $df = 1, 18$, $P > 0.05$) or in the interaction between application timing and method ($F=0.65$, $df = 4, 18$, $P > 0.05$). Those trees treated with horticultural oil, tree injection, and control were not significantly different (LSD test; $P > 0.05$).

Transient phytophaga guild species abundance was found to be significantly effected ($F = 3.56$, $df = 4, 18$, $P < 0.05$) by treatment. Those trees treated with horticultural oil, soil drench, soil injection, and tree injection were not significantly different (LSD test; $P > 0.05$); however, they did differ significantly (LSD test: $P < 0.05$) from the control trees (Figure 19). No significant differences were found for the timing of application or in the interaction between application timing and treatment method ($F = 0.04$, $df = 4, 18$, $P > 0.05$).

Scavenger guild species abundance was found to be significantly effected ($F = 2.41$, $df = 4, 18$, $P < 0.05$) by treatment (Figure 20). Those trees treated with horticultural oil had significantly lower (LSD test; $P < 0.05$) species abundance than those treated with soil injection, tree injection, and control, and soil drench and soil injection were significantly lower (LSD test; $P < 0.05$) than the control. No significant differences were found for the timing of application and ($F = 0.06$, $df = 1, 18$, $P > 0.05$) or in the interaction between application timing and treatment method ($F = 0.45$, $df = 4, 18$, $P > 0.05$).

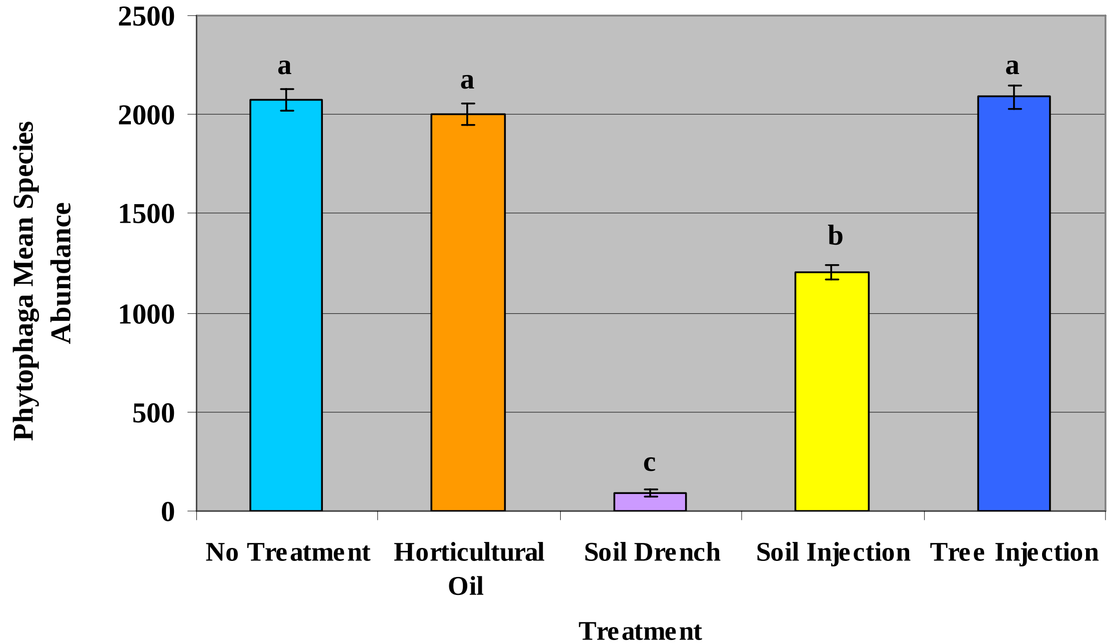

Figure 18. Phytophaga mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

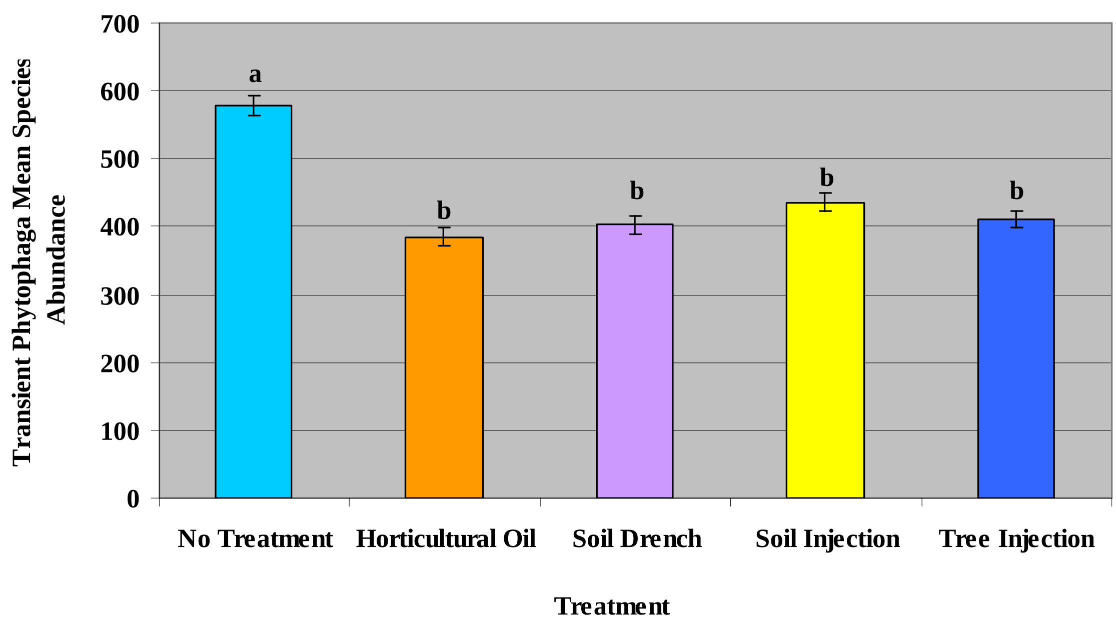

Figure 19. Transient phytophaga mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

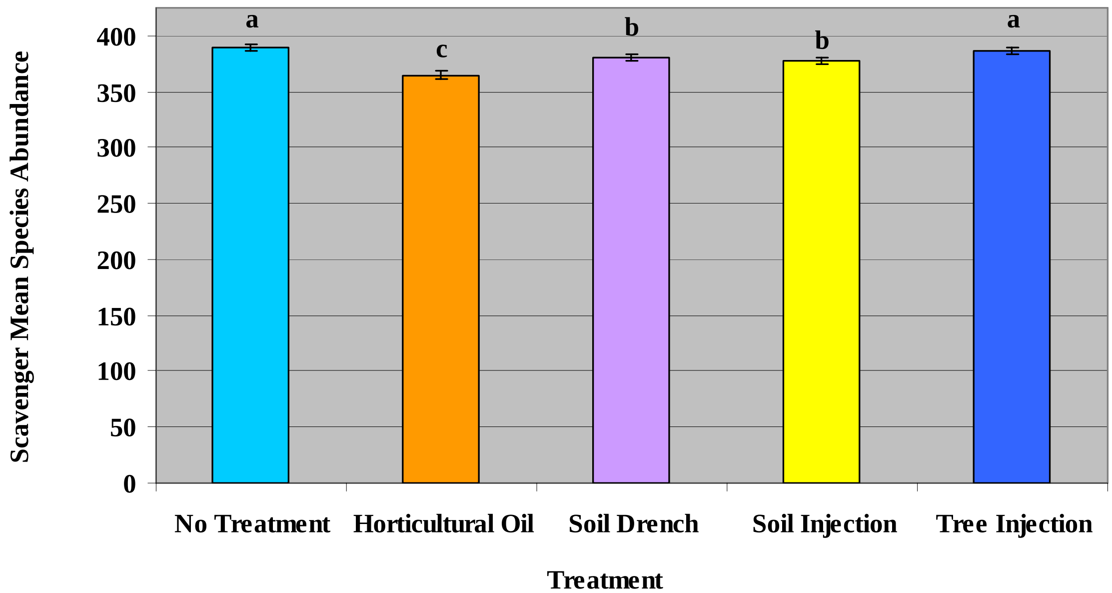

Figure 20. Scavenger mean species abundance. Means (n = 6) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

Treatment methods have an effect on species richness and abundance within guilds. This shifts guild structure to varying degrees based on the type of treatment being applied. Those trees treated with a soil drench show significant decreases in species richness in detritivore and phytophaga guilds, and in species abundance in detritivore, fungivore, phytophaga, transient phytophaga, and scavenger guilds. Treatment timing has not been a significant factor in effecting species richness and abundance within guilds.

In addition to a decrease in non-target insects species richness and abundance, the shift of guild structure may have indirect effects. Insect herbivores control nutrient turnover and leaf area (Janzen 1981; Wiegert and Evans 1967) and function as the primary herbivores in forest ecosystems removing between 3–20% of photosynthetic biomass in temperate deciduous and tropical evergreen forests (Coley and Aide 1991; Landsberg and Ohmart 1989; Odum and Ruiz-Reyes 1970; Schowalter and Ganio 1999; Schowalter et al. 1986; Van Bael et al. 2004). The significant shift in phytophaga species richness and abundance found in those trees treated with soil drench has the potential to change the rate of nutrient turnover and leaf area. Reduction in the detritivore guild may lead to a reduction in nutrient cycling, greater disease incidence, and reduction in the biodiversity of ground-dwelling species. Insect scavengers and detritivores aid ecosystem function by breaking down organic material and recycling nutrients into their surrounding environments, reductions in these guilds would reduce the rates of the latter.

Effect on Species

Independent t-tests on the differences of least squares means for the 293 insect species identified in this study indicate that 35 species are significantly effected by imidacloprid (Table 6). These species significantly belong to phytophaga and detritivore guilds. The phytophagous species belonged to the order Lepidoptera in the families Gelechiidae, Geometridae, Lymantriidae, Noctuidae, and Tortricidae, while the detritivore species belong to the order Psocoptera in the families Caeciliidae, Peripsocidae, Philotarsidae, and Psocidae.

Soil drench had the greatest effect on all these species and was significantly different (t-test; $P < 0.0001$) from the control and horticultural oil treatments in all 35 observed species. For most species tested, no significant differences (t–test; $P > 0.0006$) were found when using the Bonferroni corrected alpha of 0.0006 among those treated with horticultural oil, tree injection, and the control.

Insects in the phytophaga guild feed directly on eastern hemlock and so uptake of imidacloprid through feeding is expected. Additionally, imidacloprid works by direct contact as well as ingestion. Because all the lepidopteran species listed pupate in the soil, usually at the base of a tree, application of the soil drench may well be the reason for the significant reduction in specimen numbers. The detritivorous psocopterans feed primary on decaying organic material; however, the species listed will also feed on decaying microfungi present on the ventral side of leaves or needles (Mockford 1993). The microfungi have hyphae that penetrate the plant tissue and absorb material from the plant tissue. As such, it has the potential to uptake imidacloprid therefore exposing feeding Psocoptera to lethal concentrations of imidacloprid.

Table 6. Insect species potentially effected by insecticide treatment.

Order	Family	Genus	Species	Author	Treatment*	Mean ± SD
Lepidoptera	Gelechiidae	*Coleotechnites*	*apicitripunctella*	(Clemens)	HO	15.11 ± 6.53a
					NT	15.39 ± 6.42a
					SD	1.33 ± 0.49b
					SI	8.06 ± 2.34a
					TI	15.94 ± 7.07a
Lepidoptera	Geometridae	*Caripeta*	*divisata*	Walker	HO	9.88 ± 2.20a
					NT	9.88 ± 2.19a
					SD	1.44 ± 0.53b
					SI	4.15 ± 2.34b
					TI	9.11 ± 3.20a
Lepidoptera	Geometridae	*Cladara*	*limitaria*	(Walker)	HO	12.00 ± 0.95a
					NT	12.75 ± 2.18a
					SD	1.29 ± 0.49b
					SI	6.08 ± 1.51b
					TI	13.08 ± 1.44a
Lepidoptera	Geometridae	*Ectropis*	*crepuscularia*	(Denis & Schiffermüller)	HO	18.69 ± 14.9a
					NT	21.44 ± 8.13a
					SD	1.62 ± 0.11c
					SI	10.94 ± 4.76b
					TI	20.67 ± 6.29a
Lepidoptera	Geometridae	*Eufidonia*	*notataria*	(Walker)	HO	30.17 ± 6.96ab
					NT	46.11 ± 10.1a
					SD	1.83 ± 0.79c
					SI	24.67 ± 7.44b
					TI	43.78 ± 9.38b
Lepidoptera	Geometridae	*Eupithecia*	*lariciata*	(Freyer)	HO	10.73 ± 1.93a
					NT	10.97 ± 1.43a
					SD	1.50 ± 0.52b
					SI	4.70 ± 1.42b
					TI	11.43 ± 1.36a
Lepidoptera	Geometridae	*Eupithecia*	*palpata*	Packard	HO	11.30 ± 1.18a
					NT	11.80 ± 1.16a
					SD	1.00 ± 0.00c
					SI	4.60 ± 1.33b
					TI	11.87 ± 1.25a

Table 6 continued. Insect species potentially effected by insecticide treatment.

Order	Family	Genus	Species	Author	Treatment*	Mean ± SD
Lepidoptera	Geometridae	*Hydriomena*	*divisaria*	(Walker)	HO	9.88 ± 2.20a
					NT	9.88 ± 2.19a
					SD	1.44 ± 0.53b
					SI	4.15 ± 2.34b
					TI	9.11 ± 3.20a
Lepidoptera	Geometridae	*Hypagyrtis*	*piniata*	(Pack)	HO	13.11 ± 1.23a
					NT	13.00 ± 0.97a
					SD	1.17 ± 0.38b
					SI	5.22 ± 1.31c
					TI	13.39 ± 1.42a
Lepidoptera	Geometridae	*Lambdina*	*athasaria*	Walker	HO	26.17 ± 6.28a
					NT	36.17 ± 11.5a
					SD	1.78 ± 0.81c
					SI	17.06 ± 6.91b
					TI	30.83 ± 12.1a
Lepidoptera	Geometridae	*Lambdina*	*fiscellaria fiscellaria*	Hulst	HO	23.33 ± 3.63a
					NT	25.75 ± 2.67a
					SD	1.58 ± 0.51c
					SI	12.25 ± 2.93b
					TI	24.42 ± 2.19a
Lepidoptera	Geometridae	*Macaria*	*fissinotata*	Hübner	HO	22.90 ± 5.40a
					NT	24.27 ± 5.99a
					SD	1.63 ± 0.49c
					SI	12.47 ± 3.96b
					TI	25.33 ± 5.33a
Lepidoptera	Geometridae	*Macaria*	*signaria dispuncta*	Hübner	HO	18.47 ± 6.05a
					NT	19.13 ± 6.20a
					SD	1.47 ± 0.51b
					SI	7.80 ± 3.72b
					TI	18.70 ± 7.73a
Lepidoptera	Geometridae	*Nepytia*	*canosaria*	(Walker)	HO	12.33 ± 1.53a
					NT	12.17 ± 1.72a
					SD	1.08 ± 0.29c
					SI	6.78 ± 1.66b
					TI	12.72 ± 1.78a

Table 6 continued. Insect species potentially effected by insecticide treatment.

Order	Family	Genus	Species	Author	Treatment*	Mean ± SD
Lepidoptera	Geometridae	*Protoboarmia*	*porcelaria*	(Guenée)	HO	15.75 ± 2.63ab
					NT	14 ± 2.37b
					SD	1.42 ± 0.51d
					SI	7.25 ± 1.14c
					TI	19.74 ± 2.45a
Lepidoptera	Lymantriidae	*Dasychira*	*plagiata*	(Walker)	HO	10.08 ± 3.99a
					NT	10.90 ± 0.88a
					SD	1.00 ± 0.00b
					SI	4.83 ± 0.00b
					TI	11.33 ± 1.07a
Lepidoptera	Noctuidae	*Elaphria*	*versicolor*	(Grote)	HO	23.60 ± 1.81a
					NT	23.20 ± 1.45a
					SD	2.00 ± 0.63c
					SI	15.60 ± 3.15b
					TI	24.23 ± 1.91a
Lepidoptera	Noctuidae	*Feralia*	*comstocki*	(Grote)	HO	22.92 ± 3.56a
					NT	23.21 ± 1.79a
					SD	1.53 ± 0.52c
					SI	13.88 ± 3.69b
					TI	24.17 ± 2.66a
Lepidoptera	Noctuidae	*Feralia*	*jocosa*	(Guenée)	HO	8.72 ± 1.45a
					NT	8.72 ± 2.11a
					SD	1.00 ± 0.00c
					SI	4.22 ± 3.21b
					TI	8.94 ± 1.55a
Lepidoptera	Noctuidae	*Lithophane*	*innominata*	(Smith)	HO	7.67 ± 2.10a
					NT	6.92 ± 2.27a
					SD	1.00 ± 0.00b
					SI	2.92 ± 2.35a
					TI	6.83 ± 2.37a
Lepidoptera	Noctuidae	*Morrisonia*	*confusa*	Hübner	HO	6.72 ± 1.58a
					NT	6.44 ± 1.59a
					SD	1.00 ± 0.04b
					SI	1.56 ± 0.65b
					TI	6.47 ± 1.92a

Table 6 continued. Insect species potentially effected by insecticide treatment.

Order	Family	Genus	Species	Author	Treatment*	Mean ± SD
Lepidoptera	Noctuidae	*Morrisonia*	*latex*	(Guenée)	HO	5.78 ± 1.17a
					NT	6.22 ± 1.26a
					SD	1.00 ± 0.00b
					SI	1.67 ± 0.69b
					TI	6.06 ± 1.26a
Lepidoptera	Noctuidae	*Panthea*	*acronyctoides*	(Walker)	HO	22.83 ± 1.98a
					NT	23.11 ± 1.57a
					SD	3.33 ± 2.27c
					SI	11.44 ± 1.98b
					TI	23.22 ± 1.83a
Lepidoptera	Noctuidae	*Polypogon*	*cruvalis*	(Walker)	NT	12.17 ± 1.34a
					SD	1.00 ± 0.00c
					SI	5.17 ± 1.19b
					TI	11.83 ± 3.76a
Lepidoptera	Noctuidae	*Xestia*	*badicollis*	Grote	HO	22.63 ± 1.06a
					NT	23.00 ± 1.29a
					SD	2.09 ± 0.68c
					SI	14.42 ± 1.89b
					TI	23.83 ± 1.55a
Lepidoptera	Tortricidae	*Amorbia*	*humerosana*	Clemens	HO	19.67 ± 4.59a
					NT	22.33 ± 2.11a
					SD	1.50 ± 0.55c
					SI	10.67 ± 2.00b
					TI	21.50 ± 3.37a
Psocoptera	Caeciliidae	*Valenzuela*	*flavidus*	(Stevens)	HO	40.22 ± 5.55a
					NT	35.50 ± 2.57a
					SD	2.0 ± 0.54b
					SI	38.44 ± 5.77a
					TI	37.31 ± 2.76a
Psocoptera	Caeciliidae	*Xanthocaecilius*	*sommermanae*	(Mockford)	HO	14.13 ± 3.87a
					NT	15.22 ± 3.02a
					SD	2.22 ± 0.78b
					SI	14.55 ± 2.67a
					TI	15.33 ± 1.77a

Table 6 continued. Insect species potentially impacted by insecticide treatment.

Order	Family	Genus	Species	Author	Treatment*	Mean ± SD
Psocoptera	Peripsocidae	*Peripsocus*	*maculosus*	Mockford	HO	87 ± 6.77a
					NT	89 ± 7.12a
					SD	4.23 ± 1.32c
					SI	56.75 ± 4.55b
					TI	91.23 ± 7.98a
Psocoptera	Peripsocidae	*Peripsocus*	*subfasiatus*	(Rambur)	HO	31 ± 3.45a
					NT	28.45 ± 4.56a
					SD	2.13 ± 0.78b
					SI	26.88 ± 5.56a
					TI	32.22 ± 4.77a
Psocoptera	Philotarsidae	*Aeroniella*	*badonneli*	(Danks)	HO	22.3 ± 3.22a
					NT	24.34 ± 2.45a
					SD	1.22 ± 0.55b
					SI	21.44 ± 4.33a
					TI	22.9 ± 4.45a
Psocoptera	Philotarsidae	*Aeroniella*	*maculosa*	(Danks)	HO	91 ± 6.67a
					NT	87 ± 7.32a
					SD	3 ± 1.34b
					SI	85 ± 9.34a
					TI	89.34 ± 6.44a
Psocoptera	Psocidae	*Blaste*	*opposita*	(Banks)	HO	37.23 ± 5.34a
					NT	35.76 ± 6.44a
					SD	2.22 ± 1.30b
					SI	29.33 ± 8.34a
					TI	39.34 ± 7.23a
Psocoptera	Psocidae	*Metylophorus*	*novaescotiae*	(Walker)	HO	16.56 ± 4.34a
					NT	18.23 ± 6.35a
					SD	1.07 ± 0.56b
					SI	17.56 ± 5.34a
					TI	18.34 ± 5.45a

Means ± SD (n = 6) within species grouping followed by the same letter are not significantly different based on least squares means t-test with a Bonferroni corrected alpha.

* HO = horticultural oil, NT = no treatment, SD = soil drench, SI = soil injection, TI = tree injection.

IV. Conclusions

Application timing (fall versus spring) had no significant effect on the translocation of imidacloprid across various treatment methods, and was shown not to have an effect on non-target phytophagous and transient insects. This information will allow for broader application time providing more flexibility to the individual regarding when they can apply control measures. The application method was shown to be a significant factor in determining the concentration and translocation of imidacloprid.

Imidacloprid has been shown to translocate throughout the canopy of eastern hemlock in varying concentrations and tends to progressively decrease from the bottom strata to the top strata. Eastern hemlocks treated with soil drenches have been shown to produce significantly higher concentrations of imidacloprid in comparison to other methods, and maintained significantly higher residual levels that have been correlated to effective control of hemlock woolly adelgid by Cowles et al. (2005) throughout all strata for one year. Soil injection applications produced lower concentrations than the soil drench, but concentrations across all strata still fell within the range needed for effective control of hemlock woolly adelgid for one year. Tree injection produced the lowest concentrations of imidacloprid being translocated, these concentrations were well below the range of effective control (<120 ppb).

This significantly higher concentration of imidacloprid translocated in trees that were soil drenched has an effect on overall species richness and abundance, guild species richness and abundance, and on specific species. In most instances, tree injection effect was similar to that of the control having a minimal to no effect on observed species richness, species abundance, guild species richness, guild species abundance, and specific species abundances. This minimized effect is probably due to the non-uniform distribution and extremely low concentrations and residuals of imidacloprid throughout the canopy, relative to other treatment methods throughout the tree, especially after 3 months post-treatment. Effects of soil injection was sometimes comparable with the effects of the soil drench on overall species richness and abundance, guild species richness and abundance, and on specific species by soil injection, but not always.

The effect of chemical treatments on specific species is evident and there appears to be specific species that are more sensitive to chemical treatment than others. The lepidopteran species effected in this study are polyphagous and can feed on other trees and the psocopteran species effected in this study have a broad range of host trees that they reside on, as such the effects on forest populations of these species are unknown. In addition to these direct impacts, indirect impacts, such as the reduction of phytophagous insects, may alter the rate of nutrient turnover and other ecological processes. A reduction in the number of phytophagous and transient species may result in a reduction in the number of predators associated with this tree as the result of a lower number of available or preferred prey.

The differences between soil drench and soil injection imidacloprid concentrations and respective effect on non-target canopy insects may represent a threshold of tolerance; however, the correlation between imidacloprid concentration and LC_{50} of non-target insects is not known and the LC_{50} of imidacloprid on hemlock woolly adelgid is loosely correlated with existing estimates varying from 120 ppb-300 ppb. This is an area in need of future research. Additionally, some trees were infested with hemlock woolly adelgid and others were not. While invasive species have been shown to displace native species, the impact of the hemlock woolly adelgid on native canopy insects is unknown. Because of the small time frame remaining before the widespread establishment and potential dominance of the hemlock woolly adelgid on eastern hemlocks in the southern Appalachians, it is imperative such information be obtained prior to the displacement of those native species now inhabiting the region.

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Appendix

Appendix A. Specialists assisting in verification and identification of specimens from Cherokee National Forest for 2005, 2006, and 2007.

Specialist's Name	Address
Chris Dietrich, Ph.D. Membracidae	Illinois Natural History Survey Section for Biodiversity 1816 S. Oak Street Champaign, IL 61820
Paris Lambdin, Ph.D. Heteroptera	130 Biotechnology Bldg. 2505 E. J. Chapman Dr. University of Tennessee Knoxville, TN 37996-4560
Edward Mockford, Ph.D. Psocoptera	Department of Biological Sciences Campus Box 4120 Illinois State University Normal, IL 61790-4120
David Paulsen Diptera and Lepidoptera	147 Biotechnology Bldg. 2505 E. J. Chapman Dr. University of Tennessee Knoxville, TN 37996-4560

Appendix B. Insect species found in association with eastern hemlock at Indian Boundary, in Cherokee National Forest, Tennessee 2005-2007.

Order	**Family**	**Genus**	**Species**	**Author**	**# of specimens**	**Collecting Method***
Orthoptera	Acrididae	*Dichromorpha*	*viridis*	(Scudder)	149	M,D
Orthoptera	Gryllidae	*Allonemobius*	*socius*	(Scudder)	20	M
Orthoptera	Gryllidae	*Orocharis*	*saltator*	Uhler	16	M,D
Orthoptera	Tettigoniidae	*Scudderia*	sp.		8	M,D
Blattodea	Blatellidae	*Ischnoptera*	*deropeltiformis*	Brunner	38	M
Blattodea	Blatellidae	*Parcoblatta*	*pennsylvanica*	(DeGeer)	725	M,D
Blattodea	Blattidae	*Periplaneta*	*americana*	(L.)	218	M,D
Psocoptera	Caeciliidae	*Valenzuela*	*flavidus*	(Stevens)	992	V,S,B
Psocoptera	Caeciliidae	*Valenzuela*	*pinicola*	(Banks)	199	V,S,B
Psocoptera	Caeciliidae	*Xanthocaecilius*	*sommermanae*	(Mockford)	295	V,S
Psocoptera	Ectopsocidae	*Ectopsocus*	*cryptomeriae*	(Enderlein)	829	V,S,B
Psocoptera	Ectopsocidae	*Ectopsocus*	*meridionalis*	Ribaga	860	V,B
Psocoptera	Dasydemellidae	*Teliapsocus*	*couterminus*	(Walsh)	227	V,S,B
Psocoptera	Lachesillidae	*Lachesilla*	*contraforecepta*	Chapman	620	V,S,B
Psocoptera	Lachesillidae	*Lachesilla*	*rufa*	(Walsh)	1012	V,S,B
Psocoptera	Lepidopsocidae	*Echmepteryx*	*hageni*	(Packard)	608	V,S
Psocoptera	Myopsocidae	*Lichenomima*	sp.1		314	V,S,B
Psocoptera	Peripsocidae	*Peripsocus*	*alboguttatus*	(Dalman)	796	V,S,B
Psocoptera	Peripsocidae	*Peripsocus*	*maculosus*	Mockford	2163	V,B
Psocoptera	Peripsocidae	*Peripsocus*	*madidus*	(Hagen)	1035	V,S,B
Psocoptera	Peripsocidae	*Peripsocus*	*subfasiatus*	(Rambur)	730	V,S,B
Psocoptera	Philotarsidae	*Aeroniella*	*maculosa*	(Aaron)	1934	M,S,B
Psocoptera	Psocidae	*Blaste*	*opposita*	(Banks)	876	V,S,B
Psocoptera	Psocidae	*Blaste*	*quieta*	(Hagen)	1513	V,S,B
Psocoptera	Psocidae	*Blastopsocus*	*lithinus*	(Chapman)	149	V,S,B
Psocoptera	Psocidae	*Cerastipsocus*	*venosus*	(Burmeister)	642	V,S,B
Psocoptera	Psocidae	*Metylophorus*	*novaescotiae*	(Walker)	414	M,S,B
Psocoptera	Psocidae	*Metylophorus*	*purus*	(Walsh)	157	V,S,B
Psocoptera	Psocidae	*Psocus*	*leidyi*	Aaron	650	V,S,B
Hemiptera	Aradidae	*Aradus*	sp. 1		77	M,D,S
Hemiptera	Adelgidae	*Adelges*	*tsugae*	Annand	12242	B
Hemiptera	Cercopidae	*Lepyronia*	*quadrangularis*	Say	169	M,S
Hemiptera	Cercopidae	*Philaenus*	*spumarius*	(L.)	236	M,S
Hemiptera	Cercopidae	*Prosapia*	*bicinta*	(Say)	681	M,S
Hemiptera	Cicadellidae	*Empoasca*	sp.1		59	M,S
Hemiptera	Cicadellidae	*Empoasca*	sp.2		2	M,S
Hemiptera	Cicadellidae	*Graphocephala*	*coccinea*	(Forster)	105	M,S
Hemiptera	Cicadellidae	*Gyponana*	*conferta*	DeLong	219	M,S,B

Order	Family	Genus	Species	Author	# of specimens	Collecting Method*
Hemiptera	Cicadellidae	*Oncometopia*	*orbona*	(F.)	143	M,S,B
Hemiptera	Cicadellidae	*Osbornellus*	*limosus*	DeLong	124	M,D,S
Hemiptera	Flatidae	*Cyarda*	*melichari*	Van Duzee	165	M,D,S
Hemiptera	Flatidae	*Metcalfa*	*pruinosa*	(Say)	570	M,S
Hemiptera	Issidae	*Acanalonia*	*bivittata*	(Say)	276	M,D,S
Hemiptera	Membracidae	*Campylenchia*	*latipes*	Say	81	D,S
Hemiptera	Membracidae	*Platycotis*	*vittata*	(F.)	127	M,D,S
Hemiptera	Pentatomidae	*Apateticus*	*cynicus*	(Say)	440	M,D,S
Hemiptera	Pentatomidae	*Acrosternum*	*hilare*	(Say)	145	D,S
Hemiptera	Pentatomidae	*Elasmostethus*	*cruciatus*	(Say)	66	M,D,S
Hemiptera	Pentatomidae	*Parabrochymena*	*arborea*	(Say)	100	M,S
Hemiptera	Pentatomidae	*Menecles*	*insertus*	Say	7	M,D,S
Hemiptera	Thyreocoridae	*Corimelaena*	*pulicaria*	(Germar)	143	M,D,S
Hemiptera	Tingidae	*Corythuca*	*pruni*	Osborn and Drake	29	M,D,S
Coleoptera	Anobiidae	*Ptilinus*	*ruficornis*	Say	1	M,V,S
Coleoptera	Bostrichidae	*Xylobiops*	*basilaris*	(Haldeman)	8	M,V,S
Coleoptera	Buprestidae	*Melanophila*	*fulvoguttata*	(Harris)	184	M,V,S
Coleoptera	Cantharidae	*Rhagonycha*	*oriflava*	(LeConte)	4	M,S
Coleoptera	Cerambycidae	*Analeptura*	*lineola*	(Say)	99	M,V,S
Coleoptera	Cerambycidae	*Anthophylax*	*cyaneus*	Haldeman	94	M,V
Coleoptera	Cerambycidae	*Brachyleptura*	*circumdata*	(Olivier)	75	M,D,S
Coleoptera	Cerambycidae	*Callimoxys*	*sanguinicollis*	(LeConte)	12	M,V,S
Coleoptera	Cerambycidae	*Clytus*	*ruricola*	(Olivier)	59	M,V,S
Coleoptera	Cerambycidae	*Cyrtophorus*	*verrucosum*	(Olivier)	64	M,D,S
Coleoptera	Cerambycidae	*Judolia*	*cordifera*	(Olivier)	123	M,D,S
Coleoptera	Cerambycidae	*Leptura*	*emarginata*	F.	206	M,D,S
Coleoptera	Cerambycidae	*Lepturopsis*	*biforis*	(Newman)	131	M,S
Coleoptera	Cerambycidae	*Microclytus*	*gazellula*	(Haldeman)	8	M
Coleoptera	Cerambycidae	*Oberea*	*perspicillata*	Haldeman	50	M,V,S
Coleoptera	Cerambycidae	*Orthosoma*	*brunneum*	(Forster)	120	M
Coleoptera	Cerambycidae	*Pidonia*	*aurata*	(Horn)	134	M,V,S
Coleoptera	Cerambycidae	*Pidonia*	*densicollis*	(Casey)	93	M,V,S
Coleoptera	Cerambycidae	*Pidonia*	*ruficollis*	(Say)	110	M,V,S
Coleoptera	Cerambycidae	*Strangalepta*	*abbreviata*	(Germar)	136	M,V,S
Coleoptera	Cerambycidae	*Stangalia*	*bicolor*	(Swederus)	105	M,V,S
Coleoptera	Cerambycidae	*Stangalia*	*lutecornis*	F.	104	M,V,S
Coleoptera	Chrysomelidae	*Chrysochus*	*auratus*	F.	21	M,S
Coleoptera	Chrysomelidae	*Chrysomela*	*interrupta*	F.	31	M,V,S
Coleoptera	Chrysomelidae	*Kuschelina*	*suturella*	(Say)	40	M,V,S
Coleoptera	Cucujidae	*Silvanus*	sp.1		1	M

Order	Family	Genus	Species	Author	# of specimens	Collecting Method*
Coleoptera	Cucujidae	*Silvanus*	sp.2		2	M
Coleoptera	Curculionidae	*Hylesinus*	*aculeatus*	Say	11	M,V,S
Coleoptera	Curculionidae	*Odontopus*	*calceatus*	(Say)	199	M,S
Coleoptera	Curculionidae	*Curculio*	*caryae*	(Horn)	97	V,S
Coleoptera	Curculionidae	*Cyrtepistomis*	*castaneus*	(Roelofs)	47	M,S
Coleoptera	Curculionidae	*Myrmex*	*myrmex*	(Herst)	230	D,V
Coleoptera	Curculionidae	*Otiorhynchus*	*ovatus*	L.	111	M,D,V,S
Coleoptera	Curculionidae	*Otiorhynchus*	*sulcatus*	(F.)	28	M,V,S
Coleoptera	Elateridae	*Agriotes*	*oblongicollis*	(Melsheimer)	58	M,D,V,S
Coleoptera	Elateridae	*Athous*	*brightwell*	(Kirby)	169	M,S
Coleoptera	Elateridae	*Ctenicera*	*signaticollis*	(Melsheimer)	45	M,S
Coleoptera	Elateridae	*Melanotus*	*americanus*	(Herst)	124	M,D,S
Coleoptera	Elateridae	*Parallelostethus*	*attenuatus*	(Say)	10	M,D,S
Coleoptera	Elateridae	*Melanotus*	*hyslopi*	Zwaluwenberg	97	M,V,S
Coleoptera	Endomychidae	*Endomychus*	*biguttatus*	Say	38	M,D,S
Coleoptera	Erotylidae	*Triplax*	*festiva*	Lacordaire	22	M,D,S
Coleoptera	Eucnemidae	*melasis*	sp.		5	M
Coleoptera	Geotrupidae	*Bolboceras*	*simi*	(Wallis)	16	M,D,S
Coleoptera	Geotrupidae	*Geotrupes*	*hornii*	Blanchard	110	M,D
Coleoptera	Geotrupidae	*Geotrupes*	*semiopacus*	Jekel	72	M,S
Coleoptera	Geotrupidae	*Geotrupes*	*splendidus*	(F.)	47	M,D,S
Coleoptera	Histeridae	*Hololepta*	*aequalis*	Say	6	M
Coleoptera	Lampyridae	*Ellychnia*	*corrusca*	(L.)	154	M,S
Coleoptera	Lampyridae	*Photuris*	*pennsylvanica*	(Degeer)	97	M,D,S
Coleoptera	Lampyridae	*Pyropyga*	*decipiens*	(Harris)	99	M,D,S
Coleoptera	Cerambycidae	*Leptura*	*subhamata*	Randall	73	M,S
Coleoptera	Lucanidae	*Pseudolucanus*	*capreolus*	L.	8	M,D,S
Coleoptera	Lycidae	*Plateros*	*centralis*	Green	7	M,,D,S
Coleoptera	Meloidae	*Lytta*	*vesicatoria*	L.	47	M,D, S
Coleoptera	Mordellidae	*Mordellistena*	*ornata*	(Melsheimer)	3	M
Coleoptera	Mordellidae	*Tomoxia*	*serval*	(Say)	20	M,D,S
Coleoptera	Mycetophagidae	*Mycetophagus*	*flexuosus*	Say	892	M,D,S
Coleoptera	Nitidulidae	*Cryptarcha*	*ampla*	Erichson	18	M,D,S
Coleoptera	Nitidulidae	*Epuraea*	sp.		9	M,S
Coleoptera	Nitidulidae	*Glischrochilus*	*fasiatus*	(Olivier)	2279	M,D,S
Coleoptera	Nitidulidae	*Glischrochilus*	*quadrisignatus*	(Say)	1489	M,D,S
Coleoptera	Nitidulidae	*Glischrochilus*	*sanguinolenta*	(Olivier)	2182	M,D,S
Coleoptera	Nitidulidae	*Stelidota*	*octomaculata*	(Say)	3	M,S

Order	Family	Genus	Species	Author	# of specimens	Collecting Method*
Coleoptera	Phengodidae	*Phengodes*	sp.1		1	M
Coleoptera	Pyrochroidae	*Dendroides*	*concolor*	(Newman)	12	M,D
Coleoptera	Pyrochroidae	*Neopyrochroa*	*flabellata*	(F.)	198	M,V,S
Coleoptera	Scarabaeidae	*Anomala*	*marginata*	(F.)	28	M,D,S
Coleoptera	Scarabaeidae	*Dichelonyx*	*subvittata*	LeConte	307	M,S
Coleoptera	Scarabaeidae	*Euphoria*	*inda*	L.	487	M,S
Coleoptera	Scarabaeidae	*Melanocanthon*	sp.1		6	M
Coleoptera	Scarabaeidae	*Phyllophaga*	sp.1		372	M,D,S
Coleoptera	Scarabaeidae	*Phyllophaga*	sp.2		611	M,D,S
Coleoptera	Scarabaeidae	*Phyllophaga*	sp.3		321	M,D,S
Coleoptera	Scarabaeidae	*Serica*	*atracapilla*	(Kirby)	107	M,S
Coleoptera	Scarabaeidae	*Serica*	*giorgiana*	Leng	168	M,D,S
Coleoptera	Scarabaeidae	*Serica*	sp.1		197	MS
Coleoptera	Silphidae	*Necrophilia*	*americana*	(L.)	683	M,S
Coleoptera	Silphidae	*Nicrophorus*	*orbicollis*	Say	884	M,S
Coleoptera	Silphidae	*Nicrophorus*	*pustulatus*	Herschel	1019	M,S
Coleoptera	Silphidae	*Nicrophorus*	*tomentosus*	Weber	635	M,S
Coleoptera	Staphylinidae	*Scaphisoma*	*favescens*	(Casey)	27	M,D,S
Coleoptera	Staphylinidae	*Scaphisoma*	*lacustris*	(Casey)	20	M,S
Coleoptera	Tenebrionidae	*Arthromacra*	*aenea*	Say	328	M,D,S
Coleoptera	Tenebrionidae	*Meracantha*	*contracta*	(Beauvois)	263	M,S
Coleoptera	Tenebrionidae	*Neomida*	*bicornis*	(F.)	11	M,S
Coleoptera	Tenebrionidae	*Helops*	*aereus*	Germar	25	M,S
Coleoptera	Tenebrionidae	*Tarpela*	*micans*	(F.)	317	M,S
Coleoptera	Tenebrionidae	*Tarpela*	*undulata*	(LeConte)	470	M,S
Hymenoptera	Apidae	*Bombus*	*bimaculatus*	Cresson	1	M
Hymenoptera	Apidae	*Bombus*	*fervidus*	(F.)	3	M,D
Hymenoptera	Apidae	*Bombus*	*impatiens*	Cresson	21	M
Hymenoptera	Apidae	*Bombus*	*pennsylvanicus*	(Degeer)	83	M
Hymenoptera	Apidae	*Bombus*	*perplexus*	Cresson	14	M
Hymenoptera	Apidae	*Bombus*	*sandersoni*	Franklin	36	M
Hymenoptera	Apidae	*Bombus*	*vagans*	Smith	3	M
Hymenoptera	Formicidae	*Aphaenogaster*	sp.1		45	V,S
Hymenoptera	Formicidae	*Aphaenogaster*	sp.2		67	D,S
Hymenoptera	Formicidae	*Formica*	sp.1		123	D,V,S
Hymenoptera	Formicidae	*Camponotus*	sp.1		101	D,V
Hymenoptera	Formicidae	*Camponotus*	sp.1		87	V,S
Hymenoptera	Formicidae	*Crematogaster*	sp.1		127	V,S
Hymenoptera	Formicidae	*Crematogaster*	sp.2		234	V,S
Hymenoptera	Formicidae	*Crematogaster*	sp.3		167	V,S

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Hymenoptera	Formicidae	*Lasius*	sp.1		127	D,V,S
Lepidoptera	Gelechiidae	*Coleotechnites*	*apicitripunctella*	(Clemens)	997	M,D,S
Lepidoptera	Geometridae	*Caripeta*	*divisata*	Walker	985	M,D,S
Lepidoptera	Geometridae	*Cladara*	*limitaria*	(Walker)	536	M,D,S
Lepidoptera	Geometridae	*Ectropis*	*crepuscularia*	Denis & Schiffermüller	3516	M,D,S
Lepidoptera	Geometridae	*Eufidonia*	*notataria*	(Walker)	2638	M,D,S
Lepidoptera	Geometridae	*Eupithecia*	*lariciata*	(Freyer)	1153	M,D,S
Lepidoptera	Geometridae	*Eupithecia*	*palpata*	Packard	1192	M,D,S
Lepidoptera	Geometridae	*Hydriomena*	*divisaria*	Walker	862	M,D,S
Lepidoptera	Geometridae	*Hypagyrtis*	*piniata*	(Pack)	826	M,D,S
Lepidoptera	Geometridae	*Lambdina*	*athasaria*	Walker	2016	M,D,S
Lepidoptera	Geometridae	*Lambdina*	*fiscellaria*	Hulst	1048	M,D,S
Lepidoptera	Geometridae	*Macaria*	*fissinotata*	Walker	2593	M,D,S
Lepidoptera	Geometridae	*Macaria*	*signaria*	Hübner	1948	M,D,S
Lepidoptera	Geometridae	*Melanolophia*	*canadaria*	(Guenée)	24	M,D,S
Lepidoptera	Geometridae	*Nematolampa*	*limbata*	(Haworth)	64	M,D
Lepidoptera	Geometridae	*Nemoria*	*mimosaria*	(Guenée)	225	M,D,S
Lepidoptera	Geometridae	*Nepytia*	*canosaria*	(Walker)	805	M,D,S
Lepidoptera	Geometridae	*Prochoerodes*	*transversata*	(Drury)	45	M,D,S
Lepidoptera	Geometridae	*Protoboarmia*	*porcelaria*	(Guenée)	698	M,D,S
Lepidoptera	Geometridae	*Tetracis*	*cachexiata*	Guenée	1297	M,D,S
Lepidoptera	Lymantriidae	*Dasychira*	*plagiata*	Walker	430	M,D,S
Lepidoptera	Lymantriidae	*Orgyia*	*leucostigma*	(Smith)	1234	M,D,S
Lepidoptera	Mimallionidae	*Lacosoma*	*chiridota*	Grote	18	M,D,S
Lepidoptera	Noctuidae	*Acronicta*	*morula*	Grt. & Rob.	84	M,D,S
Lepidoptera	Noctuidae	*Agrotis*	*ipsilon*	(Hufn.)	43	M,D,S
Lepidoptera	Noctuidae	*Catocala*	*cerogama*	(Guenée)	9	M,D,S
Lepidoptera	Noctuidae	*Cucullia*	*Intermedia*	(Speyer)	8	M,D
Lepidoptera	Noctuidae	*Elaphria*	*versicolor*	(Grote)	2611	M,D,S
Lepidoptera	Noctuidae	*Feralia*	*comstocki*	Grote	2043	M,D,S
Lepidoptera	Noctuidae	*Feralia*	*jocosa*	(Guenée)	556	M,D,S
Lepidoptera	Noctuidae	*Hypena*	*baltimozalis*	(Guenée)	20	M,S
Lepidoptera	Noctuidae	*Hyppa*	*xylinoides*	(Guenée)	4	M,D,S
Lepidoptera	Noctuidae	*Lithophane*	*innominata*	Grote	294	M,D,S
Lepidoptera	Noctuidae	*Lithophane*	*petulca*	(Grote)	2	M,D,S

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Lepidoptera	Noctuidae	*Morrisonia*	*confusa*	(Hübner)	765	M,D,S
Lepidoptera	Noctuidae	*Morrisonia*	*latex*	(Guenée)	355	M,D,S
Lepidoptera	Noctuidae	*Polypogon*	*cruvalis*	(Walker)	508	M,D,S
Lepidoptera	Noctuidae	*Sunira*	*bicolorago*	(Guenée)	103	M,D,S
Lepidoptera	Noctuidae	*Tarachidia*	*erastrioides*	(Guenée)	7	M,D,S
Lepidoptera	Noctuidae	*Xestia*	*badicollis*	(Guenée)	2059	M,D,S
Lepidoptera	Nymphalidae	*Boloria*	*selene*	(Denis & Schiffermuller)	21	M,D,S
Lepidoptera	Nymphalidae	*Libytheana*	*carinenta*	Streckeri	40	M,D,S
Lepidoptera	Nymphalidae	*Polygonia*	*interrogationis*	(F.)	24	M,D,S
Lepidoptera	Papilionidae	*Papilio*	*marcellus*	Cramer	11	M,D
Lepidoptera	Papilionidae	*Papilio*	*troilus*	L.	6	M,D
Lepidoptera	Pyralidae	*Condylolomua*	*participialis*	Grote	20	M,D,S
Lepidoptera	Pyralidae	*Herpetogramma*	*thestealis*	(Walker)	62	M,D,S
Lepidoptera	Saturniidae	*Citheronia*	*sepulcralis*	Grt. & Rob.	8	M
Lepidoptera	Tortricidae	*Amorbia*	*humerosana*	Clemens	1344	M,DS
Lepidoptera	Tortricidae	*Choristoneura*	*fumiferana*	(Clemens)	145	M,D,S
Lepidoptera	Tortricidae	*Eucosma*	*tocullionana*	Heinrich	166	M,D,S
Mecoptera	Panorpidae	*Panorpa*	*appalachia*	Byers	30	M
Diptera	Anthomyiidae	*Anthomyia*	*pluvialis*	(L.)	21	M
Diptera	Anthomyiidae	*Emmesomyia*	*socialis*	(Stein)	51	M
Diptera	Anthomyiidae	*Hydrophoria*	sp.1		33	M,S
Diptera	Anthomyiidae	*Pegomya*	sp.1		158	M
Diptera	Bibionidae	*Bibio*	sp.1		43	M,D
Diptera	Bombyliidae	*Bombylius*	sp.1		8	M
Diptera	Bombyliidae	*Bombylius*	sp.2		250	M
Diptera	Calliphoridae	*Calliphora*	*vomitoria*	(L.)	758	M
Diptera	Calliphoridae	*Lucilia*	*coevuleiviridis*	(Macquart)	414	M,D
Diptera	Calliphoridae	*Lucilia*	*pallescens*	(Shannon)	230	M
Diptera	Calliphoridae	*Pollenia*	*rudis*	(F.)	180	M
Diptera	Ceratopogonidae	*Atrichopoogon*	sp.1		334	M,V,B
Diptera	Ceratopogonidae	*Culicoides*	*sanguisuga*	(Coquillet)	662	M,V
Diptera	Chironomidae	*Parametriocnemus*	*lundbeckii*	Johannsen	331	M
Diptera	Drosophilidae	*Drosophila*	sp.		1225	M
Diptera	Drosophilidae	*Paramycodrosophila*	sp.1		234	M
Diptera	Drosophilidae	*Paramycodrosophila*	sp.2		414	M,D
Diptera	Dryomyzidae	*Dryomyza*	*simplex*	Loew	30	M
Diptera	Empididae	*Euthyneura*	*bucinator*	Melander	36	M
Diptera	Heleomyzidae	*Allophyla*	*atricornis*	(Meigen)	102	M

Order	**Family**	**Genus**	**Species**	**Author**	**# of specimens**	**Collecting Method***
Diptera	Heleomyzidae	*Amoebaleria*	sp.1		21	M
Diptera	Heleomyzidae	*Amoebaleria*	sp.2		106	M,D
Diptera	Heleomyzidae	*Suillia*	sp.1		68	M
Diptera	Lauxaniidae	*Camptoprosopella*	sp.1		20	M
Diptera	Lonchaeidae	*Lonchea*	sp.1		49	M,B,S
Diptera	Lonchaeidae	*Lonchea*	sp.2		17	M,D
Diptera	Micropezidae	*Rainieria*	*antennaepes*	(Say)	25	M
Diptera	Muscidae	*Helina*	*hel1*		15	M,V
Diptera	Muscidae	*Mesembrina*	*latreillii*	Robineau – Desvoidy	77	M
Diptera	Muscidae	*Mydaea*	sp.1		14	M
Diptera	Muscidae	*Phaonia*	sp.1		174	M
Diptera	Muscidae	*Thricops*	*rufisquama*	(Schnabl)	98	M
Diptera	Mycetophilidae	*Boletina*	sp.1		14	M
Diptera	Mycetophilidae	*Boletina*	sp.2		13	M,D
Diptera	Mycetophilidae	*Boletina*	sp.3		31	M
Diptera	Mycetophilidae	*Brevicornu*	sp.1		152	M,S
Diptera	Mycetophilidae	*Docosia*	*dichroa*	Loew	2990	M
Diptera	Mycetophilidae	*Dynatosoma*	*fulvidum*	Coquillet	232	M
Diptera	Mycetophilidae	*Dynatosoma*	*placidum*	Johannsen	187	M
Diptera	Mycetophilidae	*Leptomorphus*	*subcaerula*	(Coquillet)	91	M,S
Diptera	Mycetophilidae	*Monoclona*	*rufilatera*	Walker	2243801	M
Diptera	Mycetophilidae	*Mycomya*	sp.1		87	M
Diptera	Mycetophilidae	*Mycomya*	sp.2		83	M
Diptera	Mycetophilidae	*Mycetophilia*	sp.1		83	M
Diptera	Mycetophilidae	*Mycetophilia*	sp.2		42	M
Diptera	Mycetophilidae	*Mycetophilia*	sp.3		14	M
Diptera	Mycetophilidae	*Orfelia*	sp.1		36	M
Diptera	Mycetophilidae	*Saigusaia*	*cincta*	(Johannsen)	203	M
Diptera	Mycetophilidae	*Synapha*	*tibialis*	(Coquillett)	65	M
Diptera	Mycetophilidae	*Zygomyia*	*ornata*	(Loew)	55	M,S
Diptera	Mycetophilidae	*Zygomyia*	sp. 1		4	M
Diptera	Mycetophilidae	*Zygomyia*	sp.2		21	M,D,S
Diptera	Sarcophagidae	*Blaesoxipha*	*atlanis*	Aldrich	265	M
Diptera	Sarcophagidae	*Boettcheria*	*cimbicis*	(Townsend)	586	M
Diptera	Sarcophagidae	*Fletcherimyia*	sp.1		405	M
Diptera	Sarcophagidae	*Fletcherimyia*	sp.2		183	M
Diptera	Sarcophagidae	*Sarcophaga*	sp.1		33	M
Diptera	Sarcophagidae	*Sarcophaga*	sp.2		198	M
Diptera	Sarcophagidae	*Tripanurga*	sp.1		12	M
Diptera	Sarcophagidae	*Udamopyga*	*niagarana*	(Parker)	134	M,S
Diptera	Scathophagidae	*Scathophaga*	*nigrolimbata*	Cresson	10	M
Diptera	Scathophagidae	*Scathophaga*	*stercoraria*	(L.)	44	M
Diptera	Sciaridae	*Bradysia*	sp.1		203	M
Diptera	Sciaridae	*Bradysia*	sp.2		422	M
Diptera	Sciaridae	*Bradysia*	sp.3		245	M

Order	Family	Genus	Species	Author	# of specimens	Collecting Method*
Diptera	Sciaridae	*Bradysia*	sp.4		554	M
Diptera	Simuliidae	*Prosimilium*	*mixtum*	Syme and Davies	180	M,S
Diptera	Syrphidae	*Ferdinandea*	*buccata*	(Loew)	79	M,S
Diptera	Syrphidae	*Ferdinandea*	*dives*	Osten Sacken	164	M
Diptera	Syrphidae	*Mllota*	*bautias*	(Walker)	53	M,B
Diptera	Syrphidae	*Syrphus*	sp.1		13	M
Diptera	Syrphidae	*Syrphus*	sp.2		11	M,D
Diptera	Syrphidae	*Syrphus*	sp.3		18	M
Diptera	Syrphidae	*Toxomerus*	sp.1		56	M
Diptera	Syrphidae	*Toxomerus*	sp.2		186	M
Diptera	Tabanidae	*Chrysops*	*geminatus*	Wiedemann	54	M,D
Diptera	Tachinidae	*Siphosturmia*	sp.1		238	M
Diptera	Tephritidae	*Trupanea*	sp.1		131	M
Diptera	Tipulidae	*Austrolimnophila*	*toxoneura*	(Ostensacken)	29	M,D,V
Diptera	Tipulidae	*Tipula*	*duplex*	Walker	51	M, D
Diptera	Tipulidae	*Elephantomyia*	*westwoodi*	Osten Sacken	87	M
Diptera	Tipulidae	*EpiphragM*	*fasciapennis*	(Say)	69	M
Diptera	Tipulidae	*Limonia*	*indigena*	(Osten Sacken)	37	M,S
Diptera	Xylophagidae	*Dialysis*	sp.1		109	M,D,S

*** M = malaise trap, D = direct observation, S = beat–sheet, B = branch sample, and V = vacuum.**

VITA

Carla Irene Dilling was born in Mechanicsburg, Pennsylvania, on December 3, 1975. She grew up in Mechanicsburg, Pennsylvania, where she graduated from Mechanicsburg Area High School in 1994. Carla earned a Bachelor of Science in ecology and evolutionary biology and a Bachelor of Arts in anthropology from Ohio State University in December 2004. She came to the University of Tennessee in January of 2005, where she worked as a research assistant/lab technician in the Department of Forestry, Fisheries, and Wildlife. In August 2005 she started her Masters of Science program at the University of Tennessee in the Department of Entomology and Plant Pathology under the direction of Dr. Paris Lambdin. During her time in the masters program at the University of Tennessee, she gave numerous oral and poster presentations, received an award for outstanding masters poster presentation at the 2006 Southeastern Branch of the Entomological Society of America, and won best in show by an Entomological Society of America member for a photograph of a spicebush swallowtail caterpillar at the 2006 national ESA photo salon. Carla Irene Dilling is a member of the Entomological Society of America, Ecological Society of America, Tennessee Entomological Society, Pennsylvania Entomological Society, Association of Southeastern Biologists, and Gamma Sigma Delta Agricultural Honor Society.