

**Impact of Insecticide on Pollinator Communities in a Forested System:
A Model System Using Eastern Hemlock, *Tsuga canadensis*,
Rosebay Rhododendron, *Rhododendron maximum*, and Imidacloprid**

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

**David Bruce Bechtel
May 2020**

Dedication

To

Mary G. Isaac

and

Asher & Ambrose

My family who share the joy of life and nature with me

Acknowledgements

I would like to thank Dr. Jerome Grant for his effort to get to know me and my interests to help identify an interesting and inspiring topic for my research, and for his continued guidance and feedback as the process advanced. Thank you to the rest of my committee, Dr. Becky Nichols, Jesse Webster, and Dr. Greg Wiggins, who provided technical assistance and insights to help my research proceed more methodically.

I also wish to thank the undergraduate students (Brianna Alred, Kailee Alred, Laura Garrett, Davis Harmon, and Forest Palmer) who assisted with making observations in the field, processing rhododendron seed samples, and helping to navigate. I am especially grateful to Davis Harmon for his steadfast support many days in the woods in challenging landscapes seeking out specimens for my research and providing many constructive ideas and assists. A special thanks to the USDA Forest Service for monetary support and to the staff at the Great Smoky Mountains National Park for advice, input, and permission.

Abstract

Mortality of eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinales: Pinaceae), has occurred at a high rate since the arrival of the invasive hemlock wooly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). The systemic neonicotinoid pesticide imidacloprid is soil-applied to hemlocks for effective control of HWA. However, is this pesticide translocated by incidental non-target plants under hemlock trees and, if so, does it impact non-target insects, such as pollinators? One commonly encountered flowering understory associate is *Rhododendron maximum* L. (Ericales: Ericaceae). Research has demonstrated that imidacloprid is translocated to leaves, nectar and pollen of *R. maximum*. The goal of this research was to assess if quantifiable differences in pollinators are associated with imidacloprid-treated HWA-infested hemlocks in Great Smoky Mountains National Park (GRSM). Research objectives were to: 1) determine the influence of imidacloprid treatment of hemlocks on pollinators of non-target *R. maximum*, 2) assess the influence of imidacloprid treatment of hemlock on seed production and germination of seed collected from *R. maximum* growing near treated hemlocks, and 3) determine species incidence, species composition, and seasonality of pollinators of *R. maximum* in GRSM.

Insect visitors to *R. maximum* flowers were observed and some specimens were collected. High variability in pollinator abundance and diversity was documented at the four sites and no significant difference was identified between pollinators in treated and untreated areas. Seedpods and seedfall collected from rhododendrons had high seed number variability, and no significant differences in seed characteristics were identified between treated and untreated sites. A moderate positive correlation between pollinator visitation rate and number

of seeds collected in buckets was observed. Through 64.5 hours of cumulative observations of *R. maximum* in GRSM, a total of 711 insects were observed visiting flowers. Hymenoptera comprised 89% of visitors with *Bombus* spp. the most common taxa observed. *Bombus* spp. were more active in the evening than first morning period (8am – 10am). Halictidae were more active in the two midday periods than morning. More sites and better classification of the sites by assessing landscape factors that impact pollinator populations would help obtain more definitive results to discern possible pesticide induced responses.

Table of Contents

CHAPTER I INTRODUCTION	1
Eastern Hemlock and Hemlock Woolly Adelgid	1
Imidacloprid	7
<i>Rhododendron maximum</i>	16
Research Objectives	18
 CHAPTER II INFLUENCE OF IMIDACLOPRID TREATMENT TO EASTERN HEMLOCK, <i>TSUGA CANADENSIS</i>, ON POLLINATORS OF ADJACENT, NON-TARGET ROSEBAY RHODODENDRON, <i>RHODODENDRON MAXIMUM</i>	19
Introduction	19
Materials and Methods	24
Selecting Sites and Rhododendrons	24
Data Collection	28
Data Analysis	31
Results and Discussion	33
Summary	34
 CHAPTER III IMPACT OF IMIDACLOPRID TREATMENT TO HEMLOCK ON PRODUCTION AND GERMINATION OF SEED COLLECTED FROM <i>RHODODENDRON MAXIMUM</i> IN CLOSE ASSOCIATION WITH TREATED HEMLOCKS	37
Introduction	37
Materials and Methods	43
Study Design and Data Collection	43
Data Analysis	46
Results and Discussion	47
Summary	50
 CHAPTER IV SPECIES INCIDENCE, SPECIES COMPOSITION, AND SEASONALITY OF POLLINATORS OF <i>RHODODENDRON MAXIMUM</i> IN GREAT SMOKY MOUNTAINS NATIONAL PARK	53
Introduction	53
Materials and Methods	57
Data Analysis	58
Results and Discussion	59
Summary	64

CHAPTER V CONCLUSIONS	67
REFERENCES	76
APPENDIX.....	95
VITA	140

List of Tables

Table 2.1 Overstory density measured using a concave spherical densiometer model C (Robert E. Lemmon, Forest Densiometers, 5733 Cornell Drive, Bartlesville, Oklahoma). Values represent average of measurements taken on four sides of each rhododendron at the four cardinal directions at tagged rhododendrons at the four study sites, Great Smoky Mountains National Park, 22 August and 7 September 2018.....	109
Table 3.1 Number, weight, and percent germination of <i>Rhododendron maximum</i> L. seed from seedpods collected on tagged rhododendrons at four study sites, Great Smoky Mountains National Park, 2018.....	115
Table 3.2 Number, weight, and percent germination of bucket-collected <i>Rhododendron maximum</i> L. seedfall; and cumulative number of pollinators and time spent observing with the average rate of pollinator visitation at flowers of tagged rhododendrons at the four study sites over the five-week flowering period, Great Smoky Mountains National Park, 2018.	119
Table 3.3 Average seed number per seedpod, cumulative number of pollinators and time spent observing flowers of tagged <i>Rhododendron maximum</i> L. at each of the four study sites over the five-week flowering period, with the average rate of pollinator visitation for each site, Great Smoky Mountains National Park, 2018.....	125
Table 3.4 Pollinator visitation rates by orders and families based on cumulative time observing flowers of tagged <i>Rhododendron maximum</i> L. at each of the four study sites over the five-week flowering period and cumulative visitors observed, and average seedpod seed number at each tagged rhododendron, Great Smoky Mountains National Park, 2018.....	126
Table 4.1 Taxa observed (n = 711) and collected on <i>Rhododendron maximum</i> L. flowers at selected sites in the Great Smoky Mountains National Park, 2018.	129
Table 4.2 Diversity indices for species of pollinators collected on <i>Rhododendron maximum</i> L. flowers by site with time spent observing and cumulative number of flowers observed, Great Smoky Mountains National Park, 2018.....	136
Table 4.3 Diversity indices for species of pollinators collected on <i>Rhododendron maximum</i> L. flowers by week with time spent observing and cumulative number of flowers observed, Great Smoky Mountains National Park, 2018.....	137

List of Figures

Figure 1.1 Distribution of eastern hemlock and hemlock woolly adelgid in North America, 2018 (Wisconsin Department of Natural Resources 2018).....	96
Figure 1.2 Hemlock woolly adelgid life cycle in North America (Cheah et al. 2004).....	97
Figure 1.3 Flowering <i>Rhododendron maximum</i> L. in a hemlock-dominated forest, Groundhog Creek, Great Smoky Mountains National Park, 11 July 2018.	98
Figure 1.4 Distribution of <i>Rhododendron maximum</i> L. in the United States (Little 1981).....	99
Figure 1.5 Close-up of raceme inflorescence of <i>Rhododendron maximum</i> L.; expanding flower bud in upper right of photo (see arrow), Spruce Flats, Great Smoky Mountains National Park, 25 June 2018.	100
Figure 1.6 <i>Rhododendron maximum</i> L. blossom with poricidal anthers and pink stigma visible in lower right of photo; close-up view of the raceme-form inflorescence on rhododendron; Lepturinae (flower longhorn) beetle in top middle of photo, Lynn Camp, Great Smoky Mountains National Park, 10 July 2018.....	101
Figure 2.1 Study site locations in Great Smoky Mountains National Park (1 = Spruce Flats; 2 = Gilliland Creek; 3 = Lynn Camp; 4 = Groundhog Creek).	102
Figure 2.2 Treated hemlock marked with paint at time of imidacloprid treatments represent two treatment times: 1) 2014 (June) – upper faded red paint and 2) 2009 – blue paint, Gilliland Creek, Great Smoky Mountains National Park, 9 April 2018.	103
Figure 2.3 Flowering <i>Rhododendron maximum</i> L. located 35 cm from treated hemlock, Gilliland Creek, Great Smoky Mountains National Park, 28 June 2018.....	104
Figure 2.4 Map of Gilliland Creek area showing the area of treated hemlock (yellow shading), <i>Rhododendron maximum</i> L. understory (blue shading), and overlapping areas of hemlock and rhododendron, Great Smoky Mountains National Park.	105
Figure 2.5 Map of Spruce Flats area showing the area of treated hemlock (yellow shading), <i>Rhododendron maximum</i> L. understory (blue shading), and overlapping areas of hemlock and rhododendron, Great Smoky Mountains National Park.	106
Figure 2.6 Map of Lynn Camp area showing the area of <i>Rhododendron maximum</i> L. understory (blue shading), Great Smoky Mountains National Park.	107

Figure 2.7 Map of Groundhog Creek area showing the area of *Rhododendron maximum* L. understory (blue shading), Great Smoky Mountains National Park..... 108

Figure 2.8 Adjusted mean ($\pm$ SE) number of pollinators on *Rhododendron maximum* L. flowers and interaction of treatment and distance from hemlock, Great Smoky Mountain National Park, 2018. (Adjusted means reflect numbers of pollinators observed during a typical 25-minute observation.) 110

Figure 2.9 Adjusted mean ($\pm$ SE) number of pollinators observed on *Rhododendron maximum* L. flowers located away from and beside hemlocks, Great Smoky Mountains National Park, 2018. (Adjusted means reflect numbers of pollinators observed during a typical 25-minute observation.) 111

Figure 2.10 Visitors by order and by hymenopteran family observed on *Rhododendron maximum* L. flowers at treated and control sites, Great Smoky Mountains National Park, 2018. Yellow and black patterns are hymenopteran families..... 112

Figure 3.1 Bucket used to collect seed falling from *Rhododendron maximum* L. seedpods, Great Smoky Mountains National Park..... 113

Figure 3.2 Sample of *Rhododendron maximum* L. seed placed in petri dish and sealed with parafilm for germination study. 114

Figure 3.3 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed in *Rhododendron maximum* L. seedpods from hemlock treated and untreated control areas, Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean..... 117

Figure 3.4 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed in *Rhododendron maximum* L. seedpods at four study sites in Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean. 118

Figure 3.5 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed collected in buckets under *Rhododendron maximum* L. from hemlock treated and untreated control areas, Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean..... 121

Figure 3.6 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed collected in buckets under <i>Rhododendron maximum</i> L. at four study sites in Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2 nd and 3 rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean.	122
Figure 3.7 Green mold growth in seed germination sample.	123
Figure 3.8 Black mold growth in seed germination sample.	124
Figure 3.9 Correlations between rate of visits by pollinators per hour and A) seed number, B) seed weight, and C) percent germination of seed collected in buckets under <i>Rhododendron maximum</i> L., Great Smoky Mountains National Park, 2018.	128
Figure 4.1 Visitors by order and by hymenopteran family observed on <i>Rhododendron maximum</i> L. flowers, Great Smoky Mountains National Park, 2018. Yellow and black patterns represent hymenopteran families.	132
Figure 4.2 Total number of non-hymenopteran pollinators observed by week of the flowering period on <i>Rhododendron maximum</i> L. flowers, Great Smoky Mountains National Park, 2018.	133
Figure 4.3 Total number of hymenopteran pollinators observed on <i>Rhododendron maximum</i> L. flowers by week of the flowering period and time of day, Great Smoky Mountains National Park, 2018.	134
Figure 4.4 Adjusted mean number of hymenopteran pollinators observed at each sampling period (visitation rate), 25 minutes of observation, for Apidae, Halictidae, and <i>Andrena cornelli</i> ; letter groups indicate significant differences ($p < 0.05$) across sampling time periods for each family or species separately, Great Smoky Mountains National Park, 2018.	135
Figure 4.5 Total number of <i>Rhododendron maximum</i> L. flowers observed, time spent observing, and total number of insects observed on flowers by week, Great Smoky Mountains National Park, 2018.	138
Figure 4.6 Correlations between time spent observing and total number of flowers observed, and time spent observing and total number of insects observed at <i>Rhododendron maximum</i> L. flowers by sampling week, Great Smoky Mountains National Park, 2018.	139

CHAPTER I

INTRODUCTION

Eastern Hemlock and Hemlock Woolly Adelgid

Eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinales: Pinaceae), is one of 9 or 10 (depending on the authority) hemlock species found worldwide (Havill et al. 2008, Farjon 2017, Earle 2019). Hemlocks are members of the pine family and, unlike other pine genera, hemlocks have a drooping lead shoot. Four hemlock species are native to North America. Western hemlock, *T. heterophylla* (Rafinesque) Sargent, and mountain hemlock, *T. mertensiana* (Bongard) Carrière, are found in the northwest, and Carolina hemlock, *T. caroliniana* Engelmann, is found in pockets in Virginia, Tennessee, North Carolina, South Carolina, and Georgia, with its range overlapping the range of eastern hemlock. Eastern hemlock ranges from Wisconsin east to Nova Scotia, then south to northern Alabama and Georgia, preferring areas with moist well-drained soils (Fig. 1.1) (Havill et al. 2014). Eastern hemlock typically grows to 30 m with exceptional trees growing to 53 m (Nesom 2002, Blozan 2007). Eastern hemlock has been known to live over 500 years and is sometimes referred to as the “redwood of the east” (Whiting 2018).

In the northeastern states during the 1800’s and early 1900’s, eastern hemlock was exploited for its bark which was used to tan hides for shoe leather, harnesses, and belts to drive the machines of the industrial revolution (McMartin 1992). Although not considered a prime timber species, eastern hemlock has been utilized for lumber for general construction, e.g., houses and barns, pallets, furniture, crates and boxes (Hough 1960). Today eastern hemlock

cultivars are popular as ornamentals, and there is some limited harvest for timber and pulpwood (Farjon 2017). Because of its importance and aesthetic qualities, in 1931, eastern hemlock was designated as the official state tree of Pennsylvania (National Association of State Foresters 2011).

Eastern hemlock is valued for its ecological significance (Ellison et al. 2005). It is considered a foundation species providing many ecosystem services, such as shading and cooling streams, reducing erosion and balancing nutrient cycling, and stabilizing stream flows to the benefit of many aquatic species, such as salamanders, fish, and invertebrates intolerant of seasonal drying. It creates diversity in the forest structure, enhancing the forest ecosystem and providing habitat for a variety of organisms (Howe and Mossman 1996). Being shade tolerant, it maintains live scaffold branches providing habitat niches at different levels in the canopy for a variety of species (Ellison et al. 2005).

Populations of this foundation species of our eastern forests throughout much of its native range in the U.S. have been devastated by an introduced pest, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Havill et al. 2016). HWA was discovered in Richmond, VA in 1951 having been accidentally brought to the area from southern Japan (Havill et al. 2014). Genetic markers indicate that the eastern North America population of HWA originated from a single introduction from a lineage found in southern Japan that specializes on southern Japanese hemlock, *Tsuga seiboldii* Carrière, and tiger-tail spruce, *Picea torano* (Siebold ex K. Koch) Koehne (Havill et al. 2016). Eastern hemlock and Carolina hemlock have been dying in eastern North America at a high rate since HWA began to spread rapidly in the mid-1980s (Earle 2019). In the last three decades HWA has spread

virtually unchecked to the north, south, and west after being considered a minor pest in pockets of Virginia, Pennsylvania, New York, New Jersey and Connecticut. HWA infestation has led to some foresters opting for preemptive salvage cutting in anticipation of extensive mortality (Brooks 2004).

In North America, two populations of HWA exist; a long established native population in western North America and the population that was accidentally introduced from Japan into eastern North America (Havill et al. 2016). Both of these adelgid populations are parthenogenetic and oviparous, with two generations (sistens and progrediens) completing their lifecycles in succession on host hemlock trees each year (McClure 1987a, 1989). The term sistens refers to the wingless adelgid generation that has a long aestival diapause during the first nymphal instar and the progrediens generation begins to feed and develop without delay (Havill and Footitt 2007). The two western hemlock species tolerate populations of the native HWA, while the two eastern species are devastated by populations of the introduced HWA (McClure 1992, Havill et al. 2006).

The lifecycle of HWA is complex and closely aligned to hemlock growth (Fig. 1.2). Sistens eggs, laid by progrediens in early summer, hatch into first-instar nymphs known as crawlers that wander to find a suitable site at the base of the hemlock needles to insert their stylets (Young et al. 1995). Crawlers may be blown by wind or carried by birds or other animals to new trees (McClure 1990). These first-instar sistens nymphs then settle and undergo aestival diapause for three to four months before beginning to feed in fall (McClure 1987a, 1989, 1996). As HWA feed they produce a woolly wax thread covering and molt four times through fall and early winter, maturing to adult sistens in February. All sistens are parthenogenetic females and

produce as many as 300 progrediens and sexuparae eggs under their woolly covering in March and April (Cheah et al. 2004). These eggs hatch and the offspring are the progrediens and sexuparae crawlers beginning in April and quickly develop through four nymphal instars maturing to adults in June. Adult progrediens produce as many as 125 sistens eggs under their woolly covering beginning in June (Cheah et al. 2004); these eggs become the next sistens generation.

Sexuparae develop simultaneously with the progrediens, and their first-instar nymphs are indistinguishable (McClure 1989). Sexuparae are a winged generation, found in some adelgid species or populations, that fly from the secondary host (in the case of HWA, hemlock) to their primary host which is always a spruce species (Havill and Footitt 2007). Sexuparae reproduce parthenogenetically and their eggs become a sexual generation. In eastern North America the sexuparae produce eggs on spruce but the nymphs die within a few days, not advancing past the first instar (McClure 1987a). All adelgids produced on 15 spruce species in Connecticut died in a few days suggesting that the Japanese lineage of HWA established in eastern North America requires tiger-tail spruce, native to Japan, to reproduce sexually (McClure 1987a, 1991, Havill et al. 2016).

Once the crawler inserts its stylets at the base of a needle, it stays in place for the rest of its life (Young et al. 1995). With its stylet bundle inserted, it feeds on xylem ray parenchyma cells and secretes a salivary sheath which may be toxic to the hemlock. HWA feeding results in reduced terminal and lateral growth, decreased photosynthesis, and symptoms of water stress (Gonda-King et al. 2014). HWA infestation can lead to needle loss, tree decline, and mortality in as few as four years (McClure 1991).

Forested landscapes are a mosaic of ecosystem types which include eastern hemlock ecosystems in eastern forests (Abella et al. 2003). Hemlock forests are in decline due to HWA infestation leading to structural changes in the eastern forests (Orwig et al. 2013). Loss of the dense hemlock canopy increases light availability which increases understory vegetation species richness within, while decreasing the diversity in the forest mosaic (Abella 2018). Soil and air temperatures in the understory can become higher in summer and lower in winter in these once closed canopy forests (Lustenhouwer et al. 2012). As hemlocks are lost, the forest landscape becomes less diverse with hemlock ecosystems becoming more like mesic hardwood ecosystems (Abella 2018).

The drastic changes to forest structure, composition, and diversity due to hemlock mortality have become widespread. As of 2018, HWA had spread to one-half the eastern hemlock range and was found in 20 states and Nova Scotia in Canada (Fig. 1.1). Impacts to hemlock vary across its range. A study in the Delaware Water Gap National Recreation Area found 73% hemlock survivorship after 10 years of HWA infestation (Eschtruth et al. 2013). At the Coweeta Hydrologic Laboratory in western North Carolina, HWA was first noticed on trees in 2003, by 2005 100% of the trees were infested and <1% of hemlocks were dead (Elliott and Vose 2011). In 2008, 33% of trees were dead and average crown loss was 88%. At Jocassee Gorges in South Carolina, 60 km south of Great Smoky Mountains National Park (GRSM; Park), forested plots were inventoried in 2001, before the invasion of HWA, and again in 2016 (Abella 2018). HWA infestation was first observed in the study area in 2002 and by 2006 HWA had spread throughout the area. In 2016, hemlock density had declined 90% with 100% mortality in hemlocks >60 cm dbh.

HWA was discovered in GRSM in 2002 where it has spread throughout the Park and wreaked havoc. In 2003, in areas not yet infested with HWA, plots in GRSM were sampled for woody vegetation and results were tallied (Mulroy et al. 2019). Resampling of 33 plots representing five ecogroups with hemlock as a dominant species was performed in 2017 and changes in importance value (IV) $\{IV = [(relative\ density + relative\ basal\ area)/2] \times 100\}$ in the 14-year interval were calculated. In the three plots where hemlock had received imidacloprid treatment, hemlock IV declined 22%. In the other 30 plots hemlock IV decline ranged from 70% - 96%, with 19 plots declining more than 93%.

As hemlocks are lost, hemlock-associated species decline or disappear, thus the forests become less diverse. In the Delaware Water Gap National Recreation Area, the total basal area of hemlock stands has declined from 56% to 46%, which has led to declining species richness of hemlock-associated birds (Toenies et al. 2018). Most affected was the black-throated green warbler, *Setophaga virens* (Gmelin) (Passeriformes: Parulidae), a species closely associated with hemlock.

To protect hemlocks and the forest, a variety of methods to manage HWA are investigated, implemented, and evaluated. Efforts at biological control of HWA have incorporated the introduction of predatory beetles, including lady beetles *Sasajiscymnus tsugae* (Sasaji and McClure) and *Scymnus sinuanodulus* Yu et Yao (Coleoptera: Coccinellidae), and tooth-necked fungus beetles, *Laricobius nigrinus* Fender and *L. osakensis* Montgomery and Shiyake (Coleoptera: Derodontidae), from parts of the native range of HWA (i.e., Japan, China, and western North America) (Davis et al. 2012, Hakeem 2013, Vieira et al. 2013, Havill et al. 2014). Studies continue to evaluate the establishment and efficacy of these introduced beetles

and potential introductions of new predators, such as populations of silver flies, *Leucopis argenticollis* (Zetterstedt) and *Leucopis piniperda* (Malloch) (Diptera: Chamaemyiidae), from northwestern U.S. (Havill et al. 2018). Investigations to incorporate these predators as part of an integrated pest management strategy with chemical controls and silvicultural practices, such as thinning to increase sun exposure, continue to be developed (Havill et al. 2014, Brantley et al. 2017, Sumpter et al. 2018).

Imidacloprid

Currently, the most effective method of control of HWA has been the use of the systemic pesticide imidacloprid applied as a soil drench or soil injection around the roots of susceptible and infested hemlock trees (Silcox 2002). Imidacloprid was introduced in 1991 by Bayer Crop Science and has been one of the most commonly used pesticides (Nauen and Bretschneider 2002). It is in the class of insecticides known as neonicotinoids that target the nicotinic acetylcholine receptors in the insects' central nervous system. It has been highly effective against piercing-sucking insects, such as aphids, whiteflies, planthoppers and HWA.

To control HWA, imidacloprid is applied around the base of the infested or susceptible hemlock where it is absorbed by the roots and translocated throughout the tree (Cowles 2009). Inside the tree imidacloprid is metabolized and one of the pesticidal metabolites is olefin (Nauen et al. 1998). Olefin has been shown to be at least 14 times more effective than the parent compound, and the combination of imidacloprid, olefin, and other metabolites may be synergistic. The imidacloprid concentration in the sap peaks in about one year, and, as it is metabolized, olefin concentration peaks in about three years (Coots 2012). Olefin is more readily transported to the top stratum of the tree with higher imidacloprid concentrations

found lower in the tree. Imidacloprid and olefin are present in branchlets for up to seven years, and remains an effective control of HWA for at least five years after treatment (Benton et al. 2015, Benton and Cowles 2016).

Imidacloprid is reported to have a half-life in soil ranging from 28 - 1,250 days (Goulson 2013). Depending on the soil composition and rate of application it can accumulate in the soil, for example, in agricultural settings where it may be applied annually. When used as a seed dressing most of the active ingredient is not taken up by the plant but remains in the soil where it can persist depending on the rates of application and degradation (Sur and Stork 2003). At two sites in England, imidacloprid-treated winter wheat seed was sown six years in a row and the soil was tested for imidacloprid concentration one year after each application (Goulson 2013). Imidacloprid accumulated in the soils at rates of about 3 - 10 ppb/yr depending on the site and application rate, with accumulated concentrations of 18 - 60 ppb after the sixth year. The accumulated imidacloprid could be available to be translocated by subsequent crops and possibly impact non-target species.

Imidacloprid is water soluble and highly adsorbed by organic material (Cox et al. 1998). Mobility of imidacloprid in soil depends on atmospheric precipitation, soil structure, and soil composition. Heavy rain events will tend to mobilize imidacloprid in soils and organic matter in soils tend to retain it in place. Depending on these conditions imidacloprid will remain where applied or leach and mobilize to non-target plants or waterways (Goulson 2013). In the high organic content soils around hemlocks in GRSM it is expected that imidacloprid will be adsorbed by the soil and remain available to the trees longer with less expected mobility (Knoepp et al. 2012).

To determine the collateral effects of treating hemlock trees with imidacloprid, studies have been conducted to assess responses of associated arthropod communities. These studies have included examining effects of the imidacloprid treatments on the soil arthropod community (Reynolds 2008), non-target phytophagous and transient canopy insects (Dilling 2007, Dilling et al. 2009), predatory arthropod communities in canopy strata (Hakeem 2008, Hakeem et al. 2018), and aquatic macroinvertebrates in streams near treated trees (Benton et al. 2017).

A set of cooperative studies was conducted at Indian Boundary Recreation Area within Cherokee National Forest in Monroe County, Tennessee (Dilling 2007, Hakeem 2008, Reynolds 2008, Dilling et al. 2009, 2010, Hakeem et al. 2018). As part of these studies, imidacloprid was applied using three methods: trunk injection, soil injection, and soil drench. The greatest impact on arthropods was observed with soil drench.

The soil drench used for these studies, which was different from standard applications at the time and different than the current standard protocol recommended in 2016 (Benton and Cowles 2016), was a “worst-case” scenario (with a high volume drip line to trunk drench), that was intended to exaggerate the effect and clarify impacts, if any. Current soil drench protocol for a 28 cm dbh hemlock, as was a typical tree in these studies, require 7 g imidacloprid in 200 ml water. Before applying the imidacloprid, the surface layer of plant litter around the base of the hemlock is brushed or scraped away from the tree. The soil drench is applied to the soil around the tree within 45 cm of the trunk. When the solution has been absorbed by the soil, the plant litter can be replaced. However, in the studies conducted at Indian Boundary Recreation Area the same sized tree received 16.5 g imidacloprid in 125 l

water around the tree from trunk to dripline applied using a high pressure sprayer. Soil injections for this 28 cm tree used 11 g imidacloprid applied in two 30 ml injections within 45 cm of the trunk, 7 cm below the soil surface. Trunk injections involved application of imidacloprid to the xylem tissue through holes in the tree, and a 28 cm dbh tree received 1.65 g imidacloprid. Trunk injections of imidacloprid are not as consistently reliable for controlling HWA as soil application but are preferable when trees are close to a waterway.

Following imidacloprid applications at Indian Boundary Recreation Area, populations of Collembola declined in soil drench-treated sites (Reynolds 2008). Other microarthropods in the soil including mites, which were approximately half of the microarthropods, were not impacted by the soil drench. Collembola are common in the soil and would be expected to recolonize following degradation of the imidacloprid, but repeated applications of imidacloprid would be expected to protect the tree and consequently, if applied according to the “worse-case” scenario used in these studies, suppress Collembola populations. Soil arthropods are known for ecological functional redundancy which may decrease the consequences of Collembola decline (Petersen and Luxton 1982). Additionally, the current soil drench protocol differs from the “worse-case” soil drench scenario used in these studies. In these studies, more than twice as much imidacloprid as used in the current standard protocol was applied from trunk to dripline and would be expected to impact soil dwellers in the entire application area. Current soil drench protocol involves a localized application of the imidacloprid to the soil around the tree within 45 cm of the trunk, a much smaller area, impacting many fewer soil organisms directly with the imidacloprid (Benton and Cowles 2016). Some mobility of the imidacloprid in the soil

may lead to impacts directly adjacent to the application, but it is believed to be much less than the effects from the method used in these studies.

In the assessment of canopy-dwelling insects at Indian Boundary Recreation Area, 293 phytophagous and transient insect species were found in the hemlock canopy (Dilling 2007, Dilling et al. 2009). Phytophagous and transient insect abundance and species richness were significantly lower in soil drench-treated trees than all other treatments in the study. Of those 293 species identified, short-term populations of 25 Lepidoptera and 8 Psocoptera species were directly affected by soil-applied imidacloprid treatments. Imidacloprid works by direct contact, along with its systemic mode of action, and may have killed lepidopterans pupating under the treated trees causing localized decline (Elbert et al. 1998, Dilling 2007, Dilling et al. 2009). Psocoptera species affected are detritivores that feed on decaying organic material that may have translocated some of the imidacloprid before senescing and decaying (Dilling 2007, Dilling et al. 2009). Impact of imidacloprid on Psocoptera species was more evident on soil drench-treated trees, where significant differences were observed among densities of Psocoptera. Psocoptera were predominantly impacted only by the soil drench treatment suggesting a lethal threshold was exceeded. Significant impact on only one of the Psocoptera species was observed in trees treated by soil injection. For comparison, a 28 cm dbh tree received 11 g imidacloprid by soil injection, therefore it is expected that the 7 g imidacloprid applied according to the current soil drench protocol would have minimal impact on Psocoptera.

The study assessing impacts to spiders and predatory insects at Indian Boundary Recreation Area found short-term impacts of imidacloprid treatments to populations of these predators (Hakeem 2008, Hakeem et al. 2018). Predatory insect and spider densities were

lower following imidacloprid treatments. After treatments, spider populations were greater in the top two thirds of the canopy, and predatory insects became more abundant in the top two thirds one year after treatments. Predatory insects increased in density the second year of the study, suggesting a rebound 1 - 1½ years following treatment. Predatory insect decline may be due to declines in prey abundance or through acquiring insecticide by feeding on contaminated prey (Hakeem 2008). Imidacloprid does not affect spiders directly, therefore their decline is likely due to decreased prey abundance (Elbert et al. 1998).

When leaf tissue of hemlocks treated at Indian Boundary Recreation Area were analyzed, the imidacloprid soil drench method produced higher concentrations of imidacloprid throughout the tree and had greater impact than soil injections or trunk injections (Dilling et al. 2010). In these studies more imidacloprid was applied by the soil drench method, followed by the soil injections and finally trunk injections. Levels of imidacloprid applied correspond to the relative levels of imidacloprid observed in hemlock sap. Soil injections used 67%, and trunk injections used 10% of the amount of imidacloprid that was used in the soil drench. Current protocol for soil drench would require less than 50% of the imidacloprid used for soil drench in these studies, applied in roughly 2 m² around the trunk, and is expected to control HWA for at least 5 years (Benton and Cowles 2016). In the previously discussed studies, the soil drench was “worse-case” scenario applied from trunk to dripline and would be expected to impact a larger population of soil microarthropods and pupating lepidopterans. Current protocol for soil drench treatment would be expected to ameliorate these impacts to a large extent.

Research conducted to determine impacts of imidacloprid treatments of hemlocks adjacent to streams to aquatic macroinvertebrates was performed in GRSM (Benton et al.

2016). Imidacloprid was detected in six of the nine streams sampled. When found, imidacloprid was detected at concentrations below USEPA benchmarks (Benton et al. 2017). Aquatic macroinvertebrate communities were assessed using baseline data obtained before treatments, and sampling above and below the treatment areas. Five different metrics assessing macroinvertebrate populations identified no negative impacts of imidacloprid treatments.

Loss of a hemlock may have greater impact on the soil and canopy arthropod communities than repeated imidacloprid applications for control of HWA. Aquatic studies showed that imidacloprid treatments of hemlock trees cause no measurable negative impacts to aquatic systems. Imidacloprid treatment for control of HWA provides the obvious benefit of maintaining healthy hemlocks that can maintain their role as a foundation species and provide the demonstrated ecological benefits. But what is the impact of imidacloprid on pollinators foraging in treated hemlock systems?

Imidacloprid is widely used in agriculture and non-target impacts on pollinators are of concern. Imidacloprid is toxic to bees and sub-lethal doses have been shown to have effects that are likely to reduce colony success. Bumble bee colonies given field realistic doses gained less weight and produced significantly fewer queens (Whitehorn et al. 2012). Exposed bees were found to have reduced pollen foraging efficiency, returning to the colony with 31% less pollen per hour than control bees (Feltham et al. 2014). In microcolonies of *Bombus terrestris* L. (Hymenoptera: Apidae) fed syrup with 1.27 ppb imidacloprid, fecundity was reduced 42% (Laycock et al. 2012). Imidacloprid reduced feeding which led to the reduced brood production.

Imidacloprid acts on the insects' central nervous system (Nauen and Bretschneider 2002) and appears to impact cognition which may be the culprit of the reduced foraging and related colony success. Sub-lethal doses of imidacloprid can impair *Apis cerana* F. (Hymenoptera: Apidae) decision-making as was demonstrated by failure of exposed bees to avoid a hornet predator (Tan et al. 2014). Bumble bees given different nectar rewards based on flower color took longer to acquire a preference for the most rewarding flower color (Phelps et al. 2018). *B. terrestris* exposed to 1% imidacloprid had reduced foraging motivation as demonstrated by being slower to start foraging, and visiting fewer flowers (Lämsä et al. 2018). Reduced foraging motivation caused by imidacloprid exposure may be the basis for negative colony effects observed in other studies.

Small-scale surveys to collect pollen and nectar for chemical analysis in 2015 showed imidacloprid is translocated by rosebay rhododendron, *Rhododendron maximum* L. (Ericales: Ericaceae), growing within 30 cm of treated hemlocks (Wiggins et al., unpublished data). Using soil drench protocol, imidacloprid was applied evenly around the tree within 45 cm of the trunk (Benton and Cowles 2016). Therefore the imidacloprid applied to the hemlocks was coincidentally applied to soil at the base of the surveyed rhododendrons. These surveys detected imidacloprid in two of eight pollen samples (at concentrations of 19.9 and 24.8 ppb) and in two of three nectar samples (11.2 and 1.3 ppb). One nectar sample (1.3 ppb imidacloprid) also had the metabolite olefin (1.4 ppb) (Wiggins et al., unpublished data). The detection of imidacloprid in these pollen and nectar samples confirms that imidacloprid can be translocated by non-target rhododendron, a common flowering plant in the hemlock understory.

Soil arthropods, aquatic macroinvertebrates, and canopy arthropods on treated trees have been investigated (Dilling 2007, Hakeem 2008, Reynolds 2008, Dilling et al. 2009, Benton et al. 2017, Hakeem et al. 2018). Hemlocks host a variety of arthropod species and provide habitat and diversity in the forest system. Loss of the hemlock would alter the habitat and impact species communities. Impacts to soil and canopy arthropods were considered localized and short term in duration. Soil arthropods affected are cosmopolitan and can be expected to persist in abundance outside the treatment areas and impacted canopy-dwelling insect species are known to feed on other host tree species.

Another piece of the puzzle to deduce is if imidacloprid translocated by flowering plants rooted under treated hemlocks may affect pollinators and other flower visitors to those neighboring plant species. Pollinators in the hemlock forest are expected to visit flowering plants for nectar and pollen, food for them and for their brood. It was demonstrated that rhododendron can translocate imidacloprid when growing beside treated hemlock trees (Wiggins et al., unpublished data). Imidacloprid has some mobility in GRSM soils, as can be inferred from the detections of imidacloprid in streams in areas of treated trees (Benton et al. 2017), and may be transported through the soil from treated hemlocks to rhododendron or other flowering plants and translocated.

Does imidacloprid impact populations or behaviors of pollinators of flowering plants, specifically rosebay rhododendron, in the treated hemlock forest areas? This question is the motivation for the study reported herein.

Rhododendron maximum

Rosebay rhododendron is a common and abundant flowering broadleaf evergreen shrub of the forest understory (Leach 1961) (Fig. 1.3). *R. maximum* is indigenous from Ontario and Nova Scotia southward through New York and New England and then along the Appalachian Mountains to Georgia (Fig. 1.4, Little 1981). In the extreme north of its range *R. maximum* grows slowly to about 1.2 m tall. Heading southward, the plant becomes lusher and the leaves larger, reaching its peak around southwest North Carolina where it can take the form of a tree up to 12 m tall. *R. maximum* often grows in impenetrable thickets that are sometimes locally referred to as “laurel slicks” or “laurel hells” (Romancier 1971).

Rhododendron maximum can be found flowering from late May through early August depending on local conditions, especially altitude and latitude (Williams et al. 1990). Flowers are white to pink, or white with pink margins, growing on one stalk in round, umbel-like compact terminal clusters, known as racemes, typically with 20 to 30 flowers (Figs. 1.5, 1.6). One unique trait of many ericaceous flowers, including *R. maximum*, is the presence of long sticky viscin threads in the pollen. Viscin threads help to hold pollen together in clumps that are dislodged from the poricidal anthers (Fig. 1.6) by pollinators (Sarwar and Takahashi 2014). The viscin threads may prevent pollen falling from the anthers in the dehiscing flowers. Viscin threads of *R. maximum* are correlated with insect pollination especially by bumble bees and possibly flies.

Rhododendron maximum is a common associate of hemlock, preferring mesic soils on north slopes and near streams (Johnson and Kovner 1956). Forest structure may be highly influenced by *R. maximum*. A study at Coweeta Hydrologic Laboratory in North Carolina

showed that sapling density of canopy species was inversely related to density of *R. maximum* with the exception of hemlock (Phillips and Murdy 1985). Hemlock sapling density was found to increase with time and density of *R. maximum*. Among hardwoods, the most tolerant of the deep shade in *R. maximum* understory was red maple, *Acer rubrum* L. (Sapindales: Sapindaceae). Red maple saplings were more abundant in high density *R. maximum* stands than other hardwood canopy species.

Few studies have been conducted on the pollinators of *R. maximum* in the eastern U.S. Pollinator and flower visitation studies of *R. maximum* and other *Rhododendron* spp. at Round Bald and Gregory Bald in the southern Appalachians documented nine *Bombus* spp., six *Andrena* spp., Halictidae in the genus *Evylaeus*, and Syrphidae visiting flowers of *Rhododendron* spp. (Bauer 1986, Mayor et al. 2001). These sites are open, montane meadows known as grassy balds. *Rhododendron* species were *R. catawbiense* Michaux and *R. maximum* at Round Bald, and hybrid azaleas of the species *R. arborescens* (Pursh) Torrey, *R. viscosum* (L.) Torrey, and *R. cumberlandense* (E.L. Braun) Copeland at Gregory Bald. Syrphidae and seven *Bombus* spp. were observed on *R. maximum* at Round Bald: *B. affinis* Cresson, *B. bimaculatus* Cresson, *B. impatiens* Cresson, *B. perplexus* Cresson, *B. sandersoni* Franklin, *B. terricola* Kirby, and *B. vagans* Smith (Bauer 1986). Also at Round Bald, in addition to the Syrphidae and seven *Bombus* previously listed, *B. fervidus* (F.) and *B. nevadensis* Cresson along with *Apis mellifera* L., were observed on *R. catawbiense*. On the hybrid azaleas at Gregory Bald, four *Bombus* spp. (*B. affinis*, *B. bimaculatus*, *B. perplexus*, and '*B. sp?* near *vagans*'), *Andrena cornelli* Viereck (Hymenoptera: Andrenidae), and *Evylaeus* spp. (Hymenoptera: Halictidae) were observed repeatedly visiting, and five other *Andrena* spp. and *A. mellifera* were occasional visitors (Mayor

et al. 2001). These areas are open balds, and the composition of bee species may be different in forested areas.

Due to the broad extent of treated hemlocks in GRSM, combined with the close association with *R. maximum*, this research was designed to be an ecological study to evaluate the insect visitors, with an emphasis on pollinators, of *R. maximum* growing with imidacloprid-treated hemlocks. By collecting and observing pollinators and other insect visitors to *R. maximum* growing next to treated hemlocks, within treatment areas, and in untreated areas, species diversity, richness and evenness of pollinators is compared to assess the potential for imidacloprid impacts to the pollinator community. Seed production and germination rate in *R. maximum* was evaluated to assess if a disparity in effective pollination associated with pesticide treatments is evident. This research was conducted by observing, documenting and quantifying pollinator visits to flowers of *R. maximum*, and quantifying seed production and assessing germination rate of seed from the observed plants.

Research Objectives

To enhance the knowledge of pollinators of *R. maximum* in GRSM and to assess impacts of imidacloprid use in a forested system, the focus of this research was to:

- 1) Determine the influence of imidacloprid treatment to hemlock trees on pollinators of adjacent, non-target *R. maximum*,
- 2) Assess influence of imidacloprid treatment of hemlock on seed production and germination of seed collected from *R. maximum* growing in close association with treated hemlocks, and
- 3) Determine species incidence, species composition, and seasonality of pollinators of *R. maximum* in GRSM.

CHAPTER II

**INFLUENCE OF IMIDACLOPRID TREATMENT TO EASTERN HEMLOCK,
TSUGA CANADENSIS, ON POLLINATORS OF ADJACENT, NON-TARGET
ROSEBAY RHODODENDRON, RHODODENDRON MAXIMUM**

Introduction

Soil-applied imidacloprid insecticide has been employed throughout the range of the invasive hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), in eastern North America (Fig. 1.1) to prevent the demise of hemlock trees due to infestations (Webb et al. 2003, Blumenthal et al. 2005, Cowles et al. 2006, Coots et al. 2013, Kanoti et al. 2015, Mayfield et al. 2015, Smitley and McCullough 2017). HWA was first observed in Great Smoky Mountains National Park (GRSM; Park) in 2002, and imidacloprid has been the most effective control method since that time. HWA has no natural enemies in the eastern U.S., reproduces parthenogenetically with two generations per year, and has high fecundity (McClure 1987b, 1989). When HWA eggs hatch, the first-instar nymphs crawl to and settle at the base of the needles, where they insert their stylets and feed in the xylem ray parenchyma cells causing decline, needle loss, and often death in as few as three to five years (Young et al. 1995, Mayer et al. 2002). Some predator species have been introduced in an attempt to control HWA but currently the most effective method of control is soil-applied imidacloprid (Benton and Cowles 2016). Imidacloprid is in the class of insecticides known as neonicotinoids that work by targeting the nicotinic acetylcholine receptors in the central nervous system of insects (Nauen and Bretschneider 2002). Imidacloprid has been highly effective against piercing-sucking insects, such as aphids, whiteflies, planthoppers and HWA.

Imidacloprid, a systemic insecticide, is translocated throughout all parts of the tree to reduce populations of HWA (Cowles 2009). It is water soluble and highly adsorbed by organic material (Cox et al. 1998). Depending on soil characteristics and precipitation, imidacloprid will remain for long periods of time where applied or leach and mobilize to non-target plants or waterways (Goulson 2013). In the high organic content soils around hemlocks in GRSM it is expected that imidacloprid will be adsorbed by the soil and remain available to the trees longer with less expected mobility (Knoepp et al. 2012). Imidacloprid does have some mobility in GRSM soils as demonstrated by research that detected imidacloprid at low concentrations in seven of ten streams analyzed in GRSM (Benton et al. 2016).

Non-target plants may translocate imidacloprid applied to hemlocks. For example, imidacloprid has been found in low amounts in *Rhododendron maximum* growing adjacent to hemlock (Wiggins et al., unpublished data). Similar results have been found in agricultural settings. For example, along edges of agricultural fields where the neonicotinoid clothianidin was used as a seed dressing, non-target dandelion flowers were found to contain clothianidin. Dandelions may have translocated the neonicotinoid, the plants may have been contaminated by planter dust, or both mechanisms may have been involved (Krupke et al. 2012).

Translocation of imidacloprid by non-target flowering plants is important because imidacloprid has been implicated as a potential cause of pollinator decline (Lämsä et al. 2018). In enclosed studies, the buff-tailed bumble bee, *Bombus terrestris* L. (Hymenoptera: Apidae), exposed to 1% imidacloprid in its diet had reduced foraging motivation as demonstrated by being slower to start foraging and visiting fewer flowers. This reduced motivation may be the basis for negative colony effects observed in other studies of *B. terrestris*, such as reduced pollen foraging

efficiency shown by exposed bees returning to the colony with 31% less pollen per hour than control bees (Feltham et al. 2014). In another study, imidacloprid reduced feeding in *B. terrestris*, leading to reduced brood production (Laycock et al. 2012).

Little is known about the ability of insects to detect imidacloprid and avoid it, or the role of imidacloprid as an attractant to some insects. It may also alter plants in ways that make them more attractive or repellent. Yellow pan traps containing imidacloprid at concentrations of $1 \mu\text{g L}^{-1}$, $0.1 \mu\text{g L}^{-1}$, and $0.01 \mu\text{g L}^{-1}$ were placed on the ground with control (no imidacloprid) traps to assess if imidacloprid repels or attracts insects (Easton and Goulson 2013). Diptera avoided all three concentrations of imidacloprid, and Coleoptera and spiders avoided the highest concentration. The number of spiders collected increased at the two lower concentrations suggesting imidacloprid acted as an attractant. In another study using two-choice feeding tests, honey bees, *Apis mellifera* L., and *B. terrestris*, given a choice of sucrose or sucrose containing imidacloprid preferred the imidacloprid-laced solution (Kessler et al. 2015).

Rhododendron maximum is a common understory associate of hemlock. Kincaid (2007) analyzed 50 hemlock forest plots in GRSM for compositional characteristics such as associated species and distribution, and classified each as one of four forest types based on species importance values. The four forest types were: *Tsuga canadensis*/ *Betula*/ *Acer rubrum* (N = 17), *Tsuga canadensis*/ *Liriodendron tulipifera* (N = 6), *Tsuga canadensis*/ *Betula* (N = 22), and *Tsuga canadensis* (N = 5). Common tree species associates identified in the 50 plots include sweet birch (*Betula lenta* L.), yellow birch (*B. allegheniensis* Britton), red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.), and Carolina silverbell (*Halesia carolina* L.). Average *R.*

maximum understory in five *T. canadensis* plots was 6.7%, while *R. maximum* understory in the other 45 plots averaged about 29%.

Systematic aerial photography of GRSM was conducted in 1997 and 1998 to develop digital maps of the vegetation in the Park (Madden et al. 2004). The digital vegetation layers generated can be used to map habitats, provide baseline data to monitor landscape changes, and analyze fuel loads for fire risk assessment among other functions. Factors identified include forest types and understory vegetation types. Rhododendron and mountain laurel cover nearly 50% of the understory in GRSM. The digital mapping identifies areas of rhododendron and mountain laurel as light, medium or heavy based on the space between the foliage observed in the aerial photos. The density of these understory shrubs is considered critical for researchers, foresters, rescuers, and others planning to traverse the landscape because these shrubs can be challenging to nearly impossible to pass in medium and heavy densities.

Because these digital maps are based on aerial photos taken 20 years prior to this study, some differences may be observed in the field from these maps (Madden et al. 2004). Also, mapping error, such as inaccuracies caused by evergreens in the overstory obscuring the understory, can occur. Light rhododendron cover is sometimes confused with hemlock in the understory and can be indistinguishable at the level of visual acuity provided by the aerial photos.

Landscape factors, such as total tree basal area, canopy openness, plant species diversity, shrub cover, and fire frequency, affect pollinator species abundance and diversity (Grundel et al. 2010, Jackson et al. 2014, Hanula et al. 2015, 2016). Low total basal area and

plant species richness were associated with pollinator species richness (Grundel et al. 2010, Hanula et al. 2015). Greater canopy openness and low shrub cover were the best predictors of pollinator abundance (Grundel et al. 2010, Jackson et al. 2014, Mayfield et al. 2015, Hanula et al. 2016).

To determine if populations of pollinators foraging in areas with hemlocks treated with imidacloprid are different from populations of pollinators foraging in untreated areas a study was initiated in GRSM. *R. maximum* was chosen as the flowering plant species to investigate since it is a common associate of hemlock and, as mentioned earlier, has been documented to translocate imidacloprid to pollen and nectar when growing within 30 cm of treated trees (Wiggins et al., unpublished data). Imidacloprid was detected in two of eight *R. maximum* pollen samples (at concentrations of 19.9 and 24.8 ppb) and in two of three nectar samples (11.2 and 1.3 ppb) (Wiggins et al., unpublished data). One nectar sample (1.3 ppb imidacloprid) also contained the metabolite olefin (1.4 ppb).

Rhododendron flowers grow at one or more terminal buds, each bud producing one stalk in a round, umbel-like compact terminal cluster, known as a raceme, typically with 20 to 30 flowers (Figs. 1.5, 1.6). Rhododendron flowers are considered bumble bee flowers based on their zygomorphic form whereby the flower can be divided into two mirror-image halves by a single plane (Leppik 1953). Given a choice, bumble bees showed a preference for zygomorphic flowers. Other suspected pollinators of rhododendron include other wild bees, honey bees, butterflies, and possibly flies (Leppik 1974, Sarwar and Takahashi 2014).

In the one study of visitors of *R. maximum* at Round Bald in the southern Appalachians, Syrphidae and seven *Bombus* spp. were observed on *R. maximum* flowers: *B. affinis* Cresson, *B.*

bimaculatus Cresson, *B. impatiens* Cresson, *B. perplexus* Cresson, *B. sandersoni* Franklin, *B. terricola* Kirby, and *B. vagans* Smith (Bauer 1986). Round Bald is an open area and differences in pollinator species composition may be expected in forest areas. Little is known about pollinators of *R. maximum* so documenting them in GRSM is important.

This study was conducted to investigate potential non-target impacts on pollinators resulting from imidacloprid applied to hemlocks to control HWA. Insect visitors to rhododendron flowers, with an emphasis on pollinators, were observed and documented in two areas with imidacloprid treated hemlocks and in two untreated control areas. Insect visitors were compared between the treated and control areas.

Materials and Methods

Selecting Sites and Rhododendrons

Using the digital vegetation maps of GRSM and selecting vegetation layers, four sites [two treatment sites and two sites where no treatments have ever been applied (i.e., control sites)] in GRSM within Tennessee (Fig. 2.1) were selected based on similarities in forest type and structure, and density of *R. maximum* in the understory. Forest type was successional hardwood at all four sites with a portion of Gilliland Creek in cove forest. All these forest areas had a component of hemlock. The rhododendron layer selected was mostly medium density (basal area 5.5 – 11.3 m²/ha and 5,050 – 10,500 stems/ha) (Baker and Van Lear 1998). Treatment sites are areas in GRSM with remaining stands of viable hemlocks that survived the arrival and infestation of HWA in the Park and have been treated with imidacloprid to conserve these healthy groves of hemlocks. Control sites are areas similar in forest structure, although

hemlocks may be in serious decline or dead due to HWA infestation, that are at least 1 km from any insecticide treatment.

Seven potential treatment sites were initially explored. The areas had hemlocks that had been treated with a soil drench application of imidacloprid, at the recommended rate, between June 2014 and June 2016 (two to four years prior to the study). At each site, the study needed ten (two classes of five each) rhododendrons, each with five flower buds that would be conducive to observe, identify and collect insect visitors. Five of the flowering rhododendrons would need to be located within 1 m of a treated hemlock. The other five would be beyond the perimeter of any treated hemlock canopy (i.e., drip line).

The five sites that were not selected were too remote, had challenging topography, or had too few rhododendrons that met the conditions needed for the study. Few rhododendrons met the required conditions within the seven potential treatment areas and this circumstance drove the selection process of the treatment sites and the marked rhododendrons. The two sites chosen were: 1) Spruce Flats on the Middle Prong of the Little River, and 2) an area on Gilliland Creek near Cosby, TN. Spruce Flats is in the western half of GRSM between Elkmont and Cades Cove, and Gilliland is in the eastern most part of the Park (Fig. 2.1).

The intention at the inception of this study was to identify ten designated rhododendrons at each treated site within a 5 ha area bounded on all sides by imidacloprid treated hemlocks. At the Gilliland Creek site hemlocks 20 cm DBH and larger were treated in June 2014 with imidacloprid soil drench at 0.275 gai (grams active ingredient) per cm DBH, a total of 9.1 kg of chemical was used. At the Spruce Flats site hemlocks 15 cm DBH and larger were treated in summer/fall 2015 at 0.275 gai per cm DBH, a total of 2.8 kg of chemical was

used. Five years prior, fewer trees in a smaller area were identified for treatment at both sites. GRSM increased the number of trees and total treatment areas for the latest applications after identifying additional hemlocks to be protected.

Rhododendrons growing beside (within 1 m) a treated hemlock were expected to contact the treatment with their roots and translocate the imidacloprid to nectar and pollen. Treated hemlocks were identified by paint markings on the trunk within 1 m of the ground surface (Fig. 2.2), as hemlocks are marked with paint when the imidacloprid is soil-applied as documentation of the treatment. Selected rhododendrons had at least five flower buds that could be easily observed to note behaviors of the flower visitors (Fig. 2.3). Once identified, location of each rhododendron was georeferenced using a Garmin Montana 680t (Garmin International, Inc., 1200 East 151st Street, Olathe, Kansas) GPS unit. Each of the five rhododendrons that met the required conditions were tagged at the Gilliland Creek and Spruce Flats sites.

In addition, five more rhododendrons at Gilliland Creek (Fig. 2.4) and at Spruce Flats (Fig. 2.5) were selected, georeferenced using a GPS unit, and tagged. These ten rhododendrons were typically about 8 m from treated hemlocks (eight at 6.4 to 8.7 m, one at 11.9 m, and one at 19.7 m). Imidacloprid may be reaching the forest floor within the dripline of treated trees as the needles that may contain the insecticide drop. Suitable rhododendrons (with at least five easily observable flower buds) that were as distant from any hemlock canopy as possible, initially criterion was set at 10 m from canopy but hemlock spacing made that unattainable, were selected to reduce potential impacts from any soil-applied imidacloprid hemlock treatments.

If imidacloprid altered the flowers or their nectar or pollen rewards so that they were more or less appealing to pollinators, observations might include a preference for rhododendrons based on distance from the imidacloprid treatments. In addition, insect visitors to these 20 rhododendrons in treated sites would also be compared to visitors to 20 rhododendrons in untreated control sites to determine differences that might indicate an effect of the imidacloprid in the treatment areas.

Once these two treated sites were selected, two comparable control areas were identified on the Lynn Camp Prong of the Middle Prong (upstream approximately 6 km from Spruce Flats) and Groundhog Creek in the Cosby area (Fig. 2.1). Control sites were selected to be at least 1 km from any insecticide treatment and similar in forest structure to the treated sites. It was preferable to choose treated and control sites where *R. maximum* was growing under live hemlocks, but the hemlocks beside tagged rhododendrons in the Lynn Camp Prong site were all dead due to HWA and those in the Groundhog Creek site were alive but were small (3.5 cm to 10.5 cm dbh) trees.

The criteria used to choose rhododendrons at treated sites were applied to the control sites, and five rhododendrons within 1 m of a hemlock and five rhododendrons located away from any hemlock canopy at each site were selected, georeferenced using a GPS unit, and tagged. At the Lynn Camp (Fig. 2.6) site five rhododendrons beside dead hemlocks were selected (trees 9 cm to 78 cm diameter). At Groundhog Creek (Fig. 2.7), rhododendrons selected were beside small diameter live trees (3.5 cm to 10.5 cm).

Thus, a total of 40 *R. maximum* were selected for this study. At the two treated sites, ten rhododendrons (five at each treated site) had the main stem within 1 m of a hemlock that

was treated with imidacloprid in the last 2-4 years. These plants could be expected to translocate some amount of imidacloprid from associated hemlock treatments based on Wiggins et al. (unpublished data).

Ten (five at each treated site) rhododendrons beyond the dripline of any treated hemlocks were selected. These rhododendrons were 7.3, 7.6, 6.4, 8.7, and 8.7 m away from treated trees at Spruce Flats, and 8.7, 8.7, 19.7, 7.6, and 11.9 m away from treated trees at Gilliland Creek. These *R. maximum* plants are expected to be far enough away from the localized insecticide treatments that they will translocate little or none of the chemical, however, the foraging habitat of the population of pollinators is expected to include the imidacloprid-treated areas.

At the two control sites, ten rhododendrons (five at each control site) have the main stem within 1 m of a hemlock that has never been treated with imidacloprid, in areas at least 1 km from any insecticide treatments. Ten rhododendrons (five at each control site) were rooted beyond the dripline of all hemlocks and at least 1 km from any insecticide treatments.

Data Collection

Rhododendron maximum commenced flowering about mid-June and observations began on June 19, 2018 at Spruce Flats. The goal was to visit each site each week during the five-week flowering period to observe visitors to flowers of each of the ten tagged rhododendrons for ten minutes, during each of four 2-hour periods: 8:00 am - 10:00 am, 11:00 am - 1:00 pm, 2:00 pm - 4:00 pm, and 5:00 pm - 7:00 pm. In some cases, these time periods had to be expanded slightly, due to abundance of flowers, or adjusting for thunderstorms. Typically, on any given sampling date and time period, only a portion of the rhododendrons

were flowering and could be observed. During each visit, tagged rhododendrons that were flowering were observed four times, absent thunderstorms, throughout the day for 10 minutes during each observational period.

Observation of a rhododendron was performed by focusing on five flower racemes, when available, on the individual plant. In practice, all flowers that could be observed were carefully monitored for floral visitors. Many racemes may have been in full flower in close proximity (Fig. 2.3), or often fewer than five racemes had flowers. At times only one flower was observable on a plant while at other times plants had in excess of 100 flowers that could be readily observed. Flowers were counted and recorded using a range of 1 to 100+ at each observation.

Each site was visited each week for five weeks. Site visits began as flowering commenced and ended when flowering ceased. Flower visitors were observed and described or identified as specifically as possible, sometimes order only, but usually to family or genus, e.g., Diptera, Halictidae, or *Bombus*. The behaviors of the visitors, such as pollinating, pollen robbing, or nectar feeding, also were observed, when possible. Pollinating describes visitors that contact both the anthers and stigma, pollen robbing refers to an insect landing on the stamen and collecting pollen without ever touching the stigma, and nectar feeding describes insects that avoid the reproductive parts of the flower and go to the nectaries to sip nectar (Epps et al. 2015).

A representative sampling of the flower visitors was collected as they left the flowers by trapping them in 250 ml plastic pop-top sampling containers to avoid damaging the flowers. Some specimens were collected with a net as they flew away from the tagged rhododendron.

Specimens were killed by freezing. Collected specimens were taken to the laboratory for preservation and identification using standard taxonomic keys (Mitchell 1962, Ascher and Pickering 2017). Voucher specimens will be maintained in the University of Tennessee Insect Museum and in the museum of GRSM.

The date and site being visited were recorded. At each rhododendron observed for flower visitors, data recorded included: rhododendron tag number, time of day, visitor descriptions or identifications, number of flowers, and a description of the current weather. Insects collected were stored in sampling containers (250 ml) labeled with a code, and the code was also recorded on the data sheet with the visitor description.

Prior to the start of the flowering period, HOBO monitors were mounted on the north side of a dead tree 50 cm above the ground at each site to monitor air temperature throughout the sampling period. In treated areas HOBOS were located near the center of the treatment polygon, in control areas HOBOS were located near the center of the tagged rhododendrons. After completion of all observations, temperatures obtained from HOBO monitors were included with the data records for each 10-minute observation. Overstory density was measured one time between August 22 and September 7, 2018 for each tagged rhododendron using a concave spherical densiometer model C (Robert E. Lemmon, Forest Densiometers, 5733 Cornell Drive, Bartlesville, Oklahoma). A measurement was taken on four sides of each rhododendron at the four cardinal directions and averaged to obtain the overstory density measurements (Table 2.1).

Data Analysis

The experimental design used was a completely randomized split-split plot with repeated measures. The whole plot was GRSM with the four sites as sub-plot experimental units. Whole plot treatment was the imidacloprid treatment and control. Sub-plot treatment was the proximity of the rhododendrons—five beside hemlocks and five away from hemlocks at each site. Repeated measures were the four observation times per day. Fixed effects recorded consisted of treatment (treated or control), plant location (beside tree or away from tree), rhododendron ID, and time of day (8:00am—10:00am, 11:00am—1:00pm, 2:00pm—4:00pm, and 5:00pm—7:00pm). Measured covariates recorded were overstory density, air temperature, weather conditions, amount of imidacloprid applied to site, number of flowers observed, and time spent observing. Overstory density was a percentage of canopy cover. Temperature is averaged for the cumulative times in the sample. Weather is an average of the time sampled using a 1 - 5 scale with 1 clear skies to 5 rain/thunderstorm. No imidacloprid was applied at either control site, while 9.1 kg and 2.8 kg were applied at Gilliland Creek and Spruce Flats, respectively. Total flowers was the cumulative total of the numbers of flowers recorded at each observation in the time period. Time spent was the total time spent during the time interval, accumulated over the five weeks. Response variable is the number and identity of pollinators. Mixed model analysis of variance was conducted using the GLIMMIX procedure in SAS (SAS Institute Inc. 2008), and least squares means were compared using the Tukey HSD at criterion $\alpha = 0.05$.

Because this research is focused on pollinators, only species considered pollinators were used in the statistical analysis. Pollinator status was determined by species that specialize in

eating pollen and nectar (Proctor and Yeo 1972). For this study, pollinators included bees (Order Hymenoptera) in the Families Andrenidae, Apidae (excluding *Nomada*) and Halictidae; beetles (Order Coleoptera) in families regarded as pollinators, such as Cerambycidae, Elateridae, and Cantharidae; flies (Order Diptera) in the Families Syrphidae and Bombyliidae; and butterflies (Order Lepidoptera). Families and orders excluded from the statistical analysis were: Curculionidae, Vespidae, Ichneumonidae, Formicidae, Hemiptera, and a few other incidental visitors, as well as a number of insects that were difficult to observe and identify beyond order. The eight groupings evaluated in the statistical model were pollinators in the Orders Hymenoptera, Coleoptera, Diptera, and Lepidoptera, and pollinators in the Families Andrenidae, Apidae, and Halictidae.

For this analysis, the five weekly observations were combined into a cumulative abundance throughout the flowering season for each of the eight groups analyzed. During each site visit, the study design was to observe rhododendrons four times (8:00am—10:00am, 11:00am—1:00pm, 2:00pm—4:00pm, and 5:00pm—7:00pm) for ten minutes and record the insects at flowers. Some actual observations made fell outside these times and adjustments were made as follows for the repeated measures in the model. Repeated measures were analyzed with insect observations made in the four equal time periods: 8:00am—10:45am, 10:45am—1:30pm (solar noon), 1:30pm—4:15pm, and 4:15pm—7:00pm.

Two of the covariates evaluated, total flowers and time spent observing, showed a statistically significant correlation with numbers of pollinators observed and these were incorporated in the model. Effectively, more total flowers and more time spent at a rhododendron were associated with observing more visitors to the plant as would seem logical.

The time spent observing the different rhododendrons varied due to the length of time an individual plant was in flower, timing of visits during the beginning and ending of the flowering period, and interruptions by thunderstorms and rain.

Analyses compared the observed visitors to rhododendron flowers based on imidacloprid treatment and control, proximity to hemlock (beside and away from), the four time periods, and all interactions. The eight different groups of pollinating visitors—pollinators, four orders, and three families—were analyzed separately.

Results and Discussion

Imidacloprid treatment was not a significant factor ($p > 0.05$) in the observed abundance of any of the pollinator groups on *R. maximum*. Pollinators at rhododendrons beside treated trees were not significantly different ($p > 0.05$) from pollinators at other rhododendrons. Interactions between the four time periods and imidacloprid treatment showed no significant differences ($p > 0.05$) in pollinators at rhododendrons.

Mean pollinator visitation to rhododendrons in control sites ($\bar{x} = 4.6$) was not statistically different ($p > 0.05$) from treated sites ($\bar{x} = 3.9$). Groups identified as pollinators—species that specialize in eating pollen and nectar—account for 617 of the total 711 flower visitors observed and collected. Of the total 617 flower visitors identified as pollinators, 285 were from treated sites, 267 pollinators at Spruce Flats, 43% of total pollinators, and 18 pollinators at Gilliland Creek, 3% of total, were documented. The remaining 332 pollinators were from control sites, 193 pollinators at Lynn Camp, 31% of total, and 139 pollinators at Groundhog Creek, 23% of total, were documented.

At the ten rhododendrons beside treated trees, adjusted mean pollinator visitation ($\bar{x} = 3.7$) was similar to all other proximity/treatment interactions (Fig. 2.8). Also for the interaction of treatment and proximity, in control sites pollinators visited the ten rhododendrons located away ($\bar{x} = 6.0$) from hemlocks more frequently ($p < 0.05$) than those ten located beside ($\bar{x} = 3.2$) hemlocks. The difference in pollinator abundance based on proximity at the control sites drives the difference ($p < 0.05$) in pollinator visitation based only on proximity of all rhododendrons, beside ($\bar{x} = 3.5$) and away from ($\bar{x} = 5.0$) hemlocks (Fig. 2.9). Hymenoptera and Apidae visitation had the same significant interactions with regard to proximity and treatment, while other groups showed no significant ($p > 0.05$) differences.

In total, 711 insects were documented visiting rhododendron flowers in 64.5 hours of observation at the four sites (Fig. 2.10). Numbers of visitors were similar for treated and control sites. Hymenoptera was by far the predominant order of visitors and Apidae was the largest family of pollinators observed. At treated sites, 330 insects were observed, and 381 insects were observed at the control sites. Hymenoptera was the largest order with 89% of visitors. Combined for all sites, the greatest numbers of flower visitors by family were: Apidae 44% with the vast majority (97%) in the genus *Bombus*, Halictidae 24%, Andrenidae (*Andrena cornelli* Viereck) 13%, and Formicidae 6%. Other orders observed frequently include: Coleoptera 4%, Hemiptera 3%, Diptera 2%, and Lepidoptera 2%.

Summary

Although the adjusted mean pollinator visitation was lower for treated sites, no statistical ($p > 0.05$) difference was observed in pollinator populations in treated versus control sites, the variability in the data was too high to attribute differences to treatments. While the

treated site at Gilliland Creek had lower abundance, the other treated site, Spruce Flats, had higher abundance than both control sites. Visitor data to rhododendron flowers at these two treated sites produced the high variability in the statistical analysis.

The proximity of rhododendrons beside hemlocks in treated areas was used as a proxy for insect-pollinated flowers that are expected to translocate relatively high levels of imidacloprid to pollen and nectar. The adjusted mean pollinator visitation to rhododendrons located beside treated hemlocks was not statistically ($p > 0.05$) different than the adjusted mean pollinator visitation to other rhododendrons.

To attempt to control for as many variables as possible, an equal number of rhododendrons beside hemlocks was also chosen in untreated control areas. Twenty rhododendrons overall were beside hemlocks and twenty were away from hemlocks. The difference in pollinator visitation by proximity of rhododendrons to hemlocks (beside or away) was more pronounced in the control sites. Ignoring imidacloprid treatments at the four sites, pollinator visitation to rhododendrons was significantly ($p < 0.05$) lower at rhododendrons located beside hemlocks than those located away from hemlocks. It cannot be determined that imidacloprid impacted pollinator numbers. If an association between pollinators and proximity of rhododendrons to hemlocks occurs, data suggest that pollinators tend to avoid rhododendrons beside hemlocks, whether they are treated or untreated.

Although it would be logistically difficult, the statistical power of this study would have increased with more sites. More statistical power would increase the ability of the design to detect a statistical difference among treatments. Future studies that include a model with more sites and less time spent at each site may improve statistical power and provide more

definitive conclusions about the relationship between imidacloprid and non-target pollinators in a forest system.

Chemical analysis of plant materials and collected insects also would provide evidence of the movement of imidacloprid through the system. Identifying correlations between concentrations of imidacloprid and its metabolites in insects and plants by comparing spatial relationships and gradients of the chemicals in the system would help to better define the fate of imidacloprid in the hemlock woods. More data would give a more reliable conclusion of effect or lack of effect due to imidacloprid in the forest system.

CHAPTER III

**IMPACT OF IMIDACLOPRID TREATMENT TO HEMLOCK ON PRODUCTION
AND GERMINATION OF SEED COLLECTED FROM *RHODODENDRON MAXIMUM*
IN CLOSE ASSOCIATION WITH TREATED HEMLOCKS**

Introduction

Insect pollination is required for effective reproduction in many flowering plants, such as apples, blueberries, squash, tomatoes, and coffee (Webb 2008). One of every three bites of food eaten by humans is made possible by insect pollination (Webb 2008). Plants that reproduce well by wind-pollination and self-pollination often also benefit from insect pollination. For example, insect pollinators were excluded from flowers of oilseed rape to study the impact of insect pollinators on overall yield and quality (Bommarco et al. 2012). All flowers were subject to self-pollination and wind-pollination, while some were open to insect pollination. Insect pollination increased seed weight per plant 18% and seed quality improved so that market value per plant increased 20%. In another study, strawberry yield, size, and quality were significantly improved by insect pollination compared to only wind and self-pollination (Klatt et al. 2014).

While flowering plants benefit from insect pollinators, other insects are considered pests that can degrade the quality and yield of crops and forests. Insecticides are often used to control pest insects, but they often do not discriminate and may harm non-target pollinators. For example, in New Brunswick, Canada, following aerial spraying of the organophosphate insecticide fenitrothion over wide areas of forest to control the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), blueberry producers bordering the treated

forest areas suffered crop failures believed to be the result of mortality of non-target pollinating species which led to insufficient pollination (Kevan 1975). Carcasses (n = 12) of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), and one *Andrena* sp. caught alive, but later died, were analyzed for fenitrothion. Fenitrothion was detected in the *Andrena* sp. (1.05 ppm) plus 9 of the 12 honey bee (0.03 – 0.95 ppm) specimens.

Another study in a forest setting in New Brunswick, Canada looked at how the fecundity of eight native forest plants dependent on insect pollination for seed set was affected by the aerial spraying of insecticide to control spruce budworm (Thaler and Plowright 1980). The forest plants *Aralia nudicaulis* L. (Apiales: Araliaceae), *Clintonia borealis* (Aiton) Rafinesque (Liliales: Liliaceae), *Cornus alternifolia* L. (Cornales: Cornaceae), *Cornus canadensis* L., *Cornus stolonifera* Michaux, *Kalmia angustifolia* L. (Ericales: Ericaceae), *Maianthemum canadense* Desfontaines (Asparagales: Asparagaceae), and *Viburnum trilobum* Marshall (Dipsacales: Adoxaceae), which bloomed shortly after aerial spraying of fenitrothion (an organophosphate), showed significantly ($p < 0.01$) lower fecundity in treated areas than untreated areas.

Clintonia borealis and *K. angustifolia* were also sampled in areas treated with the carbamate insecticide aminocarb, and no loss of fecundity was found (Thaler and Plowright 1980). Unlike fenitrothion (applied at 210 g/ha), aminocarb (applied at 70 g/ha) did not cause significant ($p < 0.05$) mortality in bumble bees which are the primary pollinators of *C. borealis* and *K. angustifolia* (Plowright and Rodd 1980). In fact, *C. borealis* and *K. angustifolia* had significantly ($p < 0.01$) higher fecundity in aminocarb treated areas compared to untreated control areas and bumble bee densities and worker survival were higher as well (Plowright and Rodd 1980, Thaler and Plowright 1980). Possible explanations considered were reductions in

populations of spiders or dipteran parasites of bumble bees caused by aminocarb (Thaler and Plowright 1980).

Matacil aminocarb insecticide had virtually replaced fenitrothion for spruce budworm control by 1979 and a study examined aminocarb non-target impacts on fecundity of *C. canadensis*, *C. stolonifera*, and *M. canadense* (Thomson et al. 1985). Aminocarb is considered innocuous to bumble bees, but pollinators in the bee Families Andrenidae and Halictidae, and the dipteran Family Syrphidae, are sensitive. Bumble bees were a common visitor to *C. canadensis* flowers, but only one bumble bee of 82 total visitors was observed at *M. canadense*, and none was observed at *C. stolonifera* of a total 175 pollinators. Fecundity of *M. canadense* was significantly ($p < 0.01$) lower in aminocarb treated areas, and fecundity of *C. stolonifera* was considerably lower, but variation was high. Fecundity of *C. canadensis* appeared unaffected by the aminocarb. Non-target pollinator families impacted differently by insecticides led to different impacts on fecundity of plants depending on their reliance on those pollinators.

In an agricultural setting in California, hybrid onion seed yields declined five years in a row as insecticide use to control onion thrips increased (Long and Morandin 2011). Honey bees were the majority of the pollinators and a strong positive correlation (explained 77% of the variation among sites) was found between honey bee flower visitation and seed yield. Honey bee visits were negatively correlated ($R^2 = 0.24$) with the number of insecticides applied which may have repelled or killed the bees. Another study comparing effects of seven pesticides and different application protocols on onion seed production found that the variety of treatments had various impacts that could be responsible for yield declines (Gillespie et al. 2014). Effects observed included declines in flower visitation by pollinators, pollen germination and pollen

tube growth declines, and changes in seed germination rates, seed set and weight. The use of more than one of these treatments by seed producers may be compounding the effects and exacerbating yield declines.

Neonicotinoid pesticides have been used extensively as seed dressing for oilseed brassicas and sugar beets since their registration in Finland, beginning in 1997 with imidacloprid. While yields of the wind-pollinated crops barley and wheat have steadily increased nationally in Finland, yields of insect-pollinated turnip rapeseed (TRS), black currant, and caraway have been highly variable (Hokkanen et al. 2017). TRS yields steadily increased in Finland from 1980 to 1993, but have declined steadily for over 20 years to 67% of peak yields of the early 1990s. These researchers concluded that this decline was the result of declining levels of wild pollinator populations. A significant linear correlation ($R^2 = 0.34$) linked TRS yield trend decline with increasing area of crops receiving seed dressing of neonicotinoids. Thus they concluded that disruption of pollination services by reduced populations of wild pollinators resulting from use of neonicotinoids caused this decline in TRS yield.

Other potential unintended effects of imidacloprid in flowering plants, such as rhododendron, include possibly changing the attractiveness of the flower to pollinators, or imidacloprid may impact the ability of the pollinators to forage and pollinate effectively. Little is known about the ability of insects to detect imidacloprid. Yellow pan traps containing imidacloprid at concentrations of 1 µg/L, 0.1 µg/L, and 0.01 µg/L, and control traps caught 11,967 arthropods (Easton and Goulson 2013). Diptera including Syrphidae comprised 87% of specimens caught, 8% were Araneidae (orb-weaving spiders), and 3% were Coleoptera including Cantharidae (Easton and Goulson 2013). Fewer Diptera were collected in all three

concentrations of imidacloprid, and fewer Coleoptera and Araneidae were collected in the highest concentration indicating some avoidance of the imidacloprid. The number of Araneidae collected increased at the two lower concentrations suggesting imidacloprid acted as an attractant. Scents in flowers may mask imidacloprid, and sugar rewards in nectar may motivate insects to visit flowers despite sensing the imidacloprid. Widespread use of imidacloprid may be selecting for avoidance. Conversely, in another study using two-choice feeding tests, bees given a choice of sucrose or sucrose containing imidacloprid preferred the imidacloprid-laced solution (Kessler et al. 2015).

Rhododendron maximum is considered a bumble bee flower based on the zygomorphic form (bilateral symmetry) of the flower and pollen with viscin threads (Leppik 1953, Williams et al. 1990, Sarwar and Takahashi 2014). On Round Bald in the southern Appalachians, bumble bees constituted 98% of visitors to *R. maximum* collected in one flowering season (Bauer 1986). It is unclear if cross-pollination is required to set seed, but out-crossing is associated with production of larger seed capsules in rhododendron (Romancier 1971). In the study on Round Bald, 58 *R. maximum* flowers caged to prevent insect pollination produced one fruit, while 61 open control flowers produced 61 fruits suggesting a strong reliance on insect pollination (Bauer 1986).

Other heaths (Ericales: Ericaceae) have shown low fecundity as measured by fruit production when insects were excluded using fine mesh screen around inflorescences. In New Brunswick, Canada, covered flowers of sheep laurel, *K. angustifolia*, had mean fecundity 6.5% (4,813 flowers), compared to 76.9% (3,506 flowers) for open flowers (Thaler and Plowright 1980). In Giles County, Virginia, covered flowers of flame azalea, *R. calendulaceum* (Michaux)

Torrey, produced no fruit (79 flowers) compared to 19 fruit (78 flowers) for open flowers, and 21 fruit (47 flowers) hand pollinated, suggesting flame azalea may be incapable of self-pollination (Epps et al. 2015).

Different groups of visitors may not be affecting pollination despite frequent visits to rhododendron flowers. A study of pollinators of flame azalea suggested that only butterflies were transferring pollen to stigmas (Epps et al. 2015). Approximately 75 insects were observed visiting flame azalea flowers. *Papilio glaucus* L. (Lepidoptera: Papilionidae) (N > 32) was the most common visitor followed by *Andrena cornelli* Viereck (Hymenoptera: Andrenidae) (N = 18) and *Speyeria cybele* (F.) (Lepidoptera: Nymphalidae) (N = 8). No bumble bees were observed. Only the two butterfly species contacted both the anthers and stigmas, while other species contacted only the anthers or stigma. Halictidae species were observed nectaring and contacting the stigma, and *A. cornelli* only gathered pollen from the anthers without appearing to contact the stigma. Also, inflorescences that were open to all visitors had 12-fold higher fecundity than inflorescences caged with chicken wire (2.5 cm diameter openings) to exclude butterflies but allow other insects.

Although previous studies showed impacts of imidacloprid and other neonicotinoids on pollinators of agricultural crops (Goulson 2013, Hokkanen et al. 2017), little is known about its impact, if any, on pollinators in forested settings. The neonicotinoid insecticide imidacloprid is applied to the base of hemlocks to control the invasive hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Benton and Cowles 2016). Although hemlock is wind pollinated, imidacloprid in the soil has been shown to be translocated in low amounts to pollen and nectar of non-target *Rhododendron maximum* plants 30 cm from treated trees (Wiggins et

al., unpublished data). Imidacloprid treatment of hemlocks may similarly impact non-target pollinator populations or alter pollinator behavior so that plant reproduction is affected, and seed production is impacted. Because imidacloprid soil drench has provided the most effective control of HWA and is relied upon for control of HWA in Great Smoky Mountains National Park (GRSM), it is important to assess possible non-target impacts.

To assess any influence of imidacloprid treatment of hemlocks in GRSM on pollinator pollen transfer effectiveness on *R. maximum* in treated and untreated areas, a seed study was designed and conducted. The goals were to determine if measurable differences exist between seed production of rhododendron in hemlock groves treated with imidacloprid and in those areas that were not treated (i.e., control).

Materials and Methods

Study Design and Data Collection

Four sites, two sites where imidacloprid had been applied to control HWA within 2-4 years of the study and two sites where no treatments had been applied (i.e., control sites), in GRSM within Tennessee (Fig. 2.1) were selected based on similarities in forest type and structure, and density of *R. maximum* in the understory (see Chapter II for more detailed information). The two treated sites were Spruce Flats on the Middle Prong of the Little River and an area on Gilliland Creek near Cosby, TN. Two comparable control areas were identified on the Lynn Camp Prong of the Middle Prong (upstream approximately 6 km from Spruce Flats) and Groundhog Creek in the Cosby area. Control sites were selected to be at least 1 km from any insecticide treatment and similar in forest structure to the treated sites. Forest type was successional hardwood at all four sites with a portion of Gilliland Creek in cove forest. The

rhododendron layer selected was mostly medium density. All these forest areas had a component of hemlock, although hemlocks in control areas may be in serious decline or dead due to HWA infestation.

A total of 40 *R. maximum* were selected and tagged for this study. At the two treated sites, ten rhododendrons (five at each treated site) had the main stem within 1 m of a hemlock that was treated with imidacloprid in the last 2-4 years. These plants were expected to translocate imidacloprid from associated hemlock treatment. Ten (five at each treated site) were rooted beyond the dripline of any treated hemlocks. These *R. maximum* plants were expected to be far enough away from the localized insecticide treatments that they would translocate little or none of the chemical; however, the foraging habitat of the population of pollinators was expected to include the imidacloprid-treated areas.

At the two control sites, ten rhododendrons (five at each control site) were selected that had the main stem within 1 m of a hemlock that has never been treated with imidacloprid, in areas at least 1 km from any insecticide treatments. Ten rhododendrons (five at each control site) were selected that were rooted beyond the dripline of all hemlocks and at least 1 km from any insecticide treatments.

To assess if imidacloprid treatment of hemlocks may affect pollinators of rhododendron and consequently impact seed production and viability, seed was collected from the 40 tagged rhododendrons at the four locations used in the pollinator study (see Chapter II). Seedpods had matured by the second week of October. At that time, 15 seedpods were clipped from each of the marked rhododendrons, placed in 1 L paper bags, and taken to the laboratory. Three of the 15 seedpods from each plant were stripped of seed, which was counted and weighed.

When the tagged rhododendrons were visited to collect the seedpods, seed-collection buckets (28 cm) with 6.4 mm hardware cloth screens to exclude large debris were placed under each study plant, three buckets per plant (120 buckets total) (Fig. 3.1). Buckets were examined in December 2018, and seed was removed, placed in 1 L paper bags from each rhododendron and taken to the laboratory, where they were sorted from the debris, counted and weighed.

To obtain accurate seed counts and weights, the seeds from the buckets were carefully sorted from the debris and the seedpods were carefully stripped of seed and sorted from the debris using a dissecting microscope to obtain a clean seed sample. Seed samples were then weighed using a Denver Instrument Company Model A-160 Electronic Analytical Balance with weight recorded to the nearest 0.1 mg. *R. maximum* seeds are oval and somewhat flattened with fringes or wings at the ends. The largest dimension of typical *R. maximum* seed is about 1.5 mm, and average weight of one seed in these samples was 78 µg. To count the seeds in the samples, the seeds were spread onto a standard sheet of white paper and photographed. Photo files were uploaded onto a laptop computer and ImageJ image processing program was used to obtain seed counts (Schneider et al. 2012).

Seed from each of the pods (120) and the bucket collections (39, collection buckets for one rhododendron were lost) were prepared for the germination study. Generally, one third of the seed samples by weight was set aside for a germination study in the greenhouse using conditions described by Romancier (1971). In cases where there were few seeds in a sample, more than one third of the sample was used in the germination study. Seeds were placed on wet 9 cm round filter papers that were placed in 9 cm petri dishes and sealed with parafilm (Fig. 3.2). Each petri dish with the seeds was photographed and the photo was analyzed using

ImageJ software for the initial seed count. Petri dishes were placed in the greenhouse under 47% shade cloth. Petri dishes were monitored every 1 - 3 days to ensure moisture was maintained and the seal remained intact, and to monitor germination. When germination had ceased, petri dishes were removed and taken to the laboratory where germinated seeds were counted under a dissecting microscope. Germination rates as a percentage were determined by the proportion of seeds germinated in each sample, and all data analyzed to ascertain if differences in seed germination, weights, and counts existed between treatments.

Data Analysis

A completely randomized split-plot design with sampling in the sub-plot was used to analyze the seedpod samples, assessing number, weight, and percent germination of rhododendron seed, and comparing between treated and control area samples. For the statistical model, the whole plot is the GRSM, with sub-plot experimental units of the four sites. The whole plot treatment is the imidacloprid treatment of hemlocks at two sites and the absence of imidacloprid at two sites. The rhododendrons were chosen in two different proximities (five each) at each of the four sites—five located beside hemlock trees and five located away from hemlocks. Proximity of the rhododendrons is the sub-plot treatment. The three seedpods from each plant were the samples. Mixed model analysis of variance was conducted using the GLIMMIX procedure in SAS (SAS Institute Inc. 2008), and least squares means were compared using the Tukey HSD at criterion $\alpha = 0.05$.

For the bucket seed analysis, a completely randomized design was used. Analysis was conducted to assess number, weight, and percent germination of bucket-collected rhododendron seed, comparing between treated and control area samples. Mixed model

analysis of variance was conducted with GLIMMIX procedure in SAS (SAS Institute Inc. 2008), and least squares means were compared using the Tukey HSD at criterion $\alpha = 0.05$.

To investigate if one group of pollinators may be the primary pollinators of rhododendron flowers, a correlation analysis was performed using SAS. To account for different amounts of time making observations at each rhododendron (more visitors are observed with more time spent) numbers of observed visitors were divided by the time spent observing to provide a rate with the unit, visitors per hour (i.e., visitation rate). This analysis assessed the relationship between different groups of flower visitors and seed numbers from seedpods at each rhododendron. Six pollinator groups were analyzed separately: Coleoptera, Hymenoptera, Andrenidae, Apidae, Halictidae, and Andrenidae plus Apidae. Data were square root transformed to obtain normality, if square root transformed data were not normal then data were log transformed. Correlations were examined at the 5% significance level.

Another correlation analysis was performed using SAS to determine if rhododendron seed production is correlated with the rate of visits by pollinating insects. The analysis examined the relationship between total seed collected in buckets with the rate of total pollinator visitation. Seed collected was log transformed and pollinator number was square root transformed to obtain normality, and correlation was examined at the 5% significance level.

Results and Discussion

Number, weight, and percent germination of rhododendron seed from each seedpod sample are presented in Table 3.1, and the averages are summarized in Figures 3.3 and 3.4, between treatments and study sites, respectively. No significant ($p > 0.05$) differences in the

seed numbers, weights or percent germination were identified between treated and control area samples. Although not significantly different, mean number of seeds per pod (270 treated, 341 control) and weight of seed per pod (23.2 mg treated, 24.2 mg control) were numerically greater for control sites. Conversely, mean germination rates were numerically greater in treated sites (39.4%) than control sites (29.0%). No significant ($p > 0.05$) differences in seed production between rhododendrons located beside hemlocks and located away from hemlocks were observed.

Number, weight, and percent germination of rhododendron seed from each bucket sample are presented in Table 3.2, and the averages are summarized in Figures 3.5 and 3.6. No significant ($p > 0.05$) differences in seed numbers were found between treated and control buckets. Although not significantly different, mean number of seeds collected per plant (79.5 treated, 70.6 control) and weight of seed collected (7.7 mg treated, 5.0 mg control) were greater for treated sites. Conversely, mean germination rate was greater for control sites (44.5%) than treated sites (27.4%).

Large natural variability in seedpod number can occur from one plant to another and likewise, seedfall from plant to plant (Romancier 1971). Romancier (1971) counted seed in seedpods collected from two *R. maximum*, four pods from each plant. Seed number ranged from 186 to 829 in those eight pods, and the average seed numbers for the two plants were 270 and 485. Using seed traps in two different thickets in two different years, collections from twelve different traps varied by nine-fold as well. In this study, rhododendrons with poor pollination and thus low seedpod production would have had seedfall numbers more

significantly impacted by the collection of a larger proportion of the seedpods compared to rhododendrons with more complete pollination and higher seedpod production.

During both the seedpod and bucket seed germination studies, extensive contamination by fungi was observed in the seed germination samples (Figs. 3.7, 3.8). These fungi may have affected seed germination contributing to inconclusive results. The fungal growth could be attributed to possible contamination of some of the paper filters used in the studies. Filters used in this study were stored in two different laboratories, one which is primarily used for processing wood, plant, and other bulk samples. Filters from this laboratory may have been contaminated as trials conducted using filters from the 'clean' lab were not contaminated. The boxes containing the filters had been unsealed and a portion consumed, and their history of handling is unknown.

The lowest overall mean seed number per seedpod at a site ($\bar{x} = 224$) was measured at Gilliland Creek (Fig. 3.4) which also had the lowest pollinator visitation (1.2 pollinators/hr) (Table 3.3). Mean seed numbers at other sites did not correspond to their ranking of pollinator visitation counts. If the number of visits to flowers by pollinators was related to seed numbers produced, one would expect the greatest average seedpod seed production to be at Spruce Flats where total pollinator visitation rate was highest. The highest average seedpod seed production ($\bar{x} = 403$) was at Groundhog Creek where pollinator visitation (7.0 pollinators/hr) was second lowest.

The seed production per seedpod compared to total pollinator visitation rate suggests that perhaps pollination and consequently seed production may be related to the rate of visitation by one particular species or group of species while other visitors are primarily pollen

robbers and/or nectar thieves (Table 3.4) (Epps et al. 2015). For the correlation analysis of the six pollinator groups and seed numbers (Table 3.4), no group showed a significant ($p > 0.05$) relationship. Therefore, correlation coefficients were not presented. There is no indication that any group of pollinators is the main provider of pollen transfer to affect seed production. Thus, differences may just be due to natural variation.

Seed weight is strongly correlated with number of seeds as expected by uniformity of the seed. Correlation analysis found moderate positive correlation between square root rate of pollinator visitation and log seed numbers ($Rho = 0.57$, $p = 0.0002$), as well as with seed weight ($Rho = 0.58$, $p = 0.0001$), collected from buckets (Fig. 3.9). Seed collected from a plant that is reliant on insect pollination is expected to produce more seed when more pollinators visit affecting better pollination. This correlation analysis suggests that seed production is moderately correlated with pollinator visitation. The results may be biased toward showing lower seed production in poorly pollinated plants if the seedpod collecting removed a greater proportion of the seedpods produced by the plant.

Summary

Mean seed count per pod was not significantly different between treatments. The variability in the data was too high to attribute differences to treatments. Inclusion of another treated site and control site would improve the power of the statistical model to be more likely to assess a significant difference between treatments, but data from Romancier (1971) suggest high natural variability in seedpod seed numbers.

A moderate positive correlation between pollinator rate and seed collection in the buckets was observed. Based on results of this study, seed collection buckets may be an

effective way to assess the population of pollinators in a rhododendron understory. Although no significant differences were found in the seedpod seed counts, weight of seed, and germination, a correlation ($p = 0.0002$) between seed numbers in the buckets and the rate of pollinator visitation was identified. Other pollinator studies could include placing seed collection buckets and comparing seed collection to pollinator observations to determine if a general correlation for pollination and fecundity occurs with other flowering plants.

The stated goal of this chapter was to investigate the impact of imidacloprid treatment to hemlock on production and germination of seed from rhododendron in close association with treated hemlocks. Handling of the seed for quantification of seedpod seed numbers and germination was exceedingly tedious and time and labor intensive, and this study yielded no conclusive evidence that there is any relationship of seed characteristics to imidacloprid treatments to hemlocks or any factor associated with the observed pollinators. However, production of seed based on bucket collections showed a strong correlation with the observations of pollinators.

Pollination of flowers is highly associated with fruit and coincidentally seed production. A study of *R. maximum* at Round Bald demonstrated a nearly complete reliance on open pollination for fruit production (Bauer 1986). Bag and cage studies of other heaths, such as sheep laurel and flame azalea, suggest they are highly reliant on insect pollination for fruit production or may be self-infertile (Thaler and Plowright 1980, Epps et al. 2015).

Based on the association between fruit production and insect pollination, a more efficient way to examine the impact of imidacloprid on rhododendron seed production could be to research fruit production in treated and untreated areas. The large scale of variability

inherent in ecological systems necessitates large sample sizes to obtain meaningful results. The goal of this research was to determine if imidacloprid treatment of hemlocks to control HWA is impacting non-target pollinators. Examining rhododendron fruit production for an ecological study of pollinators would require identifying rhododendrons in multiple treated and untreated areas, marking raceme inflorescences, and counting the flowers initially. Later a return visit would be necessary to count the number of fruits produced. Other flowering plants could be considered for inclusion in the study.

To attempt to control for effects of some of the variability in forest systems on pollinator populations, landscape factors that are highly associated with pollinators should be documented. Tree basal area, canopy openness, plant species richness, and shrub cover are known to be correlated with pollinator abundance (Grundel et al. 2010, Jackson et al. 2014, Hanula et al. 2015, 2016). Studying fruit production of rhododendrons as outlined should answer the question, “Is imidacloprid impacting non-target pollinators?”

CHAPTER IV
SPECIES INCIDENCE, SPECIES COMPOSITION, AND SEASONALITY OF POLLINATORS OF
***RHODODENDRON MAXIMUM* IN GREAT SMOKY MOUNTAINS NATIONAL PARK**

Introduction

Rhododendron maximum L. (Ericales: Ericaceae) is a common understory species in the southern Appalachians (Monk et al. 1985) that is increasing in abundance and dominance as a result of changes in forest management practices (Van Lear and Waldrop 1989, Baker and Van Lear 1998), loss of American chestnut, *Castanea dentata* (Marshall) Borkhausen (Fagales: Fagaceae) (Van Lear et al. 2002), and the advancing and progressive decline and mortality of eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinales: Pinaceae) (Kincaid 2007, Krapfl et al. 2011, Martin and Goebel 2012). For millennia, fire was used to manage the forest until the 1920s when the U.S. Forest Service opposed using fire and the practice declined leading to ecosystem changes (Van Lear and Waldrop 1989, Van Lear et al. 2002). Fire impeded the establishment of woody undergrowth keeping the forests more open and reduced the threat of dangerous forest fires. The open forest made gathering nuts and acorns easier for people, was better habitat for bison and deer, and stimulated production of livestock forage. Historically fire confined rhododendron primarily to stream-sides.

Most of the southern Appalachian forests were heavily logged between 1880 and 1930 (Van Lear et al. 2002). The openings created by the logging and disturbance of the soils allowed rhododendron to expand upslope. Loss of American chestnut to chestnut blight, *Cryphonectria parasitica* (Murrill) Barr (Diaporthales: Cryphonectriaceae), in the 1930s also created openings for rhododendron to spread and establish.

Hemlock mortality due to hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is expected to lead to increased establishment and density of rhododendron (Kincaid 2007, Krapfl et al. 2011, Martin and Goebel 2012). Regeneration of canopy species is severely restricted by rhododendron in the understory. Hemlock is the only canopy species capable of successful regeneration in dense rhododendron thickets, and red maple, *Acer rubrum* L. (Sapindales: Sapindaceae), is the only hardwood species that has shown some regeneration in moderate to high density rhododendron thickets (Phillips and Murdy 1985). As hemlock densities decline and create canopy gaps, canopy species are unable to establish in moderate to high density rhododendron areas in the understory (Van Lear et al. 2002, Kincaid 2007, Martin and Goebel 2012). Development of heath balds dominated by *R. maximum* is possible in areas that previously had a significant hemlock overstory (Krapfl et al. 2011).

Forest management practices and changes brought on by loss of foundational tree species impact both the forest structure and plants that compose the ecosystem, as well as the fauna that rely on that ecosystem. Pollinator abundance is positively associated with recent fire frequency, lower total basal area and shrub cover, and greater canopy openness and plant species density (Van Lear and Waldrop 1989, Grundel et al. 2010, Hanula et al. 2015, 2016). Bee species richness is positively associated with plant species richness. Dense shrub understory negatively impacts herbaceous plant species cover and diversity, as well as pollinators.

Digital vegetation maps for Great Smoky Mountains National Park (GRSM; Park), generated from aerial photos taken in 1997 and 1998, indicate one-third of GRSM has either

light, medium, or heavy density of *R. maximum* in the understory (Madden et al. 2004). As rhododendron cover increased, plant species richness decreased exponentially in canopy gaps (Baker and Van Lear 1998, Van Lear et al. 2002). The effect of rhododendron cover was even greater on herbaceous species. Herbaceous species may be lost from sites dominated by rhododendron and in areas with increased density and expanding coverage.

As rhododendron has become more dominant in the forest understory as a result of fire suppression, heavy logging, and the functional loss and decline of two foundational canopy species, it is important to survey the associated pollinators. A previous study of pollinators of *R. maximum* was conducted at Round Bald in the southern Appalachians (Bauer 1986). In six hours of collecting visitors to *R. maximum* flowers on Round Bald, for at least one hour per week over the entire flowering period, 131 specimens (three Syrphidae and the remainder were *Bombus* spp.) were collected. Seven *Bombus* spp. were collected on *R. maximum* flowers: *B. affinis* Cresson, *B. bimaculatus* Cresson, *B. impatiens* Cresson, *B. perplexus* Cresson, *B. sandersoni* Franklin, *B. terricola* Kirby, and *B. vagans* Smith (Bauer 1986). Of particular interest in this group are the yellow-banded bumble bee, *B. terricola*, and the rusty patched bumble bee, *B. affinis*. *B. terricola* has not been documented in GRSM in 16 years, and has declined in numbers since the late 1990s (Xerces Society 2019b). *B. affinis* was listed as endangered under the U.S. Endangered Species Act in 2017 (Xerces Society 2019a). The distribution of *B. affinis* has declined from an estimated 87% of its historic range, found only in small numbers in recent surveys in isolated areas primarily in northern parts of its historic range.

Rhododendron flowers are considered bumble bee flowers based on the zygomorphic form (bilateral symmetry) of the flower and pollen with viscin threads, but other suspected

pollinators include other wild bees, honey bees, butterflies, and possibly flies (Leppik 1974, Sarwar and Takahashi 2014). At Round Bald, 98% of the specimens collected on *R. maximum* flowers were *Bombus* spp. (Bauer 1986). Another bee species exclusively associated with rhododendron is the azalea miner, *Andrena cornelli* Viereck (Hymenoptera: Andrenidae) (Ascher et al. 2017, Ascher and Pickering 2019). *Andrena cornelli* is oligolectic to *Rhododendron* sp., always found within flight range of its host plants.

Rhododendron maximum produces toxic chemicals known as grayanotoxins that are toxic to many animals including many insects (Tiedeken et al. 2014). Different species of rhododendron have different levels of grayanotoxins. Bumble bees are known to be tolerant of grayanotoxins, so they are expected to be the primary pollinators of rhododendrons. Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), may forage on rhododendron but the grayanotoxins can have negative effects on them and they can produce toxic honey (White Jr and Riethof 1959, Jansen et al. 2012). Honey bee, buff-tailed bumble bee, *B. terrestris* L., and a solitary mining bee, *Andrena carantonica* Perez, fed grayanotoxins showed different levels of tolerance (Tiedeken et al. 2016). Honey bees had significant mortality, buff-tailed bumble bees were not affected, and mining bees were deterred from feeding and exhibited malaise indicative of sublethal toxicity. Grayanotoxins may benefit plant pollination by screening inefficient pollinators in favor of more productive bumble bees (Pain 2015).

Little recent research specifically on pollinators of rhododendron has been conducted. Thus, the goals of this research are to document the insect visitors and pollinators of rhododendron in a forest system to provide information that can be used as a baseline for future studies of rhododendron pollinators and to enhance knowledge of pollinators of

rhododendron in the southern Appalachians. The specific objectives of this research are to: 1) document the visitors at rhododendron flowers in a forest system and 2) record temporal differences in taxa across the flowering period and by time of day.

Materials and Methods

Species observed as part of research conducted in Chapter II are presented collectively to provide a record of the visitors to and pollinators of flowers of *R. maximum* in GRSM. To compare pollinator species diversity at the different sites, Shannon's diversity index was utilized. This analysis includes determining species richness, Shannon's diversity index and evenness.

The influence of insecticide treatment on pollinators and other insect visitors to flowers of *R. maximum*, and the corresponding methodologies for that research, are presented in Chapter II. This Chapter contains a more general examination of the species that visit flowers of *R. maximum* and details their fluctuations throughout the day and across the five-week flowering period.

The research was conducted at four sites in GRSM (Fig. 2.1). The four sites were Gilliland Creek, Groundhog Creek, Lynn Camp Prong, and Spruce Flats. Ten *R. maximum* plants were identified and tagged at each site. The goal was to visit each site each week during the five-week flowering period to observe visitors in flowers of each of the ten tagged rhododendrons for ten minutes, during each of four 2-hour periods: 8:00 am - 10:00 am, 11:00 am - 1:00 pm, 2:00 pm - 4:00 pm, and 5:00 pm - 7:00 pm. Insect visitors in rhododendron flowers were documented by observing and collecting visitors in the rhododendron flowers.

Taxa of all visitors observed were identified to order, family, genus, or species depending on the proximity and time to observe. Collected specimens were taken to the laboratory for preservation and identification using standard taxonomic keys (Mitchell 1962, Ascher and Pickering 2017). Pollinator status was designated based on their reliance on pollen and nectar as adults (Proctor and Yeo 1972). Locations where insects were observed and/or captured were cataloged.

The total number of non-hymenopteran and hymenopteran pollinators observed during each time period and during each week of the flowering period in rhododendron flowers was calculated. The mean number of hymenopteran pollinators was adjusted for 25 minute periods to better compare visitation among pollinator species during each time period. The total number of rhododendron flowers observed, total time spent observing pollinators in flowers and the total number of insects observed also were calculated. Time spent was the total time spent during the time interval, accumulated over the five weeks. Total flowers was the cumulative total of the numbers of flowers recorded at each observation in the time period.

Data Analysis

A mixed model analysis of variance to interpret differences in visitation by time of day for the most abundant groups of visitors were conducted using the GLIMMIX procedure in SAS (SAS Institute Inc. 2008), and least squares means were compared using the Tukey HSD at criterion $\alpha = 0.05$. All covariates that showed statistical significance were incorporated in the model. Correlation analysis was conducted to compare flowers observed, time spent, and insects observed by week using SAS at the criterion $\alpha = 0.05$ (SAS Institute Inc. 2008).

Shannon's diversity index, Shannon's evenness, and richness were compiled for collected pollinators from the four sites and by week of the rhododendron flowering period. Cumulative flowers observed and accumulated time spent observing also were included in analysis. Following is the formula for calculating Shannon's diversity indices:

H: Shannon's diversity index

S: Richness

N: Number of individuals in sample

n_i : Number of individuals in i^{th} species

p_i : Proportion of N made up of i^{th} species

E_H : Shannon's evenness

$$p_i = n_i / N$$

$$H = -\sum p_i \ln p_i$$

$$E_H = H / \ln S$$

Shannon's diversity index gives a measure of the richness combined with the relative abundance of the species. Evenness is a measure of how even the number of individuals in a species are in the area sampled, and richness is the total number of different species found. These indices are useful for comparing populations in similar communities. Shannon's diversity indices for sites and weeks were compared statistically using Shannon diversity Hutcheson t-test calculator online spreadsheet (Hutcheson 1970, Gardener 2017).

Results and Discussion

A total of 711 insects were observed visiting flowers in 64.5 hours of cumulative observations of *R. maximum* in GRSM (Table 4.1, Fig. 4.1). Of these, 132 were collected and

identified in the laboratory, and 37 different taxa were determined with 20 identified to species. The most common species collected were the half-black bumble bee, *B. vagans* (n = 29), and the azalea miner, *A. cornelli* (n = 22).

Seven orders were collected and eight observed. From the collected specimens, 19 different genera have been identified. The most common order was Hymenoptera (89% of all insects collected), and Apidae represented 44% of all insects collected. Of all Hymenoptera collected, Apidae represented 49%, with *Bombus* comprising 97% of the Apidae, Halictidae is 27%, and 14% of Hymenoptera is *A. cornelli*. Among the Apidae were eight *A. mellifera*, all observed at Lynn Camp. This finding suggests that a feral beehive is present in the area considering the distance from the Park boundary. The locations of insect visitors, numbers collected or observed and classification (i.e., pollinator or not) of all 711 individuals are presented in Table 4.1.

After Hymenoptera, Coleoptera represented the most common order with 4% (n = 30) of the specimens observed. Of the 27 identified to family, 22 were long-horn beetles, Cerambycidae. Five of the Cerambycidae were collected and identified to species, and all are in the subfamily Lepturinae, “flower longhorns.” Lepidoptera were conspicuous as they were seen flying, and 11 were observed on the study rhododendrons. Families represented were Hesperidae and Papilionidae with four *Battus philenor* L., pipevine swallowtail, identified.

The previous study of pollinators of *R. maximum* was conducted on an open montane meadow at Round Bald in the southern Appalachians 35 years prior to this study conducted in forest areas so differences in pollinator visitation were expected (Bauer 1986). Seven *Bombus* spp. and Syrphidae were collected at Round Bald. Four of those *Bombus* spp. [*B. bimaculatus* (n

= 6), *B. impatiens* (n = 2), *B. perplexus* (n = 4), and *B. vagans* (n = 29)] and Syrphidae were collected in this study, in addition to Coleoptera, Lepidoptera, and other Diptera and Hymenoptera pollinators. In six hours of collecting on Round Bald, 128 bumble bees were collected while 300 were collected or observed in this study over 64.5 hours of observation. The two greatest predictors of pollinator abundance are canopy openness and low shrub cover. The relative abundance of bumble bees at Round Bald is likely associated with the openness of the bald compared with the dense canopy of hemlock forest. The most common bumble bee species collected at Round Bald were *B. perplexus* (n = 48) and *B. sandersoni* (n = 64), compared to only four *B. perplexus* identified in this study. In this study, the most common species collected was *B. vagans* (n = 29) while five were collected at Round Bald.

The four species of bumble bee collected during both the current and previous (1986) studies are considered common, while the additional three species collected on Round Bald (*B. affinis*, endangered; *B. sandersoni*, uncommon; and *B. terricola*, uncommon and declining) are considered uncommon although not all were historically (Colla et al. 2011, Xerces Society 2019b, a). Given the heavy overstory in this study, *B. vagans* would be expected to be the most common bumble bee observed; according to Colla et al. (2011) it readily forages in heavily shaded forest areas unlike most bumble bees. Also of note is the phenology of *R. maximum*, at Round Bald it was flowering beginning the second week of July thru early August and in GRSM flowering occurred about mid-June to mid-July possibly affecting the species composition of the flower visitors (Bauer 1986).

Incidence, by time of day and across the flowering period, of pollinators shows some trends. More coleopteran pollinators were observed during the second week of the flowering

period, and numbers observed decreased through the rest of the flowering period (Fig. 4.2). Lepidopteran pollinators were only observed on flowers the third and fourth weeks of the flowering period. Two dipteran pollinators were observed the first week, with the highest number observed at peak of the flowering period in the beginning of July with numbers observed declining through the last two weeks.

The more abundant visitors—Apidae, Halictidae, and *A. cornelli*—provide trends across time of day and through the flowering period (Fig. 4.3). Although *A. cornelli* was commonly observed, it was only observed once in the first two weeks and then was present during peak flowering in the first week of July with observations declining through the end of flowering. Halictidae were active throughout the flowering period, peaking during flowering peak. Their pattern was similar to *A. cornelli* but about twice as many were observed, and they were more frequently observed in the first two weeks of flowering than *A. cornelli*.

A difference in active species of Halictidae may have occurred throughout the flowering period. More *Augochlora pura* (Say) were collected during the first three weeks of the flowering period, and more *Lasioglossum (Dialictus)* spp. were collected during the final three weeks. Also, three *Augochloropsis metallica* F. were collected during the third week.

Apidae, which peaked with the flowering peak, was active throughout the flowering period. Adjusted mean Apidae visitation rate in the evening (5:00pm—7:00pm) ($\bar{x} = 3.1$) was significantly ($p < 0.05$) greater than the first morning (8:00am - 10:00am) ($\bar{x} = 1.5$) period. (Fig. 4.4). Apidae visitation rates during the late morning (11:00am—1:00pm) ($\bar{x} = 1.9$) and afternoon (2:00pm—4:00pm) ($\bar{x} = 2.0$) periods did not differ significantly ($p > 0.05$) from the first morning or evening periods. Hymenopteran pollinators and all pollinators observed were

also analyzed with the model and both showed activity similar to Apidae, with visitation rates higher in the evening than first morning period, and two midday periods not differing significantly from the first morning and evening. Apidae was the most abundant group among all pollinators.

Adjusted mean visitation rate for Halictidae in the first morning period ($\bar{x} = 0.4$) was significantly ($p < 0.05$) lower than during the two midday periods ($\bar{x} = 1.6$) (Fig. 4.4). Evening visitation rate ($\bar{x} = 1.1$) for Halictidae was not significantly different ($p > 0.05$) from the other three time periods analyzed. Adjusted mean visitation rates for *A. cornelli* across the day [first morning ($\bar{x} = 0.4$), late morning ($\bar{x} = 0.5$), afternoon ($\bar{x} = 0.7$), and evening ($\bar{x} = 0.7$)] did not differ significantly ($p > 0.05$).

Shannon's diversity index, Shannon's evenness, and richness were compiled for pollinators collected at the four sites and during each of the five weeks of observation through the flowering period (Tables 4.2, 4.3). Total cumulative flowers observed and time spent observing showed statistically significant correlation with numbers of pollinators observed; therefore, they were included in the tables to provide some context.

Diversity indices for the collected pollinators were calculated based on the number of species in the collection (Table 4.2). In this case, Spruce Flats (2.23) has the highest Shannon's diversity index, species richness (14), and abundance of specimens (49) collected. With only six total specimens, Gilliland had the lowest Shannon's index (1.56) but the highest evenness (0.97) with five different species accounted for in six specimens. Shannon's index for Groundhog Creek (1.93) and Lynn Camp (1.91) were nearly equal but evenness was higher for Groundhog Creek (0.93 to 0.83) because there were more species with multiple specimens. Despite the

numerical differences, Hutcheson t-tests (Hutcheson 1970) found no significant ($p > 0.05$) differences in Shannon's indices among the four sites.

Diversity indices were also calculated for collected pollinators across the five weeks of the flowering period (Table 4.3). Shannon's index was significantly ($p < 0.05$) lower during the last week (1.30) of the flowering period than during the second (2.09), third (2.14), and fourth (2.10) weeks. Shannon's index in the first week (1.68) did not differ significantly ($p > 0.05$) from the other four weeks.

Correlation analysis comparing total numbers of flowers observed, time spent observing, and total number of insects observed by week showed a strong positive correlation ($Rho = 0.95$, $p < 0.05$) between flowers observed and time spent observing (Figs. 4.5, 4.6). When rhododendrons were flowering more frequently, more flowers were available to observe and more time was spent observing. Number of insects observed peaked about one week after rhododendron flowering first peaked. Pollinators continued to actively forage as rhododendron flowering senesced in the final two weeks.

Summary

Hymenoptera was the most abundant order (89% of all visitors) observed visiting rhododendron flowers. One-half of all Hymenoptera observed were Apidae, and 97% of those were from the genus *Bombus*. Twenty-four percent of all visitors were Halictidae with two species identified, *Augochlora pura* and *Augochloropsis metallica*, and one subgenus identified, *Lasioglossum (Dialictus)*. One of eight of all visitors was *A. cornelli*, the azalea miner, which occurs only in areas with rhododendrons. The next most common order was Coleoptera at 4%

of total visitors, with 30 observed. Twenty-two cerambycids were observed, and the five collected were Lepturinae (subfamily “flower longhorns”).

Broadly, all of the orders and hymenopteran families of pollinators were observed most during the peak of rhododendron flowering, which occurred during the first week of July. Andrenidae were virtually absent until the first week of July and became a common visitor during the last half of the flowering period. Apidae, Hymenoptera, and pollinators overall were less active in the first morning period compared with evening. During the two midday periods, observations of the three groups were similar to all other times of day. Halictidae were observed less frequently in the first morning period than the two midday periods and evening observations were similar to all other times.

At Round Bald, bumble bees were observed at flowers more than four times the frequency observed in forested areas in GRSM. The most commonly identified bumble bee in this study was *B. vagans* which is better habituated to heavily shaded areas than most other bumble bees. Differences in abundance may relate to habitat type or quality, such as more plant species diversity and less total basal area which are associated with greater abundance and diversity of pollinators. Additionally, the timing of rhododendron flowering on Round Bald was three to four weeks later and the bees may have produced another generation in this time. Also noteworthy is the lack of taxa beside bumble bees and Syrphidae collected at Round Bald. The difference illustrates the difficulty comparing species between two disparate habitats, but both contribute to our understanding of pollinators.

Observations of insect visitors lagged behind the flowering observed in the rhododendrons. This lag between flowering and pollinator foraging may be caused by the

insects adapting to the seasonal change in the available forage or possibly a result of different rates of change in the phenology of rhododendron flowering and insect reproduction or abundance due to forest changes.

A greater diversity of rhododendron flower visitors was observed in the forest system than what had been found on Round Bald. Eight orders of visitors were observed in flowers in the forest system compared to two at Round Bald. The most common visitor collected, *B. vagans* is attuned to the deep forest shade more than other *Bombus* spp. The next most common visitor collected, *A. cornelli*, only occurs in areas with rhododendrons. Other taxa frequently observed include Cerambycidae, Syrphidae, Lepidoptera, and Halictidae. The data will provide a baseline information for comparison in future investigations of pollinators in a forest system as well as information for management of forest areas.

CHAPTER V

CONCLUSIONS

This research focused on examining potential non-target impacts on pollinators of rhododendron in Great Smoky Mountains National Park (GRSM; Park) from imidacloprid applied to hemlocks for control of hemlock woolly adelgid (HWA). Imidacloprid is a widely used systemic insecticide that has low toxicity to humans but is effective in controlling insects. It is applied to hemlock trees to control the invasive HWA which was discovered in GRSM in 2002 and has killed tens of thousands of hemlocks. Remaining healthy hemlocks are treated as extensively as practical with imidacloprid to conserve a species that has a vast range and provides important ecosystem services. Research has investigated other potential non-target impacts of imidacloprid treatment to hemlocks including effects on arthropod hemlock canopy dwellers (Dilling 2007, Hakeem 2008, Dilling et al. 2009, Hakeem et al. 2018), soil macroinvertebrates (Reynolds 2008), and aquatic systems from runoff (Benton et al. 2016, Benton et al. 2017). In a study conducted in Finland in a flight arena using a computer-controlled robotic flower system, imidacloprid reduced foraging motivation of bumble bees, suggesting an association between imidacloprid and negative colony effects observed in many studies (Lämsä et al. 2018), but it remains undetermined if imidacloprid impacts pollinators in forested systems where it is used to control HWA. This research was designed to assess if measurable effects of the imidacloprid treatments can be detected in non-target populations of pollinators foraging in the forest system.

Research was conducted at four sites in GRSM, two sites where hemlocks had been treated with imidacloprid and two control sites, where imidacloprid had not been applied.

Rhododendron maximum is a common associate of hemlock and plants within 30 cm of treated hemlocks have been confirmed to translocate imidacloprid to pollen and nectar (Wiggins et al., unpublished data). In this study ten rhododendrons were within 1 m of treated hemlocks and expected to translocate imidacloprid to pollen and nectar. Another ten were within the two treatment areas, but generally $8\pm$ m from treated trees, where they were expected to contact little or no imidacloprid but be within the same foraging range for pollinators as those rhododendrons within 1 m of treated hemlocks. At the two control sites, which were 1 km from any treatments, the design was replicated so that ten rhododendrons were next to hemlocks and ten away from hemlocks, with a total of 40 rhododendrons in the study.

Pollinators were observed and collected from rhododendrons throughout the flowering period. In 64.5 hours of observation, a total of 711 insects were observed in rhododendron flowers, and 132 of those were collected, preserved and identified. Representatives of eight orders were observed and members of seven orders were collected. Pollinators composed 617 of the total observed, represented by ten families in four orders—Coleoptera (25), Diptera (10), Hymenoptera (571), and Lepidoptera (11). The hymenopteran pollinators were predominantly Apidae (309), including 300 *Bombus* sp., with Halictidae (172), and *Andrena cornelli* (90) commonly observed.

Analysis was completed with an emphasis on pollinating species and by comparing pollinators at treated sites with pollinators at control sites. Mean pollinator visitation to rhododendrons at treated sites ($\bar{x} = 3.9$) was not statistically different ($p > 0.05$) from control sites ($\bar{x} = 4.6$). Pollinator visitation was highly variable among the four sites. At the two imidacloprid-treated sites, Gilliland Creek and Spruce Flats, 18 and 267 pollinators were

observed, respectively, while at control sites, Groundhog Creek and Lynn Camp Prong, 139 and 193 pollinators were observed, respectively. Among covariates in the statistical model, only the number of flowers observed and time spent observing were statistically significant ($p < 0.05$), in other words, observing more flowers on a rhododendron and spending more time observing a rhododendron was associated with a greater likelihood of observing pollinators on the plant.

Many flowering plants require insect pollination for effective reproduction, and evidence suggests that *R. maximum* and other Ericaceae are highly reliant on insects for pollination or may be self-incompatible (Thaler and Plowright 1980, Bauer 1986, Epps et al. 2015). In other studies, examples of effects of pesticides on seed production include declines in flower visitation by pollinators, pollen germination and pollen tube growth declines, and changes in seed germination rates, seed set and weight for hybrid onion seed production (Gillespie et al. 2014). In Finland, a significant linear correlation linked turnip rape seed yield decline with increasing area of neonicotinoid crops (Hokkanen et al. 2017).

To investigate if imidacloprid affected seed production or other seed characteristics of rhododendron, a seed study was conducted. Seedpods were collected from the study plants and buckets were placed under study plants to collect seedfall. Seeds from three seedpods from each rhododendron and seed from the buckets were counted, weighed, and tested for percent germination. No significant ($p > 0.05$) differences in the seed number, weights, or percent germination of rhododendron seed were identified between treated and untreated samples for seedpods or for seedfall collected in buckets. Romancier (1971) found high natural variability in seedpod seed production and it may be difficult to detect impacts to seedpod seed numbers based on environmental factors such as insecticide treatments.

The bucket seed counts had a moderately positive correlation with rhododendron pollinator visitation rate. The correlation between pollinators observed and seed counts concurs with the idea that pollination improves seed production. Perhaps this idea could be developed as an indicator to assess pollinator numbers in a system and may also be applied to other types of plants. If the links between pollinator populations and seed or seedpod production can be more conclusively correlated, then pollinator populations could potentially be assessed over a wider range with less labor by collecting seedfall or measuring seedpod production.

To assess pollinators of *R. maximum* in a forest, a more general examination of the species visiting flowering *R. maximum* detailed their fluctuations throughout the day and across the five-week flowering period. This research provided information about the species composition and abundance in the rhododendron understory in GRSM and temporal data regarding pollinators and flowering of *R. maximum*. This information can be used to monitor changes in the insect fauna in the Park and other natural areas. It provides information to improve understanding of the pollinators of *R. maximum* for the conservation and ecological management of forest systems in GRSM.

Bombus vagans (n = 29) was the most commonly collected species in the study. According to Colla et al. (2011) *B. vagans* readily forages in heavily shaded forest areas unlike most bumble bees. The second most commonly collected species was *A. cornelli* (n = 22). *A. cornelli*, the azalea miner, is oligolectic to *Rhododendron* sp., always found within flight range of its host plants (Ascher et al. 2017, Ascher and Pickering 2019).

An examination of fluctuations across time of day, revealed that Apidae were more active in the evening than in early morning. Although lower numbers of Apidae were observed in the late morning and afternoon than evening, these were not significantly different than any other time of day. Halictidae were more active in the two midday periods observed than the first morning period. Evening observations were lower than the midday periods but not significantly different than any other time of day measured. Mean numbers of *A. cornelli* showed a trend of increased activity across the day but no significant differences were found in numbers observed in the four time periods measured.

Shannon's diversity index, evenness, and richness were calculated for pollinators collected at the four sites and for the five weeks of observations. Although richness based on collected specimens ranged from a low of 5 species at Gilliland Creek to a high of 14 species at Spruce Flats, no statistically significant ($p > 0.05$) difference was found in diversity between any of the four sites using Hutcheson's t-test. Across the five weeks of observations, Shannon's index was significantly ($p < 0.05$) lower during the last week (1.30) of the flowering period than during the second (2.09), third (2.14), and fourth (2.10) weeks. Shannon's index in the first week (1.68) did not differ significantly ($p > 0.05$) from the other four weeks.

For the five weeks of observations, flowers observed, time spent, and insects observed were plotted, and a strong positive correlation ($R = 0.95$, $p < 0.05$) between flowers observed and time spent observing was demonstrated, showing that more time was spent observing when more plants were flowering. Increases in insect observations lagged behind the rhododendron flowering in abundance beginning in the second week and declines in insect observations lagged behind declines in flowering so that when the profusion of flowers began

there were few pollinators observed foraging, but they continued to actively forage as rhododendron flowering senesced in the final two weeks.

Natural systems have inherently great variability that can require extensive sampling to reliably identify an effect. To determine if fenitrothion treatments to control spruce budworm in New Brunswick were impacting fecundity of plants dependent on insect pollination a large number of widely separated sites were identified (Thaler and Plowright 1980). This study illustrated that non-target pollinators were impacted by fenitrothion, and this impact affected pollination and fecundity of study plants. The current study suggests a more efficient way to use labor in GRSM to examine differences in pollinator effectiveness in areas with imidacloprid-treated hemlocks.

First, dependence on insect pollination should be assessed using a bagging experiment whereby fecundity, measured by production of fruits per flower, of flowers bagged to exclude pollinators are compared to fecundity of unbagged flowers. Other studies have suggested high reliance of *R. maximum* on insect pollination. Bauer (1986) bagged 58 flowers and compared fruit production with 61 unbagged flowers. All 61 unbagged flowers produced fruit while 1 of the 58 bagged flowers produced fruit. Other Ericaceae have been studied including *R. calendulaceum* which produced no fruits from 79 bagged flowers suggesting self-incompatibility (Epps et al. 2015), and 6.5% of 4,813 bagged *K. angustifolia* flowers and 76.9% of 3,506 open flowers produced fruit indicating dependence on insect pollination (Thaler and Plowright 1980).

To better identify differences in fecundity associated with imidacloprid treatment, a large number of widely separated sites with different treatment histories should be identified. Different treatment histories would include untreated areas, areas that are treated with

different amounts of imidacloprid, and areas where treatments were applied in different years. One variable that may be difficult to control is having untreated areas with hemlocks in a condition similar to the treated areas. In these areas, racemes on rhododendrons would be tagged and flowers counted; later, fruits would be counted. Fruit production would correlate to pollinator effectiveness.

Covariates that have been correlated with pollinator abundance and diversity should also be assessed in the areas sampled. Total tree basal area, canopy openness, plant species diversity, shrub cover, and fire frequency are known to affect pollinator species abundance and diversity (Grundel et al. 2010, Jackson et al. 2014, Hanula et al. 2015, 2016). Low total basal area and plant species richness were associated with pollinator species richness. Greater canopy openness and low shrub cover were the best predictors of pollinator abundance. To improve the pollinator study conducted in this research, recommended changes would include a more complete assessment of these site characteristics.

Overstory density at each plant, temperature, weather conditions, amount of imidacloprid applied to site, number of flowers observed, and time spent observing also should be recorded as done in this study. Although many of these covariates were not significant, they may have more significance in a different year, such as when temperatures or weather are more variable, or when more sites are included.

The bucket study is different from the seedpod seed study conducted because the collector seeks out and collects from the seedpods that are there without consideration of the total seedpod production. Seed collected in the bucket is a result of the total seed produced by the plant. Seedpods have been shown to have high natural variability in seed number

(Romancier 1971). If few seedpods existed because of poor pollination, the low yield of seed in the buckets would be a good indicator of poor pollination. Collecting seedpods from study plants that had lower fecundity, evidenced by lower seedpod production, would have greater impact on their bucket seed yields, diminishing an already limited supply of seed. If seedpod collecting diminished seed on low fecundity plants this removal of a higher proportion of total seed on the plant could have exacerbated the relationship found between bucket seed numbers and pollinator visitation rates.

Processing the seedpod seed and bucket seed is labor intensive. A more efficient method to ascertain pollinator effectiveness may have been to take a census of flowers on each of the 40 plants while doing the weekly visits and returning after flowers senesced to count seedpods produced, avoiding the tedium and labor of handling the tiny and abundant seed.

Differences in pollinators at treated sites compared to control sites was not statistically significant ($p > 0.05$), and other environmental variables or site characteristics may be responsible for the lower abundance at Gilliland Creek. Variables known to affect pollinator abundance and diversity are total tree basal area, canopy openness, plant species diversity, shrub cover, and fire frequency (Grundel et al. 2010, Jackson et al. 2014, Hanula et al. 2015, 2016). Chemical analysis of plant and insect material for the presence of imidacloprid and its metabolites would provide confirmation that imidacloprid was translocated to non-target rhododendron and pollinators.

The development and completion of an expanded study to assess non-target impacts of imidacloprid on pollinators in a forested system that addresses design improvements discussed earlier in this chapter would help characterize the interactions among imidacloprid, pollinators,

and *R. maximum* and provide more definitive results. Imidacloprid treatments to hemlock are still a viable management option to protect this important tree from HWA and ecological extinction. Management plans should consider abundance of rhododendron when selecting trees for treatment. This management plan refinement will continue to protect eastern hemlock from HWA, while reducing potential impacts on non-target pollinators.

REFERENCES

- Abella, S. R. 2018.** Forest decline after a 15-year “perfect storm” of invasion by hemlock woolly adelgid, drought, and hurricanes. *Biological Invasions* 20: 695-707.
- Abella, S. R., V. B. Shelburne, and N. W. MacDonald. 2003.** Multifactor classification of forest landscape ecosystems of Jocassee Gorges, southern Appalachian Mountains, South Carolina. *Canadian Journal of Forest Research* 33: 1933-1946.
- Ascher, J., and J. Pickering. 2017.** Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Available online: <https://www.discoverlife.org/mp/20q?search=Apoidea> (accessed on: Various, 2018-2019).
- Ascher, J., and J. Pickering. 2019.** Discover Life *Andrena cornelli*. Available online: <https://www.discoverlife.org/20/q?search=Andrena+cornelli> (accessed on: 1 December 2019).
- Ascher, J., T. Kropiewnicki, and H. Go. 2017.** BugGuide.Net *Andrena cornelli*. Available online: <https://bugguide.net/node/view/257988> (accessed on: 1 December 2019).
- Baker, T., and D. H. Van Lear. 1998.** Relations between density of rhododendron thickets and diversity of riparian forests. *Forest Ecology and Management* 109: 21-32.
- Bauer, P. J. 1986.** Bumblebee pollination on a southern Appalachian grass bald and narcotic nectar in *Angelica triquinata* (Apiaceae). Ph.D. Dissertation, University of North Carolina, Chapel Hill, North Carolina.
- Benton, E. P., and R. S. Cowles. 2016.** Optimized insecticide dosage for hemlock woolly adelgid control in hemlock trees. WSFNR-17-01. The University of Georgia Warnell School of Forestry and Natural Resources, Tifton, Georgia.

- Benton, E. P., J. F. Grant, T. C. Mueller, R. J. Webster, and R. J. Nichols. 2016.** Consequences of imidacloprid treatments for hemlock woolly adelgid on stream water quality in the southern Appalachians. *Forest Ecology and Management* 360: 152-158.
- Benton, E. P., J. F. Grant, R. J. Nichols, R. J. Webster, J. S. Schwartz, and J. K. Baily. 2017.** Risk assessment of imidacloprid use in forest settings on the aquatic macroinvertebrate community. *Environmental Toxicology and Chemistry* 36: 3108-3119.
- Benton, E. P., J. F. Grant, R. J. Webster, R. J. Nichols, R. S. Cowles, A. F. Lagalante, and C. I. Coots. 2015.** Assessment of imidacloprid and its metabolites in foliage of eastern hemlock multiple years following treatment for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in forested conditions. *Journal of Economic Entomology* 108: 2672-2682.
- Blozan, W. 2007.** The Usis hemlock climb. Eastern Native Tree Society. Available online: http://www.nativetreesociety.org/fieldtrips/gsmnp/usis/usis_hemlock_climb.htm (accessed on: 4 September 2019).
- Blumenthal, M., K. Carlin, D. Eggen, and S. Werner. 2005.** Managing Hemlock Woolly Adelgid in Pennsylvania. Pennsylvania Department of Conservation and Natural Resources. Available online: https://www.fs.fed.us/foresthealth/fhm/posters/posters05/hwa_pa.pdf (accessed on: 7 October 2019).
- Bommarco, R., L. Marini, and B. E. Vaissière. 2012.** Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia* 169: 1025-1032.

Brantley, S. T., A. E. Mayfield, R. M. Jetton, C. F. Miniatt, D. R. Zietlow, C. L. Brown, and J. R.

Rhea. 2017. Elevated light levels reduce hemlock woolly adelgid infestation and improve carbon balance of infested eastern hemlock seedlings. *Forest Ecology and Management* 385: 150-160.

Brooks, R. T. 2004. Early regeneration following the presalvage cutting of hemlock from hemlock-dominated stands. *Northern Journal of Applied Forestry* 21: 12-18.

Cheah, C., M. E. Montgomery, S. Salom, B. L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock woolly adelgid. Forest Health Technology Enterprise Team, U.S. Department of Agriculture Forest Service, Morgantown, West Virginia.

Colla, S., L. Richardson, and P. Williams. 2011. Bumble bees of the eastern United States. U.S. Department of Agriculture. Available online:
<https://www.fs.fed.us/wildflowers/pollinators/documents/BumbleBeeGuideEast2011.pdf> (accessed on: 12 December 2019).

Coots, C., P. Lambdin, J. Grant, and R. Rhea. 2013. Spatial and temporal distribution of residues of imidacloprid and its insecticidal 5-hydroxy and olefin and metabolites in eastern hemlock (Pinales: Pinaceae) in the southern Appalachians. *Journal of Economic Entomology* 106: 2399-2406.

Coots, C. I. 2012. Spatial, temporal, and tri-trophic distribution of imidacloprid, olefin and 5-hydroxy and their effect on hemlock woolly adelgid, *Adelges tsugae* Annand, (Hemiptera: Adelgidae). Ph.D. Dissertation, University of Tennessee, Knoxville, Tennessee.

- Cowles, R., M. Montgomery, and C.-J. Cheah. 2006.** Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *Journal of Economic Entomology* 99: 1258-1267.
- Cowles, R. S. 2009.** Optimizing dosage and preventing leaching of imidacloprid for management of hemlock woolly adelgid in forests. *Forest Ecology and Management* 257: 1026-1033.
- Cox, L., W. Koskinen, and P. Yen. 1998.** Influence of soil properties on sorption-desorption of imidacloprid. *Journal of Environmental Science & Health Part B* 33: 123-134.
- Davis, G. A., S. M. Salom, C. C. Brewster, B. P. Onken, and L. T. Kok. 2012.** Spatiotemporal distribution of the hemlock woolly adelgid predator *Laricobius nigrinus* after release in eastern hemlock forests. *Agricultural and Forest Entomology* 14: 408-418.
- Dilling, C. 2007.** 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. M.S. Thesis, University of Tennessee, Knoxville, Tennessee.
- Dilling, C., P. Lambdin, J. Grant, and R. Rhea. 2009.** Community response of insects associated with eastern hemlock to imidacloprid and horticultural oil treatments. *Environmental Entomology* 38: 53-66.
- Dilling, C., P. Lambdin, J. Grant, and R. Rhea. 2010.** Spatial and temporal distribution of imidacloprid in eastern hemlock in the southern Appalachians. *Journal of Economic Entomology* 103: 368-373.
- Earle, C. J. 2019.** *Tsuga*. The gymnosperm database. Available online: <https://www.conifers.org/pi/Tsuga.php> (accessed on: 4 September 2018).

- Easton, A. H., and D. Goulson. 2013.** The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. *PLoS One* 8: e54819.
- Elbert, A., R. Nauen, and W. Leicht. 1998.** Imidacloprid, a novel chloronicotinyl insecticide: biological activity and agricultural importance, pp. 50-73. *In* I. Ishaaya and D. Degheele (eds.), *Insecticides with Novel Modes of Action*. Springer, New York, New York.
- Elliott, K. J., and J. M. Vose. 2011.** The contribution of the Coweeta Hydrologic Laboratory to developing an understanding of long-term (1934–2008) changes in managed and unmanaged forests. *Forest Ecology and Management* 261: 900-910.
- Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, and G. M. Lovett. 2005.** Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3: 479-486.
- Epps, M. J., S. E. Allison, and L. M. Wolfe. 2015.** Reproduction in flame azalea (*Rhododendron calendulaceum*, Ericaceae): a rare case of insect wing pollination. *The American Naturalist* 186: 294-301.
- Eschtruth, A. K., R. A. Evans, and J. J. Battles. 2013.** Patterns and predictors of survival in *Tsuga canadensis* populations infested by the exotic pest *Adelges tsugae*: 20 years of monitoring. *Forest Ecology and Management* 305: 195-203.
- Farjon, A. 2017.** *A Handbook of the World's Conifers*. Second Ed. Brill, Boston, Massachusetts.
- Feltham, H., K. Park, and D. Goulson. 2014.** Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 23: 317-323.

Gardener, M. 2017. Statistics for Ecologists (Edition 2) Exercise 12.1.4. Pelagic Publishing.

Available online: <https://www.dataanalytics.org.uk/comparing-diversity/#spreadsheet>

(accessed on: 11 December 2019).

Gillespie, S., R. Long, N. Seitz, and N. Williams. 2014. Insecticide use in hybrid onion seed production affects pre- and postpollination processes. *Journal of Economic Entomology* 107: 29-37.

Gonda-King, L., S. Gómez, J. L. Martin, C. M. Orians, and E. L. Preisser. 2014. Tree responses to an invasive sap-feeding insect. *Plant Ecology* 215: 297-304.

Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology* 50: 977-987.

Grundel, R., R. P. Jean, K. J. Frohnapple, G. A. Glowacki, P. E. Scott, and N. B. Pavlovic. 2010. Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. *Ecological Applications* 20: 1678-1692.

Hakeem, A. 2008. Non-target effect of imidacloprid on the predatory arthropod guild on eastern hemlock, *Tsuga canadensis* (L.) Carriere, in the southern Appalachians. M.S. Thesis, University of Tennessee, Knoxville, Tennessee.

Hakeem, A. 2013. Assessment of factors affecting establishment of biological control agents of hemlock woolly adelgid on eastern hemlock in the Great Smoky Mountains National Park. Ph.D. Dissertation, University of Tennessee, Knoxville, Tennessee.

Hakeem, A., J. Grant, P. Lambdin, F. Hale, J. Rhea, G. Wiggins, and C. Coots. 2018. Influence of imidacloprid and horticultural oil on spider abundance on eastern hemlock in the southern Appalachians. *Environmental Entomology* 47: 951-959.

- Hanula, J. L., S. Horn, and J. J. O'Brien. 2015.** Have changing forests conditions contributed to pollinator decline in the southeastern United States? *Forest Ecology and Management* 348: 142-152.
- Hanula, J. L., M. D. Ulyshen, and S. Horn. 2016.** Conserving pollinators in North American forests: a review. *Natural Areas Journal* 36: 427-440.
- Havill, N. P., and R. G. Foottit. 2007.** Biology and evolution of adelgidae. *Annual Review of Entomology* 52: 325-349.
- Havill, N. P., L. C. Vieira, and S. M. Salom. 2014.** Biology and control of hemlock woolly adelgid. FHTET-2014-05. United States Department of Agriculture, Forest Health Technology Enterprise Team, Washington, D.C.
- Havill, N. P., S. D. Gaimari, and A. Caccone. 2018.** Cryptic east-west divergence and molecular diagnostics for two species of silver flies (Diptera: Chamaemyiidae: *Leucopis*) from North America being evaluated for biological control of hemlock woolly adelgid. *Biological Control* 121: 23-29.
- Havill, N. P., M. E. Montgomery, G. Y. Yu, S. Shiyake, and A. Caccone. 2006.** Mitochondrial DNA from hemlock woolly adelgid (Hemiptera : Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America* 99: 195-203.
- Havill, N. P., C. S. Campbell, T. F. Vining, B. LePage, R. J. Bayer, and M. J. Donoghue. 2008.** Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal ITS and chloroplast DNA sequence data. *Systematic Botany* 33: 478-489.

- Havill, N. P., S. Shiyake, A. L. Galloway, R. G. Foottit, G. Y. Yu, A. Paradis, J. Elkinton, M. E. Montgomery, M. Sano, and A. Caccone. 2016.** Ancient and modern colonization of North America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. *Molecular Ecology* 25: 2065-2080.
- Hokkanen, H. M., I. Menzler-Hokkanen, and M. Keva. 2017.** Long-term yield trends of insect-pollinated crops vary regionally and are linked to neonicotinoid use, landscape complexity, and availability of pollinators. *Arthropod-Plant Interactions* 11: 449-461.
- Hough, A. F. 1960.** Silvical Characteristics of Eastern Hemlock (*Tsuga canadensis*). Northeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Upper Darby, Pennsylvania.
- Howe, R. W., and M. Mossman. 1996.** The significance of hemlock for breeding birds in the western Great Lakes region, pp. 125-140. *In* G. Mroz and J. Martin (eds.), *Hemlock Ecology and Management*. Department of Forestry, University of Wisconsin-Madison, Madison, Wisconsin.
- Hutcheson, K. 1970.** A test for comparing diversities based on the Shannon formula. *Journal of Theoretical Biology* 29: 151-154.
- Jackson, M. M., M. G. Turner, and S. M. Pearson. 2014.** Logging legacies affect insect pollinator communities in southern Appalachian forests. *Southeastern Naturalist* 13: 317-337.
- Jansen, S. A., I. Kleerekooper, Z. L. Hofman, I. F. Kappen, A. Stary-Weinzinger, and M. A. van der Heyden. 2012.** Grayanotoxin poisoning: 'mad honey disease' and beyond. *Cardiovascular Toxicology* 12: 208-215.

Johnson, E. A., and J. L. Kovner. 1956. Effect on streamflow of cutting a forest understory.
Forest Science 2: 82-91.

Kanoti, A., K. Lombard, J. Weimer, B. Schultz, J. Esden, R. Hanavan, and M. Bohne. 2015.
Managing Hemlock in Northern New England Forests Threatened by Hemlock Woolly
Adelgid and Elongate Hemlock Scale. United States Department of Agriculture Forest
Service. Available online:
https://extension.unh.edu/resources/files/Resource005573_Rep7772.pdf (accessed on:
7 October 2019).

**Kessler, S. C., E. J. Tiedeken, K. L. Simcock, S. Derveau, J. Mitchell, S. Softley, A. Radcliffe, J. C.
Stout, and G. A. Wright. 2015.** Bees prefer foods containing neonicotinoid pesticides.
Nature 521: 74.

Kevan, P. G. 1975. Forest application of the insecticide fenitrothion and its effect on wild bee
pollinators (Hymenoptera: Apoidea) of lowbush blueberries (*Vaccinium* spp.) in
southern New Brunswick, Canada. Biological Conservation 7: 301-309.

Kincaid, J. A. 2007. Compositional and environmental characteristics of *Tsuga canadensis* (L.)
Carr. forests in the southern Appalachian Mountains, USA. Journal of the Torrey
Botanical Society 134: 479-488.

Klatt, B. K., A. Holzschuh, C. Westphal, Y. Clough, I. Smit, E. Pawelzik, and T. Tschardtke. 2014.
Bee pollination improves crop quality, shelf life and commercial value. Proceedings of
the Royal Society B: Biological Sciences 281: 20132440.

- Knoepp, J. D., J. M. Vose, J. L. Michael, and B. C. Reynolds. 2012.** Imidacloprid movement in soils and impacts on soil microarthropods in southern Appalachian eastern hemlock stands. *Journal of Environmental Quality* 41: 469-478.
- Krapfl, K. J., E. J. Holzmüller, and M. A. Jenkins. 2011.** Early impacts of hemlock woolly adelgid in *Tsuga canadensis* forest communities of the southern Appalachian Mountains. *Journal of the Torrey Botanical Society* 138: 93-106.
- Krupke, C. H., G. J. Hunt, B. D. Eitzer, G. Andino, and K. Given. 2012.** Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7: e29268.
- Lämsä, J., E. Kuusela, J. Tuomi, S. Juntunen, and P. C. Watts. 2018.** Low dose of neonicotinoid insecticide reduces foraging motivation of bumblebees. *Proceedings of the Royal Society B: Biological Sciences* 285: 20180506.
- Laycock, I., K. M. Lenthall, A. T. Barratt, and J. E. Cresswell. 2012.** Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21: 1937-1945.
- Leach, D. G. 1961.** *Rhododendron maximum*, pp. 191-193. In *Rhododendrons of the World and How to Grow Them*. Scribner, New York, New York.
- Leppik, E. 1953.** The ability of insects to distinguish number. *The American Naturalist* 87: 229-236.
- Leppik, E. E. 1974.** Evolutionary interactions between rhododendrons pollinating insects and rust fungi. *Journal American Rhododendron Society* 28: No. 2.
- Little, E. L. 1981.** *Atlas of United States Trees: Supplement. Vol. 6*, U.S. Department of Agriculture, Forest Service.

- Long, R., and L. Morandin. 2011.** Low hybrid onion seed yields relate to honey bee visits and insecticide use. *California Agriculture* 65: 155-159.
- Lustenhouwer, M. N., L. Nicoll, and A. M. Ellison. 2012.** Microclimatic effects of the loss of a foundation species from New England forests. *Ecosphere* 3: 1-16.
- Madden, M., R. Welch, T. Jordan, P. Jackson, R. Seavey, and J. Seavey. 2004.** Digital Vegetation Maps for the Great Smoky Mountains National Park. Final Report to U.S. Department of Interior, National Park Service, Great Smoky Mountains National Park, Gatlinburg, Tennessee.
- Martin, K. L., and P. C. Goebel. 2012.** Decline in riparian *Tsuga canadensis* forests of the central Appalachians across an *Adelges tsugae* invasion chronosequence. *Journal of the Torrey Botanical Society* 139: 367-378.
- Mayer, M., R. Chianese, T. Scudder, J. White, K. Vongpaseuth, and R. Ward. 2002.** Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests, pp. 50-60. *In* B. Onken, R. Reardon and J. Lashomb (eds.), *Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium*. Rutgers University, New Brunswick, New Jersey.
- Mayfield, A. E., III, B. C. Reynolds, C. I. Coots, N. P. Havill, C. Brownie, A. R. Tait, J. L. Hanula, S. V. Joseph, and A. B. Galloway. 2015.** Establishment, hybridization and impact of *Laricobius* predators on insecticide-treated hemlocks: exploring integrated management of the hemlock woolly adelgid. *Forest Ecology and Management* 335: 1-10.
- Mayor, A. J., J. F. Grant, and P. L. Lambdin. 2001.** Nectar thieves, pollen robbers and pollinators of hybrid azaleas. *Proceedings of the Southern Nursery Association Research Conference* 46: 219-223.

- McClure, M. S. 1987a.** Biology and control of hemlock woolly adelgid. Bulletin 851, 7 pp.
Connecticut Agricultural Experiment Station, New Haven, Connecticut.
- McClure, M. S. 1987b.** Biology and control of hemlock woolly adelgid—Bulletin 851, pp. 7.
Connecticut Agricultural Experiment Station.
- McClure, M. S. 1989.** Evidence of a polymorphic life-cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Annals of the Entomological Society of America 82: 50-54.
- McClure, M. S. 1990.** Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environmental Entomology 19: 36-43.
- McClure, M. S. 1991.** Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environmental Entomology 20: 258-264.
- McClure, M. S. 1992.** Hemlock woolly adelgid. American Nurseryman (USA) 175(6): 82-89.
- McClure, M. S. 1996.** Biology of *Adelges tsugae* and its potential for spread in the northeastern United States, pp. 16-25. In Proceedings of the First Hemlock Woolly Adelgid Review, Charlottesville, Virginia.
- McMartin, B. 1992.** Hides, Hemlocks and Adirondack History: How the Tanning Industry Influenced the Region's Growth. North Country Books, Utica, New York.
- Mitchell, T. B. 1962.** Bees of the Eastern United States Volume II. Technical Bulletin No. 152.
North Carolina Agricultural Experiment Station, Raleigh, North Carolina.
- Monk, C. D., D. T. McGinty, and F. P. Day. 1985.** The ecological importance of *Kalmia latifolia* and *Rhododendron maximum* in the deciduous forest of the southern Appalachians.
Bulletin of the Torrey Botanical Club 112: 187-193.

Mulroy, M. L., E. J. Holzmüller, and M. A. Jenkins. 2019. Woody regeneration response to overstory mortality caused by the hemlock woolly adelgid (*Adelges tsugae*) in the southern Appalachian mountains. *Forests* 10, 717; doi:10.3390/f10090717.

National Association of State Foresters. 2011. State tree of Pennsylvania. Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry. Available online:
http://www.docs.dcnr.pa.gov/cs/groups/public/documents/document/dcnr_009327.pdf
f. (accessed on: 13 September 2019).

Nauen, R., and T. Bretschneider. 2002. New modes of action of insecticides. *Pesticide Outlook* 13: 241-245.

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

Nesom, G. 2002. Plant guide: Eastern hemlock, *Tsuga canadensis* (L.) Carr. United States Department of Agriculture Natural Resource Conservation Service. Available online:
https://plants.usda.gov/plantguide/pdf/cs_tsca.pdf (accessed on: 4 September 2019).

Orwig, D. A., A. A. B. Plotkin, E. A. Davidson, H. Lux, K. E. Savage, and A. M. Ellison. 2013. Foundation species loss affects vegetation structure more than ecosystem function in a northeastern USA forest. *PeerJ* 1: e41.

Pain, S. 2015. Sweet deceit. *New Scientist* 226: 42-45.

Petersen, H., and M. Luxton. 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* 39: 288-388.

- Phelps, J. D., C. G. Strang, M. Gbylik-Sikorska, T. Sniegocki, A. Posyniak, and D. F. Sherry. 2018.** Imidacloprid slows the development of preference for rewarding food sources in bumblebees (*Bombus impatiens*). *Ecotoxicology* 27: 175-187.
- Phillips, D. L., and W. H. Murdy. 1985.** Effects of rhododendron (*Rhododendron maximum* L.) on regeneration of southern Appalachian hardwoods. *Forest Science* 31: 226-233.
- Plowright, R., and F. Rodd. 1980.** The effect of aerial insecticide spraying on hymenopterous pollinators in New Brunswick. *The Canadian Entomologist* 112: 259-269.
- Proctor, M., and P. Yeo. 1972.** *The Pollination of Flowers*. Taplinger, New York, New York.
- Reynolds, W. N. 2008.** Imidacloprid insecticide treatments for hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), affect a non-target soil arthropod community surrounding eastern hemlock, *Tsuga canadensis* (L.) Carriere. M.S. Thesis, University of Tennessee, Knoxville, Tennessee.
- Romancier, R. M. 1971.** Ecology of the seedling establishment of *Rhododendron maximum* L. in the southern Appalachians. Ph.D. Dissertation, Duke University, Durham, North Carolina.
- Sarwar, A. K. M. G., and H. Takahashi. 2014.** Pollen morphology of the tribe Phyllodoceae (Ericoideae, Ericaceae) and its taxonomic significance. *Bangladesh Journal of Plant Taxonomy* 21: 137.
- SAS Institute Inc. 2008.** SAS/STAT® 9.2 User's Guide. Cary, NC, SAS Institute Inc.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012.** NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671-675.

Silcox, C. A. 2002. Using imidacloprid to control hemlock woolly adelgid, pp. 280-287. *In*

Proceedings Hemlock Woolly Adelgid in the Eastern United States. New Jersey

Agricultural Experiment Station, New Brunswick, New Jersey.

Smitley, D., and D. G. McCullough. 2017. How to treat hemlock trees for hemlock woolly

adelgid. Michigan State University Extension. Available online:

https://www.canr.msu.edu/uploads/resources/pdfs/hwa_tip_sheet.pdf (accessed on: 7

October 2019).

Sumpter, K. L., T. J. McAvoy, C. C. Brewster, A. E. Mayfield III, and S. M. Salom. 2018.

Assessing an integrated biological and chemical control strategy for managing hemlock

woolly adelgid in southern Appalachian forests. *Forest Ecology and Management* 411:

12-19.

Sur, R., and A. Stork. 2003. Uptake, translocation and metabolism of imidacloprid in plants.

Bulletin of Insectology 56: 35-40.

Tan, K., W. Chen, S. Dong, X. Liu, Y. Wang, and J. C. Nieh. 2014. Imidacloprid alters foraging

and decreases bee avoidance of predators. *PLoS One* 9: e102725.

Thaler, G., and R. Plowright. 1980. The effect of aerial insecticide spraying for spruce budworm

control on the fecundity of entomophilous plants in New Brunswick. *Canadian Journal of*

Botany 58: 2022-2027.

Thomson, J. D., R. Plowright, and G. Thaler. 1985. Matacil insecticide spraying, pollinator

mortality, and plant fecundity in New Brunswick forests. *Canadian Journal of Botany* 63:

2056-2061.

- Tiedeken, E. J., J. C. Stout, P. C. Stevenson, and G. A. Wright. 2014.** Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. *Journal of Experimental Biology* 217: 1620-1625.
- Tiedeken, E. J., P. A. Egan, P. C. Stevenson, G. A. Wright, M. J. Brown, E. F. Power, I. Farrell, S. M. Matthews, and J. C. Stout. 2016.** Nectar chemistry modulates the impact of an invasive plant on native pollinators. *Functional Ecology* 30: 885-893.
- Toenies, M. J., D. A. Miller, M. R. Marshall, and G. E. Stauffer. 2018.** Shifts in vegetation and avian community structure following the decline of a foundational forest species, the eastern hemlock. *The Condor: Ornithological Applications* 120: 489-506.
- Van Lear, D. H., and T. A. Waldrop. 1989.** History, uses, and effects of fire in the Appalachians. General Technical Report SE-54. U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station, Asheville, North Carolina.
- Van Lear, D. H., D. Vandermast, C. Rivers, T. Baker, C. Hedman, B. Clinton, and T. Waldrop. 2002.** American chestnut, rhododendron, and the future of Appalachian cove forests, pp. 214-220. *In* K. Outcalt (ed.), *Proceedings of the eleventh biennial southern silvicultural research conference*. General Technical Report SRS-48. U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, North Carolina.
- Vieira, L. C., S. M. Salom, M. E. Montgomery, and L. T. Kok. 2013.** Field-cage evaluation of the survival, feeding and reproduction of *Laricobius osakensis* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae). *Biological Control* 66: 195-203.
- Webb, J. K. 2008.** Beyond butterflies: gardening for native pollinators. B1349. University of Georgia Cooperative Extension, Tifton, GA.

- Webb, R. E., J. R. Frank, and M. J. Raupp. 2003.** Eastern hemlock recovery from hemlock wooly adelgid damage following imidaclopid therapy. *Journal of Arboriculture* 29: 298-302.
- White Jr, J. W., and M. L. Riethof. 1959.** The composition of honey. 3. Detection of acetylandromedol in toxic honeys. *Archives of Biochemistry* 79: 165-167.
- Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson. 2012.** Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336: 351-352.
- Whiting, T. M. 2018.** Can we save the mighty hemlock? *Appalachian Voices*. Available online: <http://appvoices.org/2018/02/19/can-we-save-the-mighty-hemlock/> (accessed on: 12 September 2019).
- Williams, E., J. Rouse, B. Palser, and R. Knox. 1990.** Reproductive biology of rhododendron. *Horticultural Reviews* 12: 1-68.
- Wisconsin Department of Natural Resources. 2018.** Hemlock Woolly Adelgid Regulated Areas. Wisconsin Department of Agriculture, Trade and Consumer Protection. Available online: <https://datcp.wi.gov/Documents/HWAMap.pdf> (accessed on: 1 September 2019).
- Xerces Society. 2019a.** Rusty patched bumble bee. The Xerces Society for Invertebrate Conservation. Available online: <https://xerces.org/endangered-species/species-profiles/at-risk-invertebrates/bumble-bees/rusty-patched-bumble-bee> (accessed on: 19 October 2019).
- Xerces Society. 2019b.** Pocket guide to identifying the yellow-banded bumble bee. The Xerces Society for Invertebrate Conservation. Available online: <https://xerces.org/publications/id-monitoring/pocket-guide-identifying-yellow-banded-bumble-bee> (accessed on: 19 October 2019).

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

APPENDIX

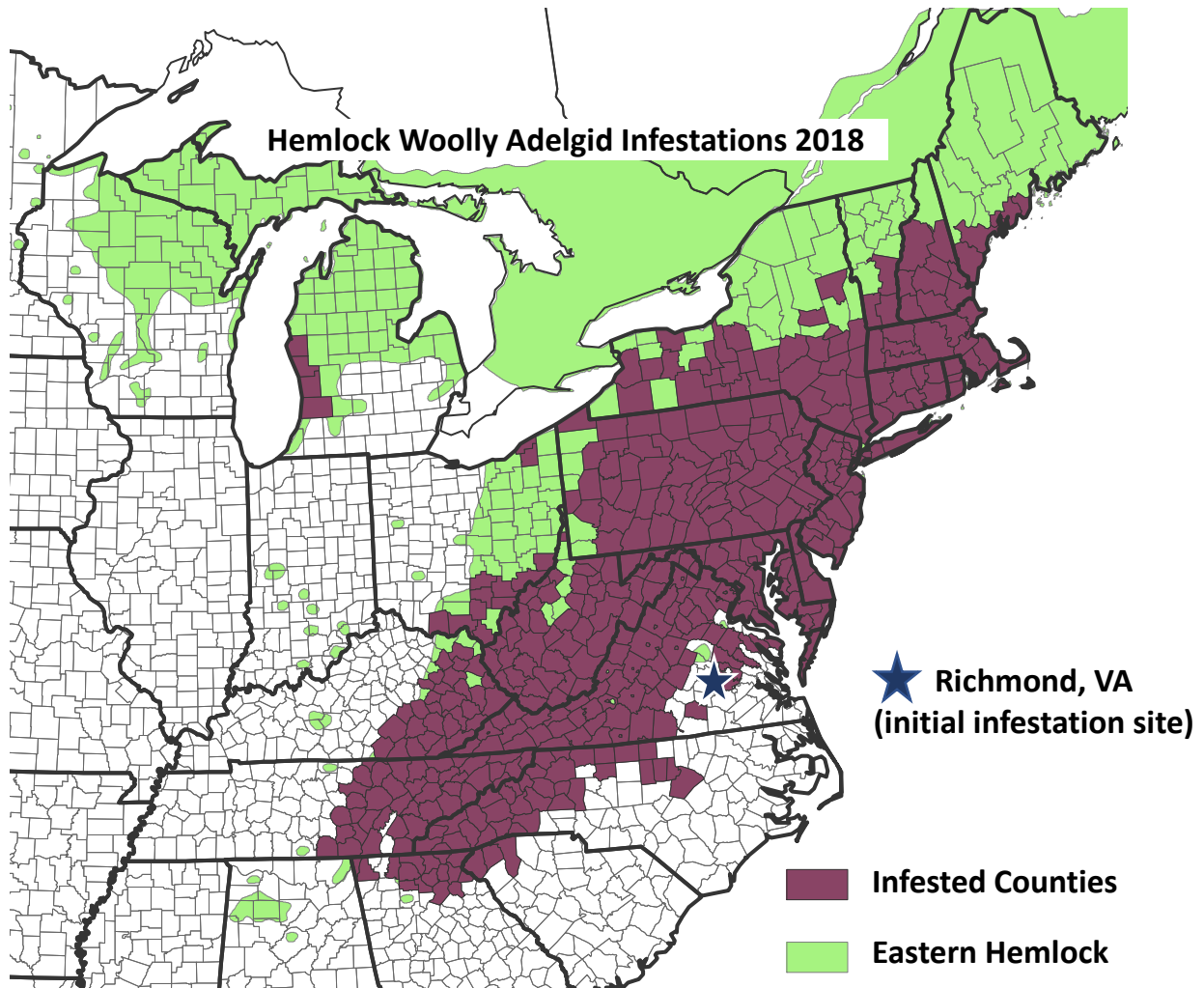


Figure 1.1 Distribution of eastern hemlock and hemlock woolly adelgid in North America, 2018 (Wisconsin Department of Natural Resources 2018).

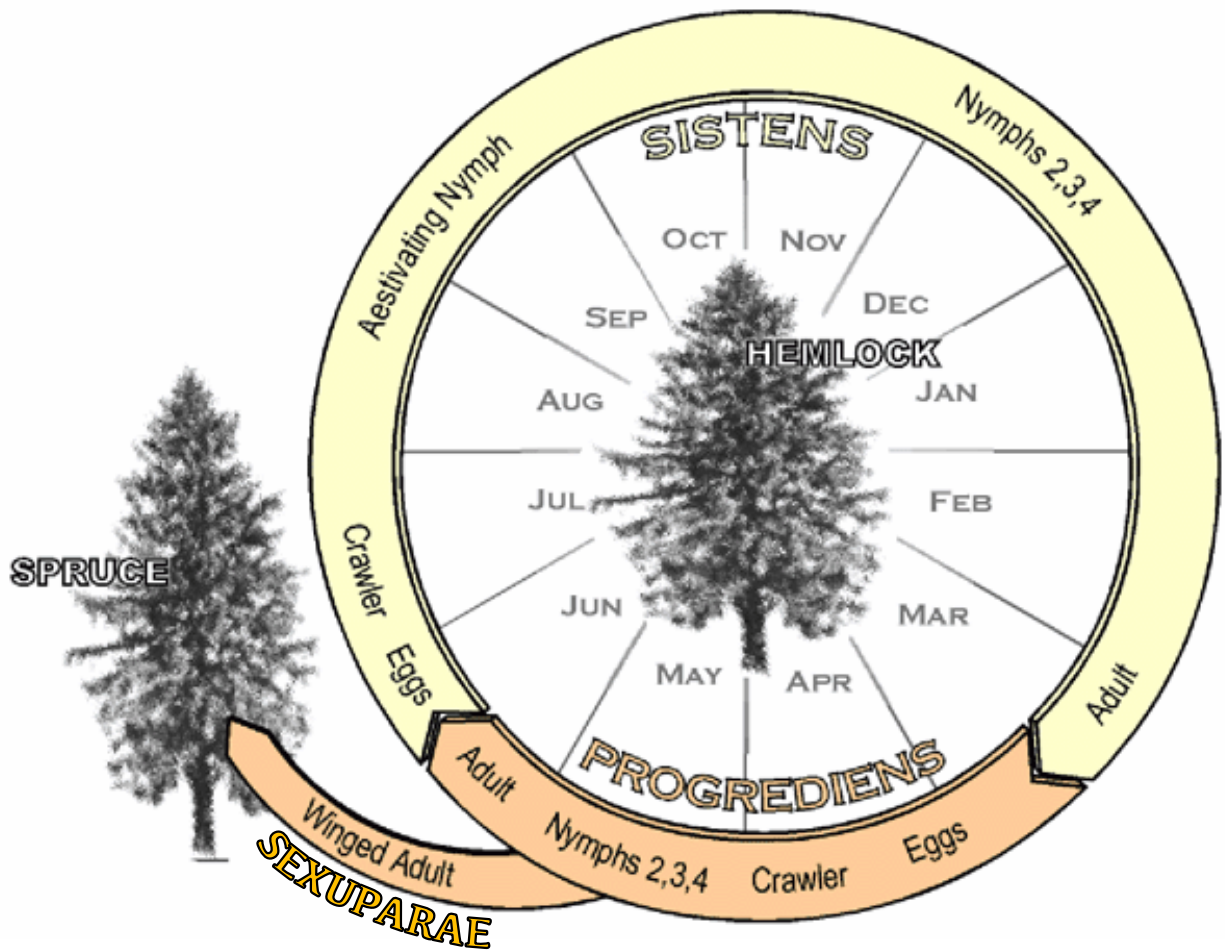


Figure 1.2 Hemlock woolly adelgid life cycle in North America (Cheah et al. 2004).



Figure 1.3 Flowering *Rhododendron maximum* L. in a hemlock-dominated forest, Groundhog Creek, Great Smoky Mountains National Park, 11 July 2018.

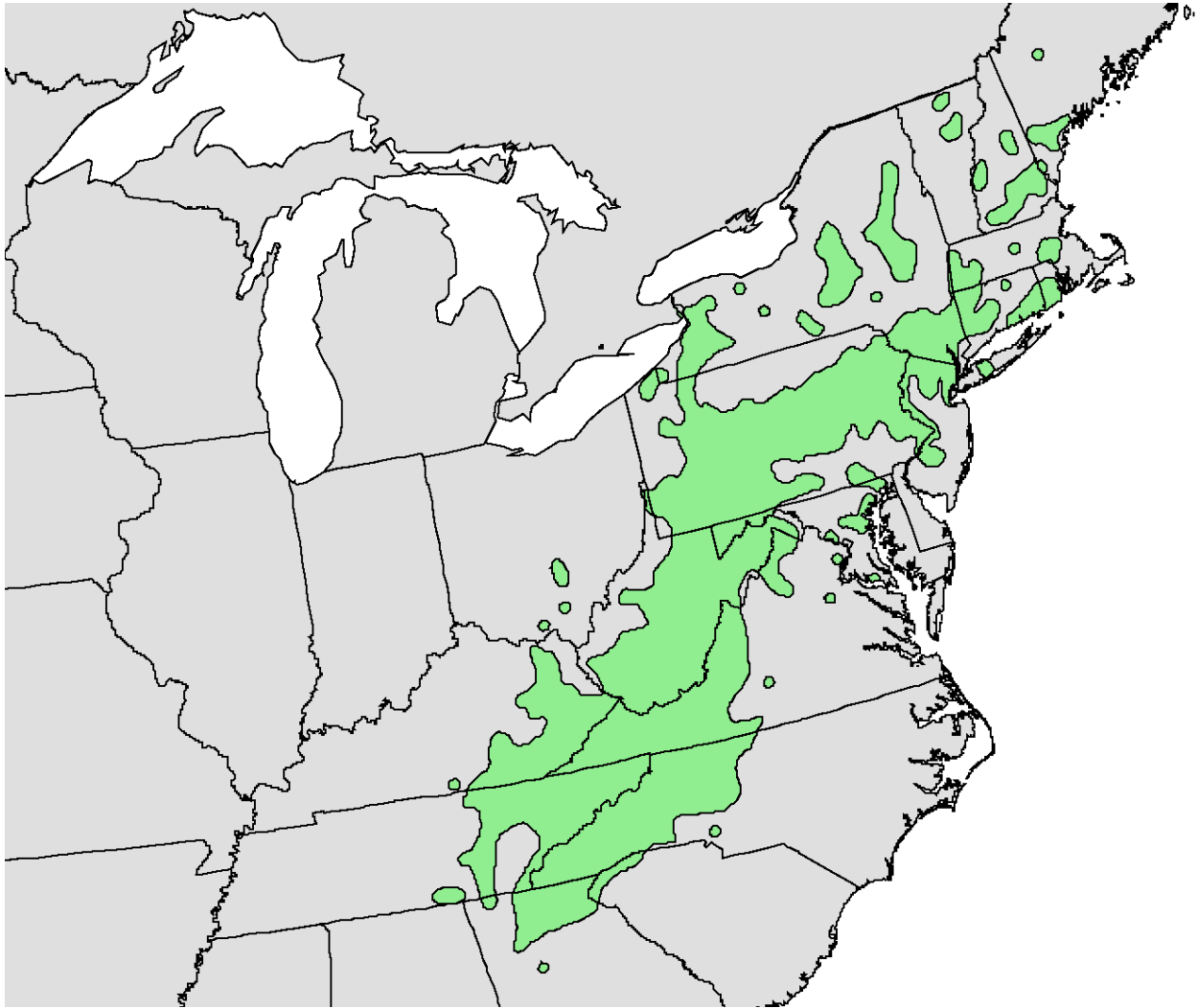


Figure 1.4 Distribution of *Rhododendron maximum* L. in the United States (Little 1981).



Figure 1.5 Close-up of raceme inflorescence of *Rhododendron maximum* L.; expanding flower bud in upper right of photo (see arrow), Spruce Flats, Great Smoky Mountains National Park, 25 June 2018.



Figure 1.6 *Rhododendron maximum* L. blossom with poricidal anthers and pink stigma visible in lower right of photo; close-up view of the raceme-form inflorescence on rhododendron; Lepturinae (flower longhorn) beetle in top middle of photo, Lynn Camp, Great Smoky Mountains National Park, 10 July 2018.

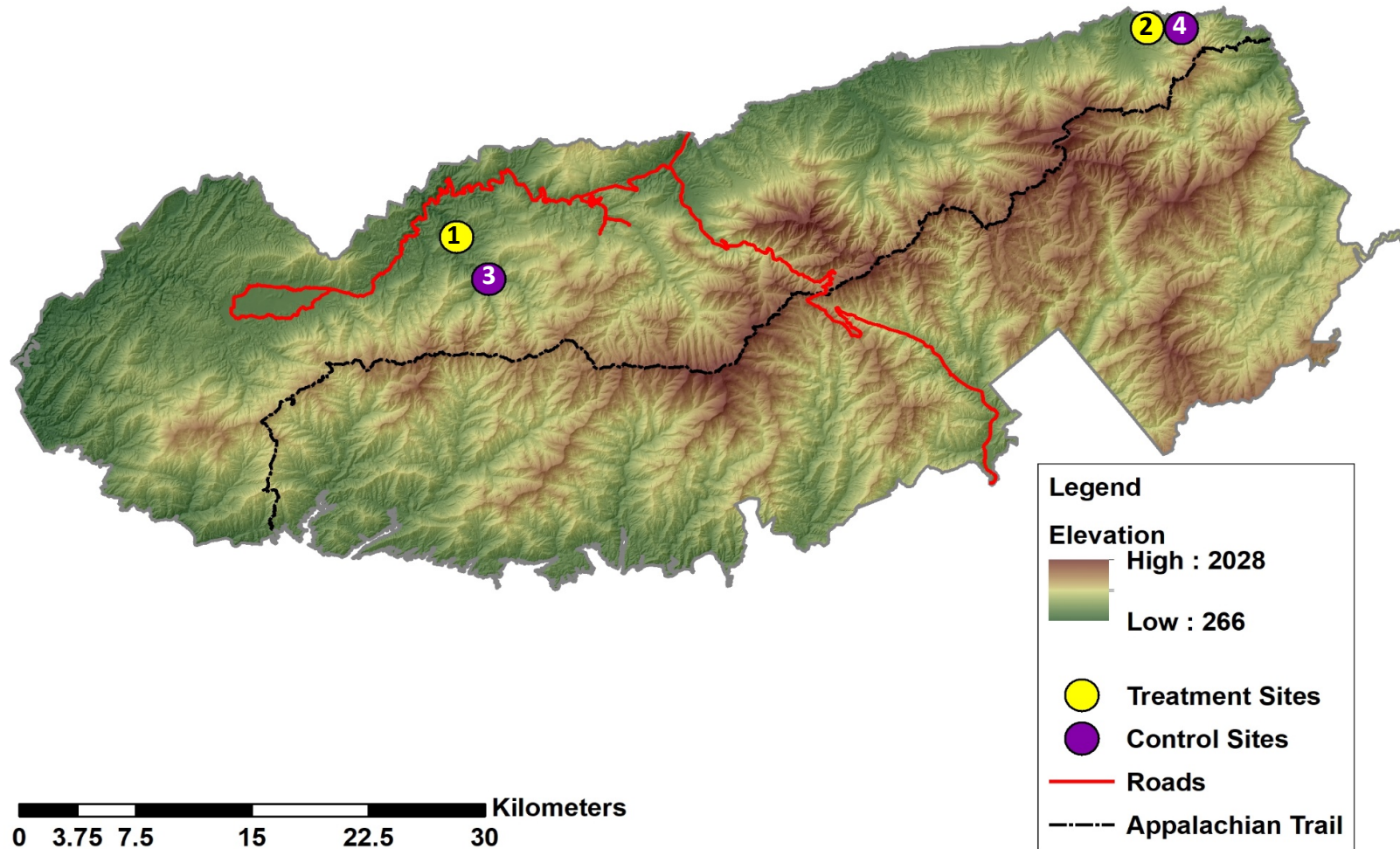


Figure 2.1 Study site locations in Great Smoky Mountains National Park (1 = Spruce Flats; 2 = Gilliland Creek; 3 = Lynn Camp; 4 = Groundhog Creek).



Figure 2.2 Treated hemlock marked with paint at time of imidacloprid treatments represent two treatment times: 1) 2014 (June) – upper faded red paint and 2) 2009 – blue paint, Gilliland Creek, Great Smoky Mountains National Park, 9 April 2018.



Figure 2.3 Flowering *Rhododendron maximum* L. located 35 cm from treated hemlock, Gilliland Creek, Great Smoky Mountains National Park, 28 June 2018.

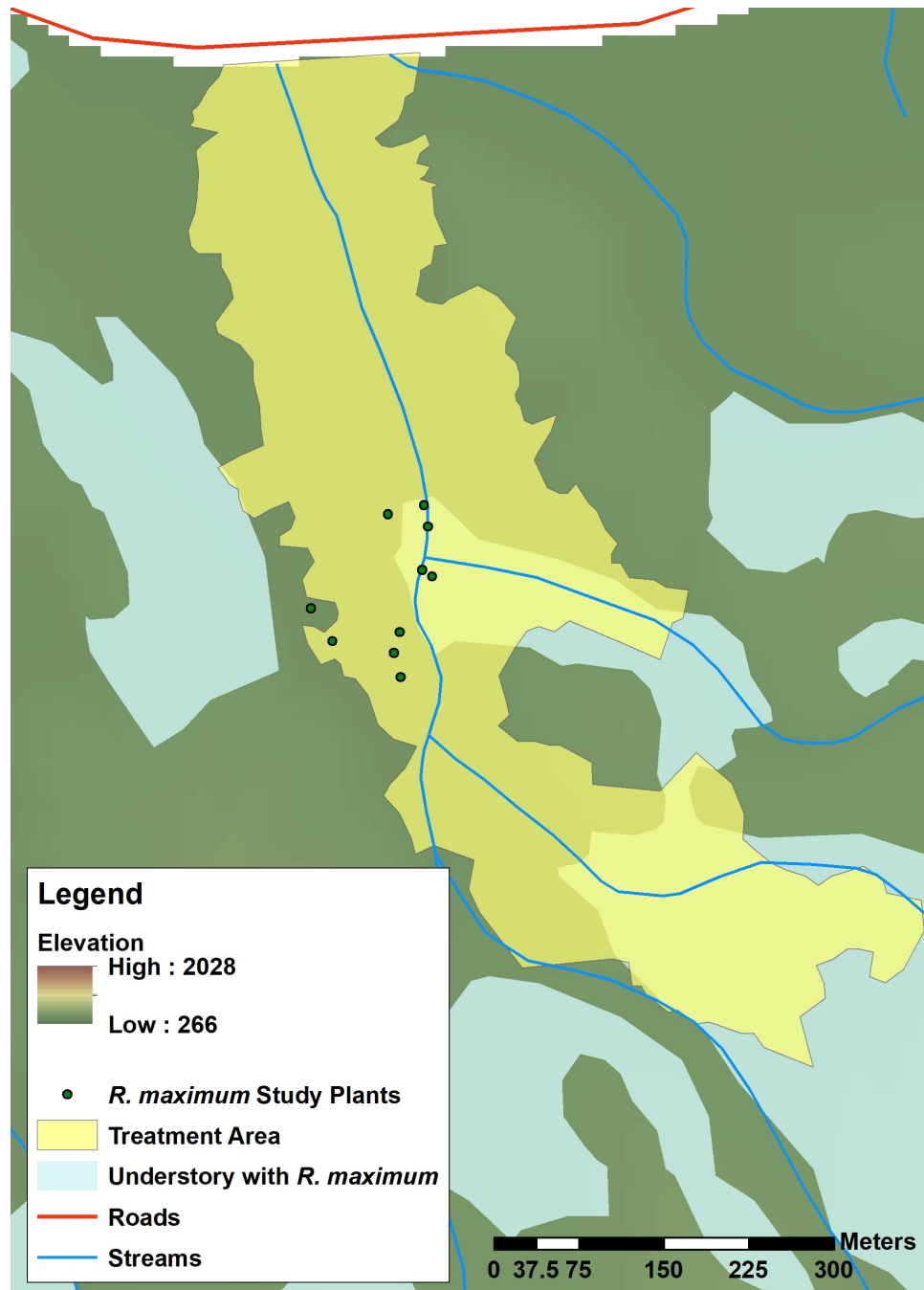


Figure 2.4 Map of Gilliland Creek area showing the area of treated hemlock (yellow shading), *Rhododendron maximum* L. understory (blue shading), and overlapping areas of hemlock and rhododendron, Great Smoky Mountains National Park.

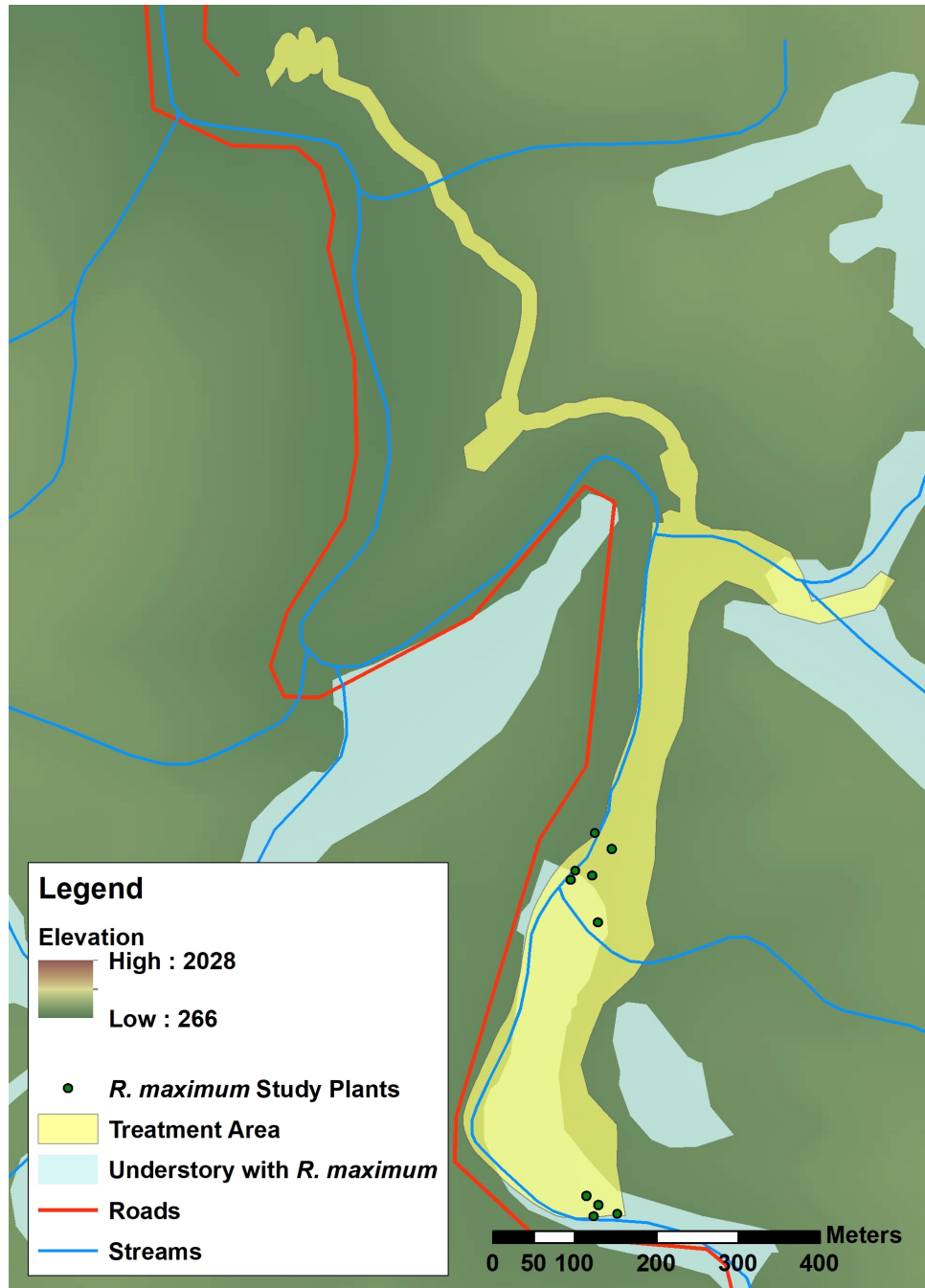


Figure 2.5 Map of Spruce Flats area showing the area of treated hemlock (yellow shading), *Rhododendron maximum* L. understory (blue shading), and overlapping areas of hemlock and rhododendron, Great Smoky Mountains National Park.

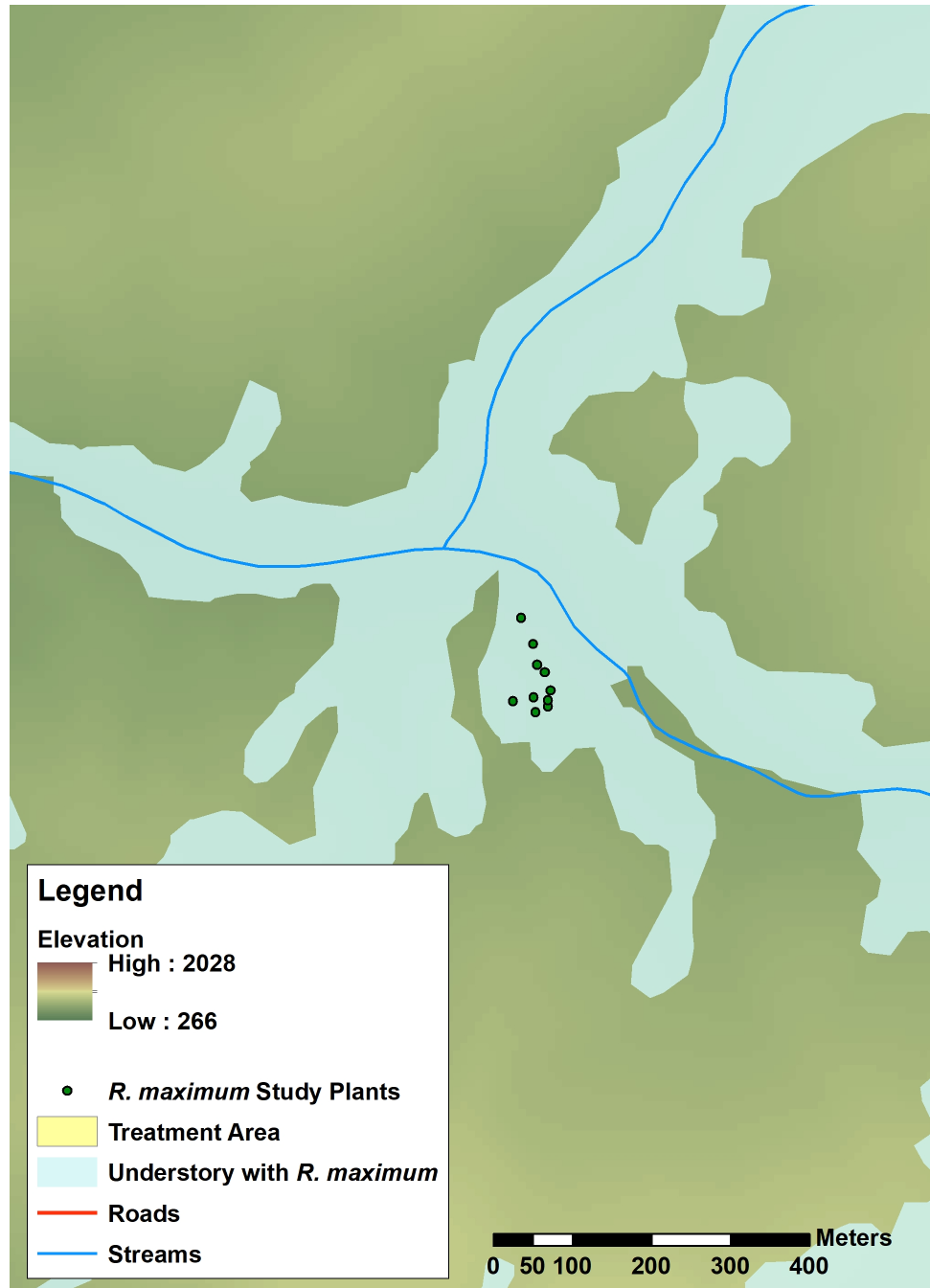


Figure 2.6 Map of Lynn Camp area showing the area of *Rhododendron maximum* L. understory (blue shading), Great Smoky Mountains National Park.

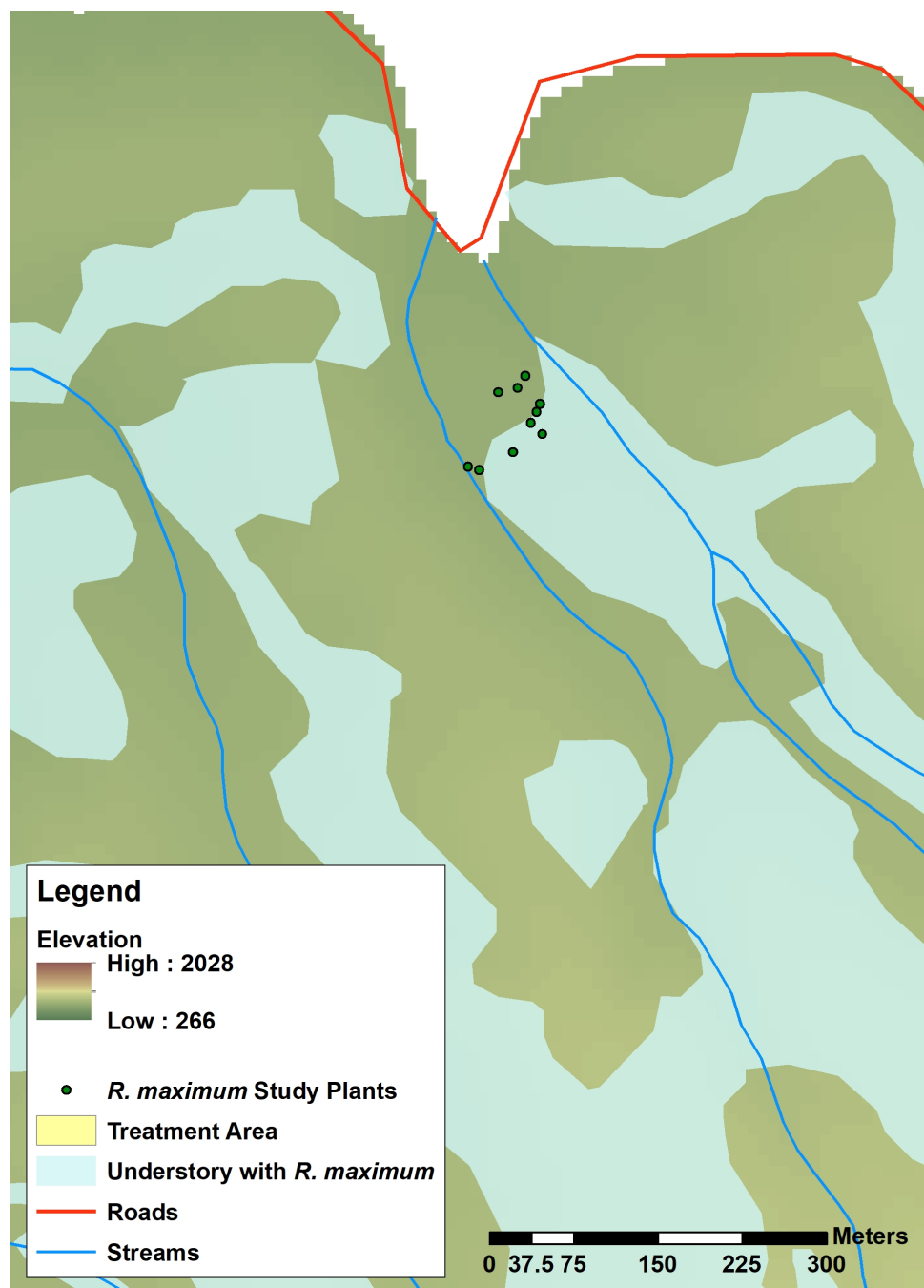


Figure 2.7 Map of Groundhog Creek area showing the area of *Rhododendron maximum* L. understory (blue shading), Great Smoky Mountains National Park.

Table 2.1 Overstory density measured using a concave spherical densiometer model C (Robert E. Lemmon, Forest Densiometers, 5733 Cornell Drive, Bartlesville, Oklahoma). Values represent average of measurements taken on four sides of each rhododendron at the four cardinal directions at tagged rhododendrons at the four study sites, Great Smoky Mountains National Park, 22 August and 7 September 2018.

Rhododendron ID Number	Overstory Density (Percent)			
	Treated Sites		Control Sites	
	Gilliland (GC)	Spruce Flats (SF)	Groundhog (GH)	Lynn Camp (LC)
01	85	83	95	89
02	80	87	89	90
03	85	90	88	92
04	70	91	92	89
05	83	82	90	90
06	86	87	92	94
07	91	90	92	84
08	90	86	92	95
09	81	77	89	95
10	83	89	94	94

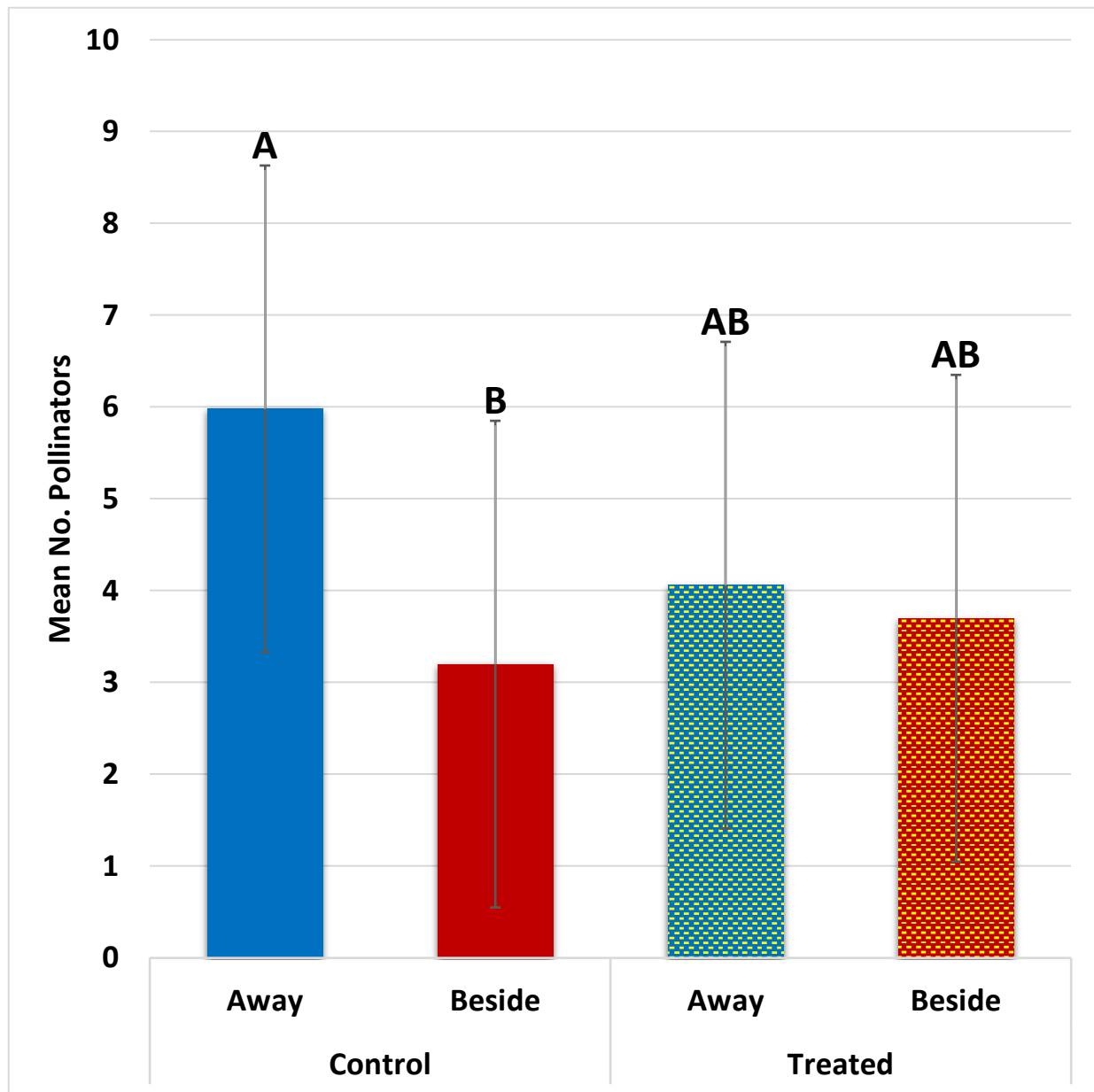


Figure 2.8 Adjusted mean ($\pm$ SE) number of pollinators on *Rhododendron maximum* L. flowers and interaction of treatment and distance from hemlock, Great Smoky Mountain National Park, 2018. (Adjusted means reflect numbers of pollinators observed during a typical 25-minute observation.)

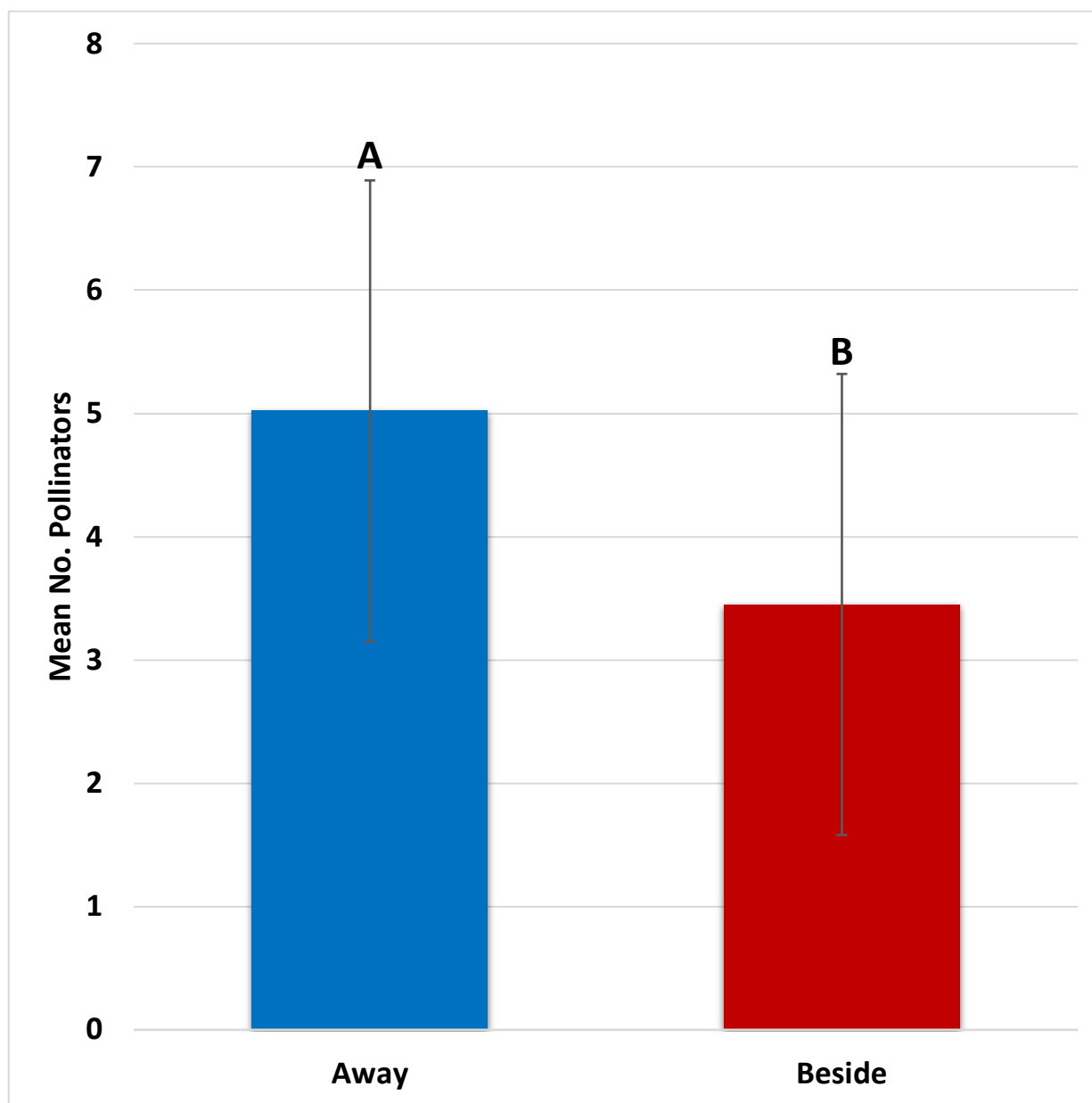


Figure 2.9 Adjusted mean ($\pm$ SE) number of pollinators observed on *Rhododendron maximum* L. flowers located away from and beside hemlocks, Great Smoky Mountains National Park, 2018. (Adjusted means reflect numbers of pollinators observed during a typical 25-minute observation.)

Visitors by Order/Family

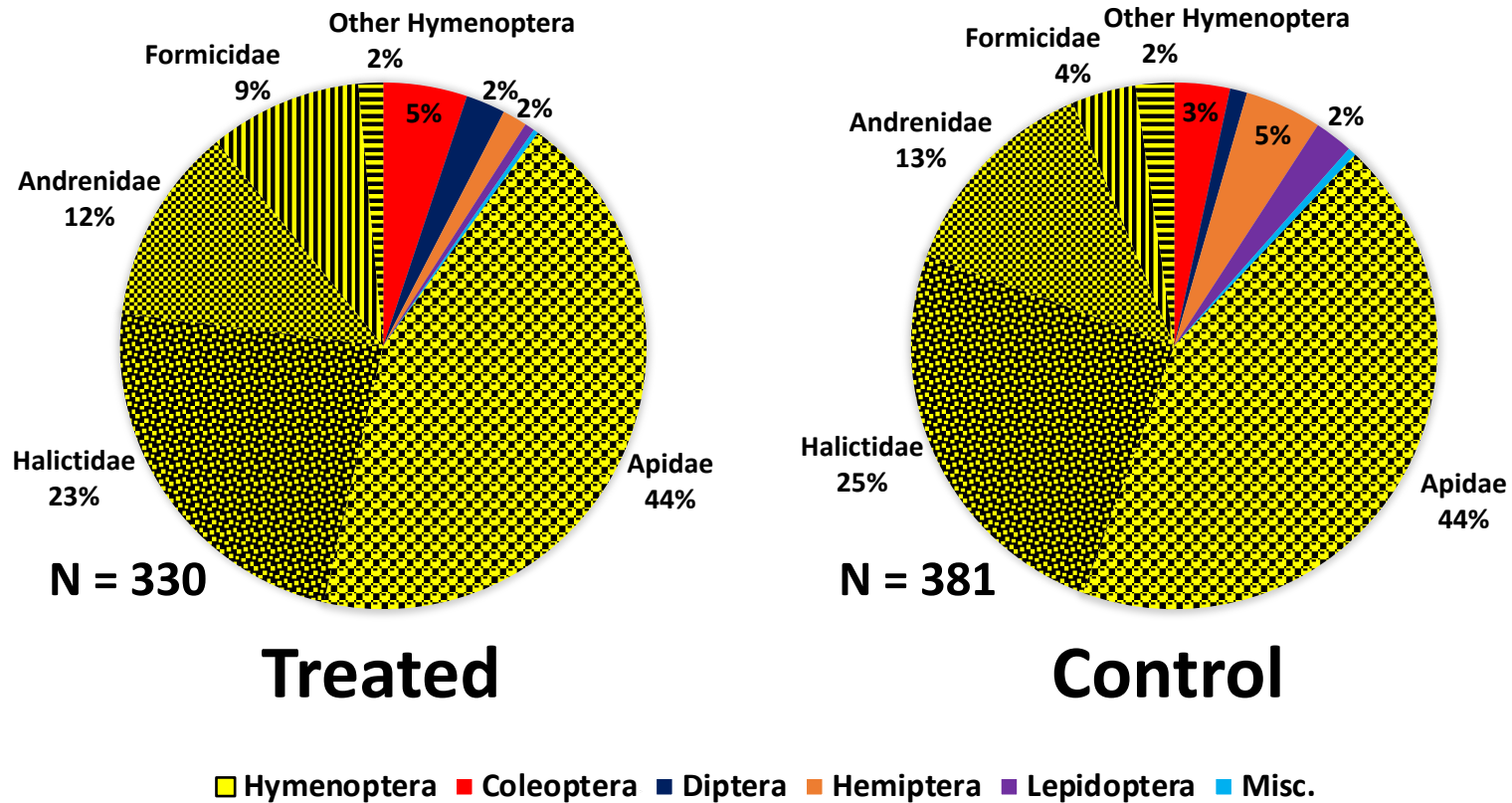


Figure 2.10 Visitors by order and by hymenopteran family observed on *Rhododendron maximum* L. flowers at treated and control sites, Great Smoky Mountains National Park, 2018. Yellow and black patterns are hymenopteran families.



Figure 3.1 Bucket used to collect seed falling from *Rhododendron maximum* L. seedpods, Great Smoky Mountains National Park.

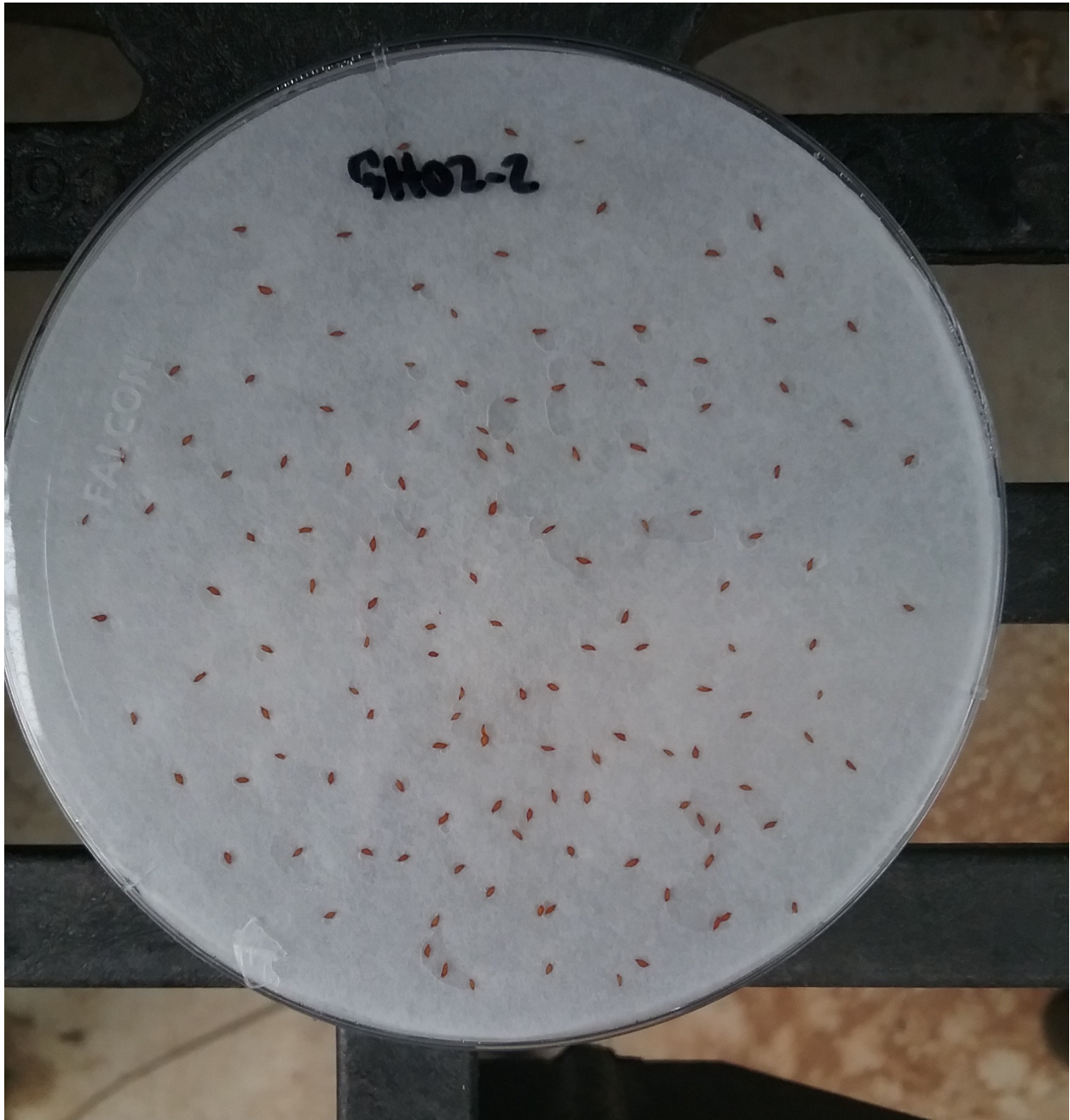


Figure 3.2 Sample of *Rhododendron maximum* L. seed placed in petri dish and sealed with parafilm for germination study.

Table 3.1 Number, weight, and percent germination of *Rhododendron maximum* L. seed from seedpods collected on tagged rhododendrons at four study sites, Great Smoky Mountains National Park, 2018.

			Seedpod Sample ^a								
			1			2			3		
Site	Treated/ Control	Rhododendron ID ^b	Number	Weight (mg)	Germ ^c (%)	Number	Weight (mg)	Germ ^c (%)	Number	Weight (mg)	Germ ^c (%)
Gilliland	Treated	GC01	240	15.5	38	115	8.1	63	237	17.7	28
Gilliland	Treated	GC02	341	25.7	69	644	45.0	19	219	19.1	54
Gilliland	Treated	GC03	86	7.7	46	108	10.7	70	512	34.6	30
Gilliland	Treated	GC04	321	29.6	34	196	17.0	31	245	19.2	22
Gilliland	Treated	GC05	25	2.8	31	12	1.2	50	35	2.8	13
Gilliland	Treated	GC06	212	15.7	54	249	17.6	64	143	11.0	60
Gilliland	Treated	GC07	461	35.6	56	420	29.9	51	234	19.8	44
Gilliland	Treated	GC08	102	8.7	69	213	15.9	64	396	29.8	86
Gilliland	Treated	GC09	125	11.4	68	248	20.1	48	321	23.0	54
Gilliland	Treated	GC10	163	15.8	81	43	4.9	52	68	8.0	45
Spruce Flats	Treated	SF01	293	29.0	39	232	22.6	22	366	37.2	34
Spruce Flats	Treated	SF02	458	43.8	1	239	21.1	13	86	10.3	37
Spruce Flats	Treated	SF03	616	60.2	16	728	75.8	64	299	30.1	25
Spruce Flats	Treated	SF04	300	34.4	26	196	22.7	43	106	13.7	53
Spruce Flats	Treated	SF05	212	27.4	17	204	22.3	39	387	37.8	22
Spruce Flats	Treated	SF06	364	23.8	34	210	13.9	66	194	15.9	26
Spruce Flats	Treated	SF07	449	34.7	15	633	47.0	23	512	41.2	38
Spruce Flats	Treated	SF08	308	27.7	12	537	40.0	5	339	34.7	14
Spruce Flats	Treated	SF09	239	20.2	60	352	30.0	46	468	34.9	9
Spruce Flats	Treated	SF10	63	8.0	32	40	5.3	13	36	4.4	25

Table 3.1 (Continued)

			Seedpod Sample ^a								
			1			2			3		
Site	Treated/ Control	Rhododendron ID ^b	Number	Weight (mg)	Germ ^c (%)	Number	Weight (mg)	Germ ^c (%)	Number	Weight (mg)	Germ ^c (%)
Groundhog	Control	GH01	397	32.3	16	230	21.2	39	101	8.7	71
Groundhog	Control	GH02	442	31.7	61	432	27.7	42	189	13.7	39
Groundhog	Control	GH03	242	20.0	45	349	25.0	36	427	30.5	41
Groundhog	Control	GH04	778	47.2	63	517	33.7	21	330	16.9	70
Groundhog	Control	GH05	586	47.1	8	665	57.1	24	631	55.4	5
Groundhog	Control	GH06	168	15.7	71	468	38.5	23	160	16.7	44
Groundhog	Control	GH07	445	30.4	11	686	37.6	10	307	19.5	27
Groundhog	Control	GH08	229	15.4	9	505	34.7	18	403	24.6	2
Groundhog	Control	GH09	427	26.2	36	322	18.7	14	364	20.0	30
Groundhog	Control	GH10	591	42.9	26	318	26.4	20	371	30.0	32
Lynn Camp	Control	LC01	295	19.4	37	242	16.1	46	327	17.1	32
Lynn Camp	Control	LC02	96	5.1	60	150	10.9	24	246	14.5	35
Lynn Camp	Control	LC03	281	23.6	27	249	16.8	24	386	25.8	3
Lynn Camp	Control	LC04	309	22.7	5	72	7.3	39	202	16.0	24
Lynn Camp	Control	LC05	330	18.8	2	172	10.8	17	70	4.1	21
Lynn Camp	Control	LC06	157	10.3	63	192	10.6	22	399	24.7	16
Lynn Camp	Control	LC07	188	15.9	40	258	19.1	24	355	29.6	5
Lynn Camp	Control	LC08	96	9.9	45	637	39.4	38	242	21.9	35
Lynn Camp	Control	LC09	533	33.0	6	526	30.5	27	251	17.0	21
Lynn Camp	Control	LC10	363	30.4	29	275	24.4	13	501	40.8	7

^a Seedpod sample was all seed from oblong woody seed capsule, pods ~1 cm long with five boat-shaped valves containing the seed.

^b Rhododendron ID: first two characters identify site and last two indicate which of the 10 tagged specimens produced seedpod (GC = Gilliland Creek, SF = Spruce Flats, GH = Groundhog, LC = Lynn Camp).

^c Germ = Percent germination; one-third of seed sample on moist filter paper in petri dish in greenhouse under 47% shade cloth.

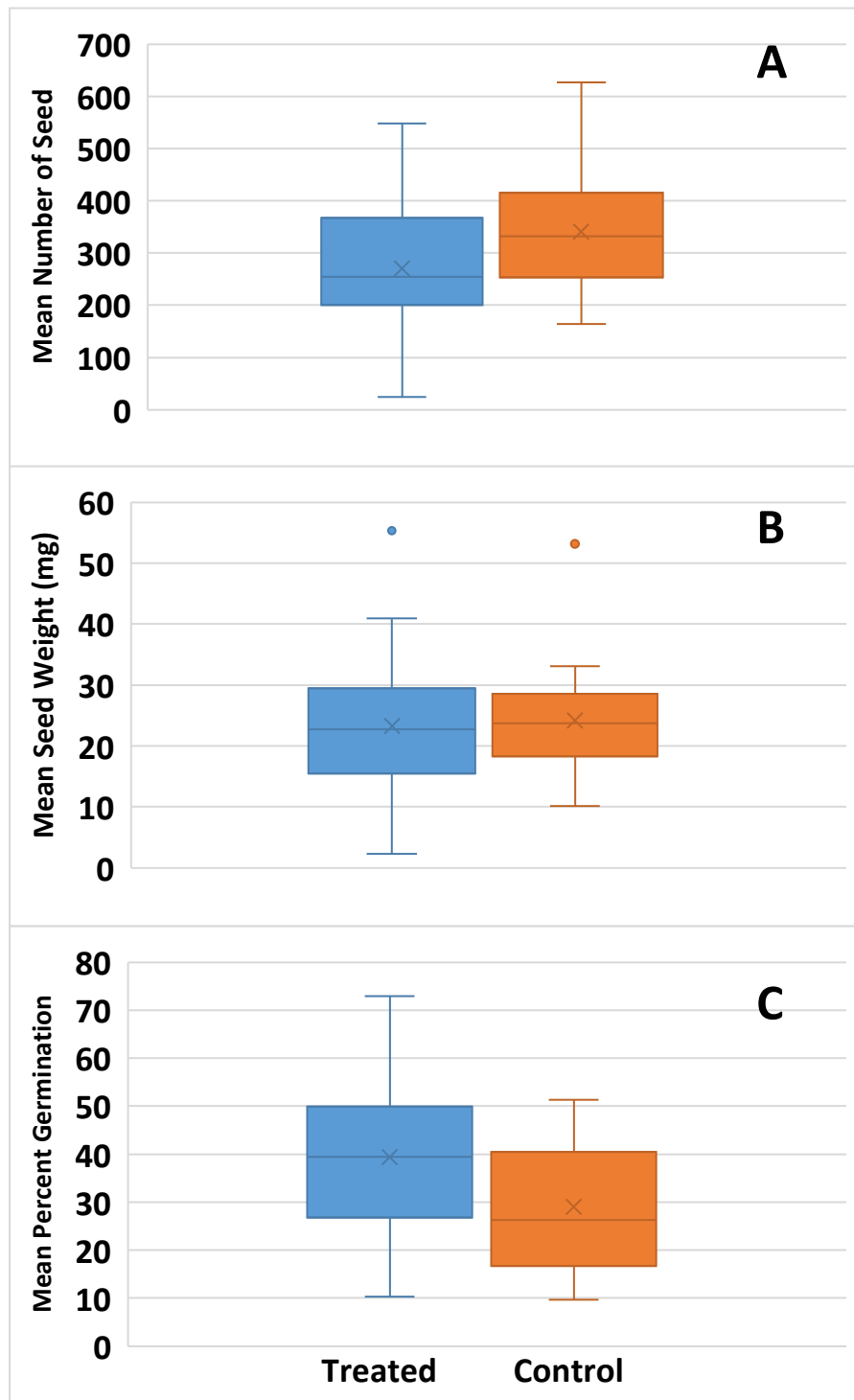


Figure 3.3 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed in *Rhododendron maximum* L. seedpods from hemlock treated and untreated control areas, Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean.

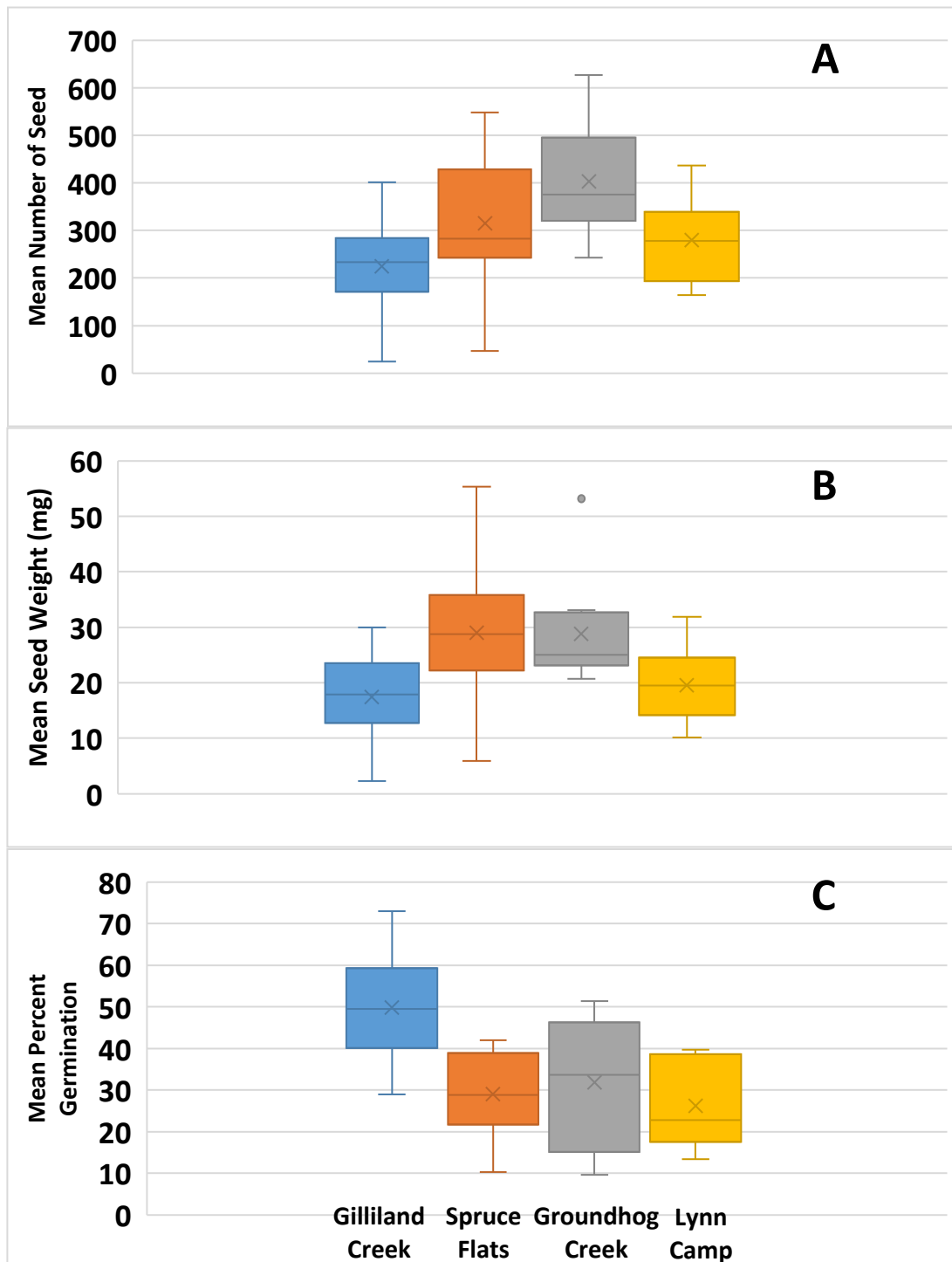


Figure 3.4 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed in *Rhododendron maximum* L. seedpods at four study sites in Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean.

Table 3.2 Number, weight, and percent germination of bucket-collected *Rhododendron maximum* L. seedfall; and cumulative number of pollinators and time spent observing with the average rate of pollinator visitation at flowers of tagged rhododendrons at the four study sites over the five-week flowering period, Great Smoky Mountains National Park, 2018.

Site	Treated/ Control	Rhododendron ID ^a	Number	Weight (mg)	Germination ^b (%)	Number of Pollinators	Time Spent ^c (minutes)	Pollinators/Hr ^d
Gilliland	Treated	GC01	2	0.2	50	1	95	0.6
Gilliland	Treated	GC02	1	0.1	0	1	115	0.5
Gilliland	Treated	GC03	16	1.7	56	2	115	1.0
Gilliland	Treated	GC04	1	0.1	0	2	115	1.0
Gilliland	Treated	GC05	58	4.5	53	0	35	0.0
Gilliland	Treated	GC06	1	0.1	0	0	35	0.0
Gilliland	Treated	GC07	15	1.4	7	0	85	0.0
Gilliland	Treated	GC08	1	0.1	0	10	135	4.4
Gilliland	Treated	GC09	59	5.6	32	0	95	0.0
Gilliland	Treated	GC10	7	0.6	57	2	115	1.0
Spruce Flats	Treated	SF01	603	62.4	17	16	50	19.2
Spruce Flats	Treated	SF02	8	1	25	63	170	22.2
Spruce Flats	Treated	SF03	6	0.6	17	17	90	11.3
Spruce Flats	Treated	SF04	115	11.7	26	7	20	21.0
Spruce Flats	Treated	SF06	120	12.2	20	82	160	30.8
Spruce Flats	Treated	SF07	48	4.2	75	3	50	3.6
Spruce Flats	Treated	SF08	268	24.5	23	50	150	20.0
Spruce Flats	Treated	SF09	150	12.3	27	23	140	9.9
Spruce Flats	Treated	SF10	32	3	36	3	40	4.5
Groundhog	Control	GH01	43	3.7	38	9	110	4.9
Groundhog	Control	GH02	10	1	10	6	130	2.8
Groundhog	Control	GH03	31	2.2	23	23	150	9.2
Groundhog	Control	GH04	27	1.9	15	3	150	1.2

Table 3.2 (Continued)

Site	Treated/ Control	Rhododendron ID ^a	Number	Weight (mg)	Germination ^b (%)	Number of Pollinators	Time Spent ^c (minutes)	Pollinators/Hr ^d
Groundhog	Control	GH05	11	0.9	73	8	80	6.0
Groundhog	Control	GH06	5	0.5	40	5	120	2.5
Groundhog	Control	GH07	30	1.9	82	41	120	20.5
Groundhog	Control	GH08	24	1.7	50	2	70	1.7
Groundhog	Control	GH09	26	1.7	88	22	140	9.4
Groundhog	Control	GH10	34	3	24	20	120	10.0
Lynn Camp	Control	LC01	24	1.7	13	0	40	0.0
Lynn Camp	Control	LC02	134	9.9	25	25	80	18.8
Lynn Camp	Control	LC03	90	7.2	39	13	120	6.5
Lynn Camp	Control	LC04	117	7.4	41	35	140	15.0
Lynn Camp	Control	LC05	23	1.6	74	5	70	4.3
Lynn Camp	Control	LC06	148	9.2	47	8	40	12.0
Lynn Camp	Control	LC07	88	6.7	45	35	130	16.2
Lynn Camp	Control	LC08	135	9.9	38	7	50	8.4
Lynn Camp	Control	LC09	96	6.5	67	52	120	26.0
Lynn Camp	Control	LC10	316	22.3	58	13	60	13.0

^a Rhododendron ID: first two characters identify site and last two indicate which of the 10 tagged rhododendrons were collected (GC = Gilliland Creek, SF = Spruce Flats, GH = Groundhog, LC = Lynn Camp).

^b Germ = Percent germination; one-third of seed sample on moist filter paper in petri dish in greenhouse under 47% shade cloth.

^c Total time spent observing flowers of tagged rhododendron over the five-week flowering period.

^d Rate of pollinators observed at flowers in an average hour based on total pollinators observed in the total time spent.

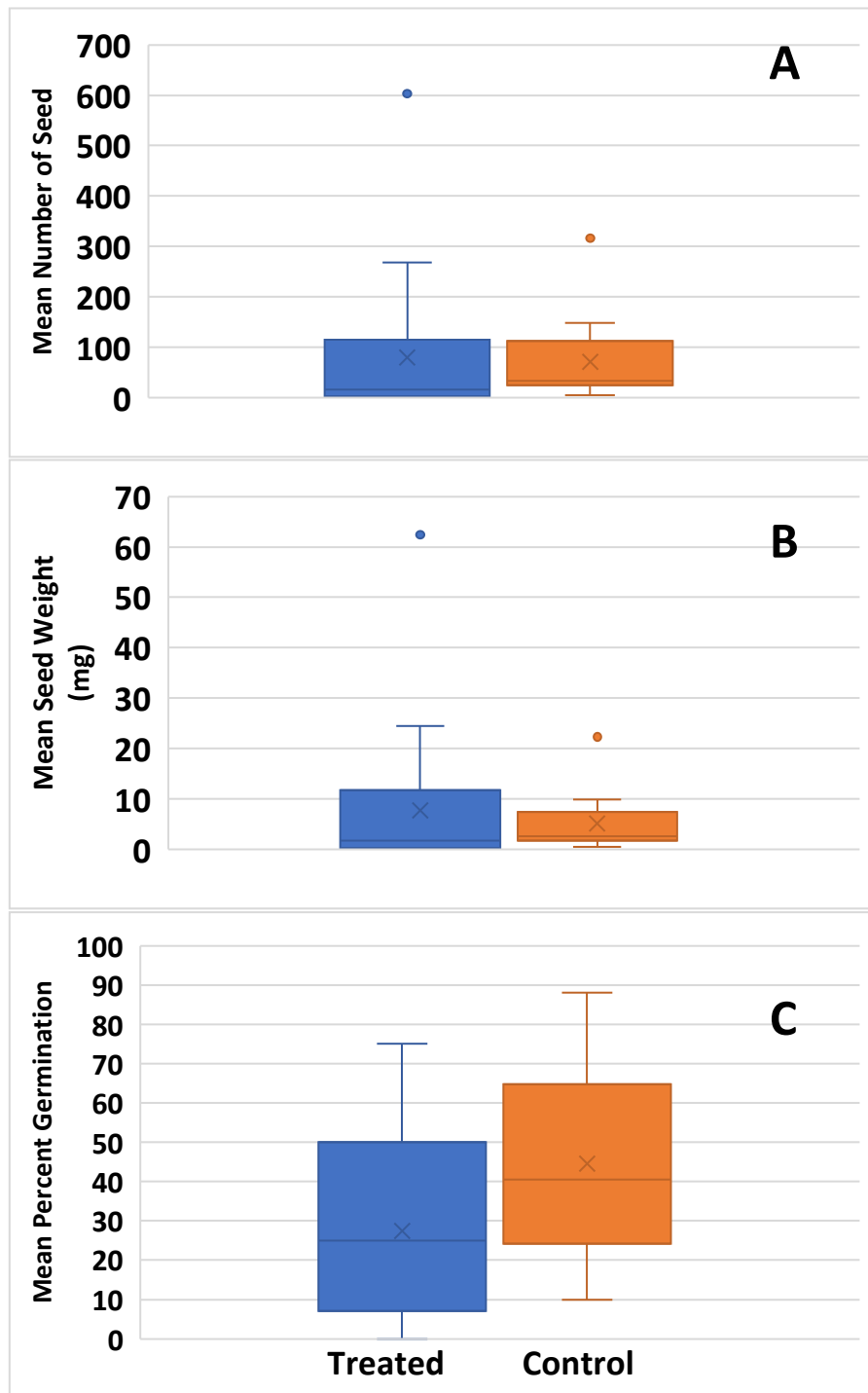


Figure 3.5 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed collected in buckets under *Rhododendron maximum* L. from hemlock treated and untreated control areas, Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean.

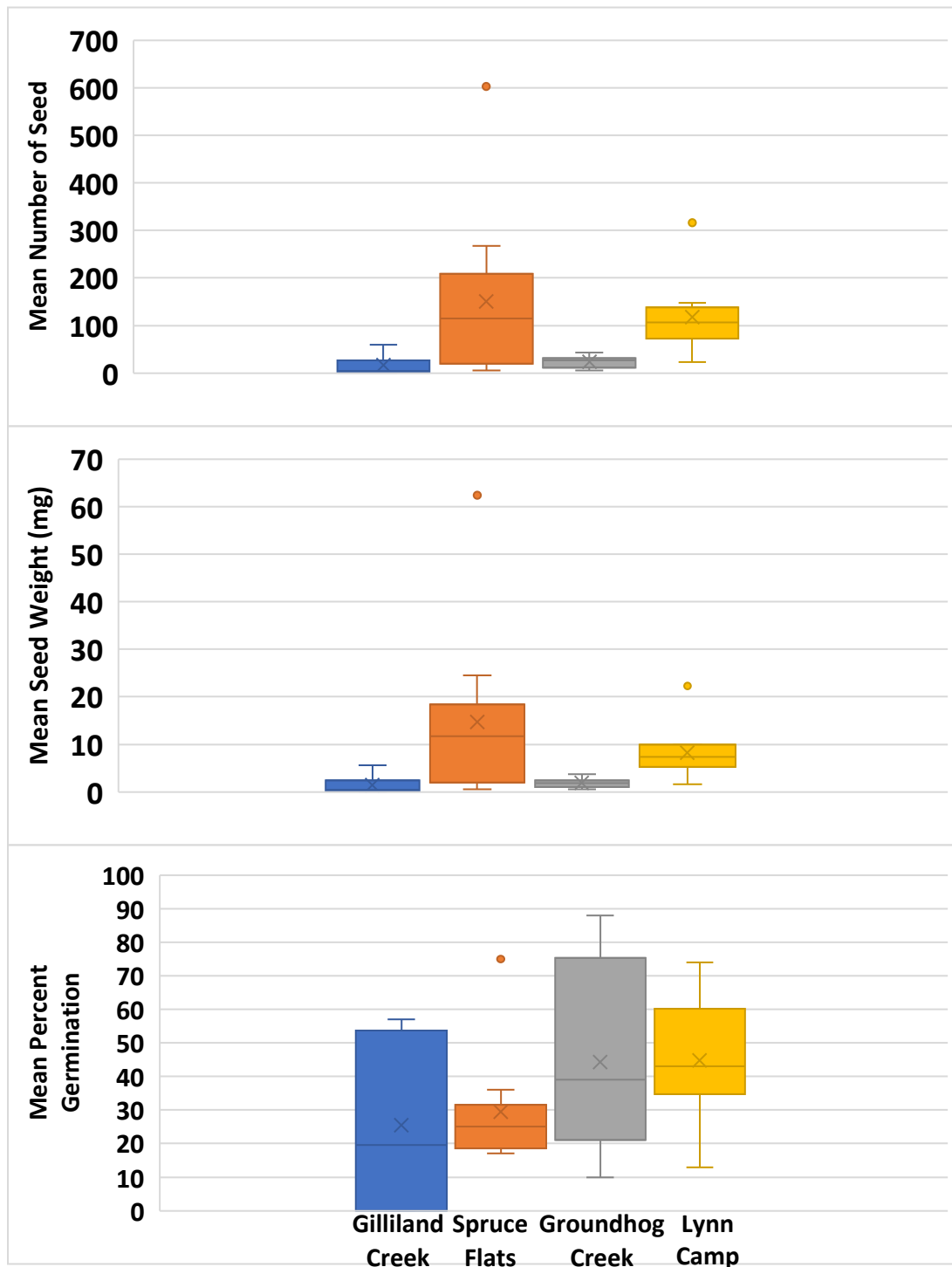


Figure 3.6 A) Mean number of seed, B) mean seed weight, and C) mean percent germination of seed collected in buckets under *Rhododendron maximum* L. at four study sites in Great Smoky Mountains National Park, 2018. Box & whisker plot -- midline inside box represents median, box represents interquartile range (IQR) comprised of 2nd and 3rd quartiles, whiskers delineate lower and upper quartiles, outliers are data points more than 1.5 X IQR below or above IQR, and X represents mean.



Figure 3.7 Green mold growth in seed germination sample.

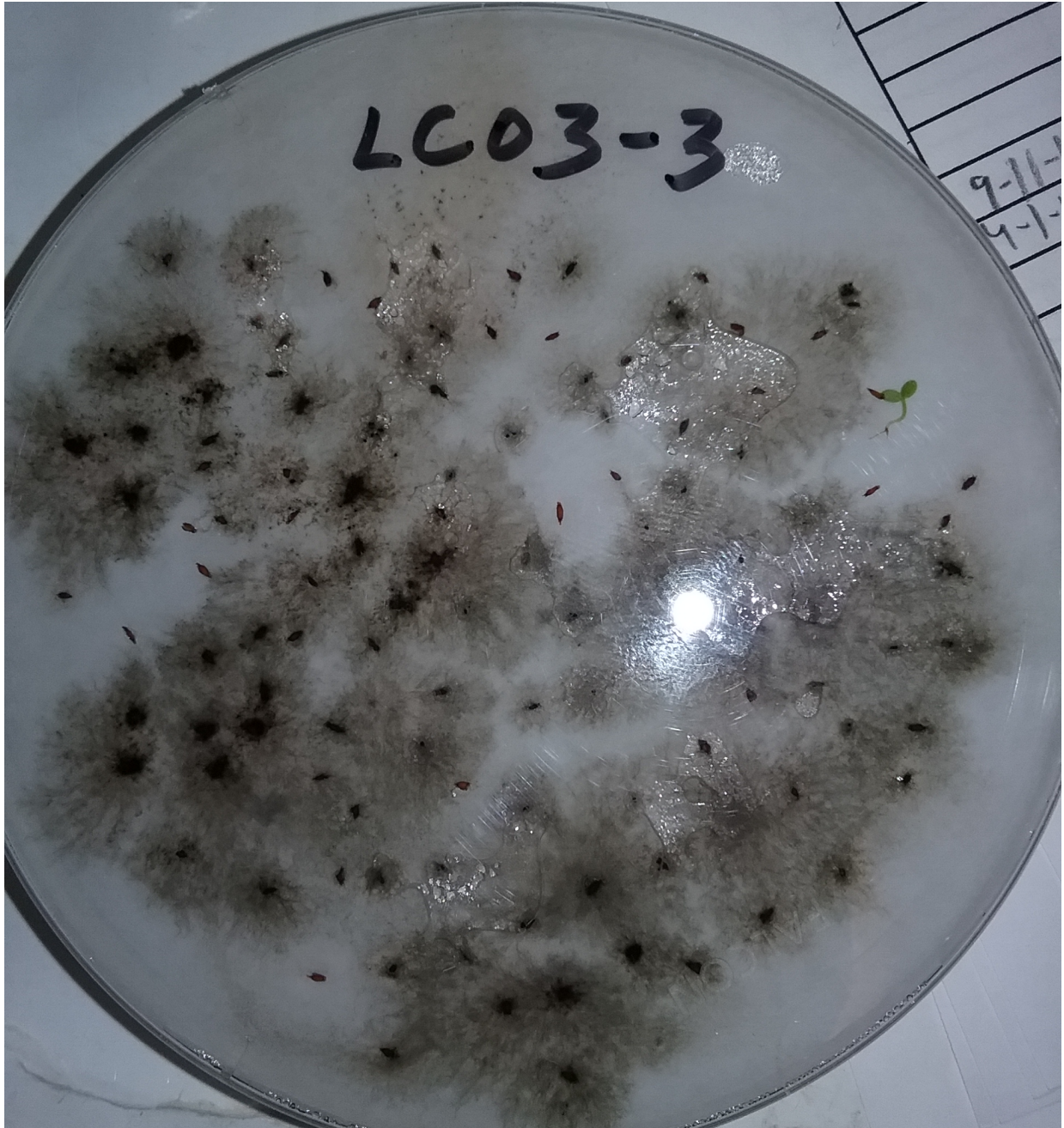


Figure 3.8 Black mold growth in seed germination sample.

Table 3.3 Average seed number per seedpod, cumulative number of pollinators and time spent observing flowers of tagged *Rhododendron maximum* L. at each of the four study sites over the five-week flowering period, with the average rate of pollinator visitation for each site, Great Smoky Mountains National Park, 2018.

Site	Average Seed Number/Pod ^a	Number of Pollinators ^b	Time Spent ^c (Hours:Minutes)	Pollinators/Hour ^d
Gilliland	224	18	15:30	1.2
Spruce Flats	316	267	15:00	17.8
Groundhog	403	139	19:50	7.0
Lynn Camp	280	193	14:10	13.6

^a Average number of seeds per seedpod for all 30 seedpods sampled at site.

^b Total number of pollinators observed visiting tagged rhododendron flowers at site.

^c Total time spent observing flowers of tagged rhododendron at the site over the five-week flowering period.

^d Rate of pollinators observed at flowers in an average hour based on total pollinators observed in the total time spent.

Table 3.4 Pollinator visitation rates by orders and families based on cumulative time observing flowers of tagged *Rhododendron maximum* L. at each of the four study sites over the five-week flowering period and cumulative visitors observed, and average seedpod seed number at each tagged rhododendron, Great Smoky Mountains National Park, 2018.

Rhododendron ID ^a	Average Seed Number/Pod ^b	Pollinator Group Visitation Rates Per Hour ^c					
		Coleoptera	Hymenoptera	Andrenidae	Apidae	Halictidae	Apidae plus Andrenidae
GC01	197	0.0	0.7	0.7	0.0	0.0	0.7
GC02	401	0.0	0.5	0.0	0.5	0.0	0.5
GC03	235	0.0	1.0	0.5	0.5	0.0	1.0
GC04	254	0.5	0.5	0.0	0.0	0.5	0.0
GC05	24	0.0	0.0	0.0	0.0	0.0	0.0
GC06	201	0.0	0.0	0.0	0.0	0.0	0.0
GC07	372	0.0	0.0	0.0	0.0	0.0	0.0
GC08	237	0.4	4.0	1.3	0.4	2.2	1.8
GC09	231	0.0	0.0	0.0	0.0	0.0	0.0
GC10	91	0.0	0.5	0.0	0.0	0.5	0.0
SF01	297	1.2	16.8	0.0	10.8	6.0	10.8
SF02	261	0.0	21.9	1.8	13.4	6.7	15.2
SF03	548	1.3	10.0	0.0	7.3	2.7	7.3
SF04	201	3.0	18.0	0.0	6.0	12.0	6.0
SF05	268	2.0	4.0	0.0	4.0	0.0	4.0
SF06	256	1.9	28.5	7.1	16.5	4.9	23.6
SF07	531	2.4	1.2	0.0	1.2	0.0	1.2
SF08	395	0.4	18.8	2.8	10.0	6.0	12.8
SF09	353	0.0	8.6	1.7	2.6	4.3	4.3
SF10	46	0.0	4.5	0.0	4.5	0.0	4.5
GH01	243	0.0	4.9	1.1	1.1	2.7	2.2
GH02	354	0.0	2.8	0.9	0.9	0.9	1.8
GH03	339	0.4	8.8	2.0	2.0	4.8	4.0

Table 3.4 (Continued)

Rhododendron ID ^a	Average Seed Number/Pod ^b	Pollinator Group Visitation Rates Per Hour ^c					
		Coleoptera	Hymenoptera	Andrenidae	Apidae	Halictidae	Apidae plus Andrenidae
GH04	542	0.0	1.2	0.4	0.8	0.0	1.2
GH05	627	0.0	6.0	0.8	1.5	3.8	2.3
GH06	265	0.0	2.0	0.5	1.5	0.0	2.0
GH07	479	1.0	18.5	4.5	9.5	4.5	14.0
GH08	379	1.7	0.0	0.0	0.0	0.0	0.0
GH09	371	0.0	9.4	2.1	6.4	0.9	8.6
GH10	427	0.0	10.0	4.0	4.0	2.0	8.0
LC01	288	0.0	0.0	0.0	0.0	0.0	0.0
LC02	164	1.5	17.3	2.3	11.3	3.8	13.5
LC03	305	0.0	6.5	0.0	2.5	4.0	2.5
LC04	194	0.0	13.7	1.3	8.1	4.3	9.4
LC05	191	0.0	4.3	0.0	2.6	1.7	2.6
LC06	249	1.5	10.5	0.0	7.5	3.0	7.5
LC07	267	0.0	14.3	0.9	10.6	2.8	11.5
LC08	325	0.0	8.4	0.0	1.2	7.2	1.2
LC09	437	0.5	24.5	4.0	13.0	7.5	17.0
LC10	380	1.0	12.0	0.0	10.0	2.0	10.0

^a Rhododendron ID: first two characters identify site and last two indicate which of the 10 tagged rhododendrons were collected and observed (GC = Gilliland Creek, SF = Spruce Flats, GH = Groundhog, LC = Lynn Camp).

^b Average number of seeds per seedpod for three seedpods sampled at site.

^c Rate of pollinators observed at flowers in an average hour based on total number of pollinators observed in the total time spent.

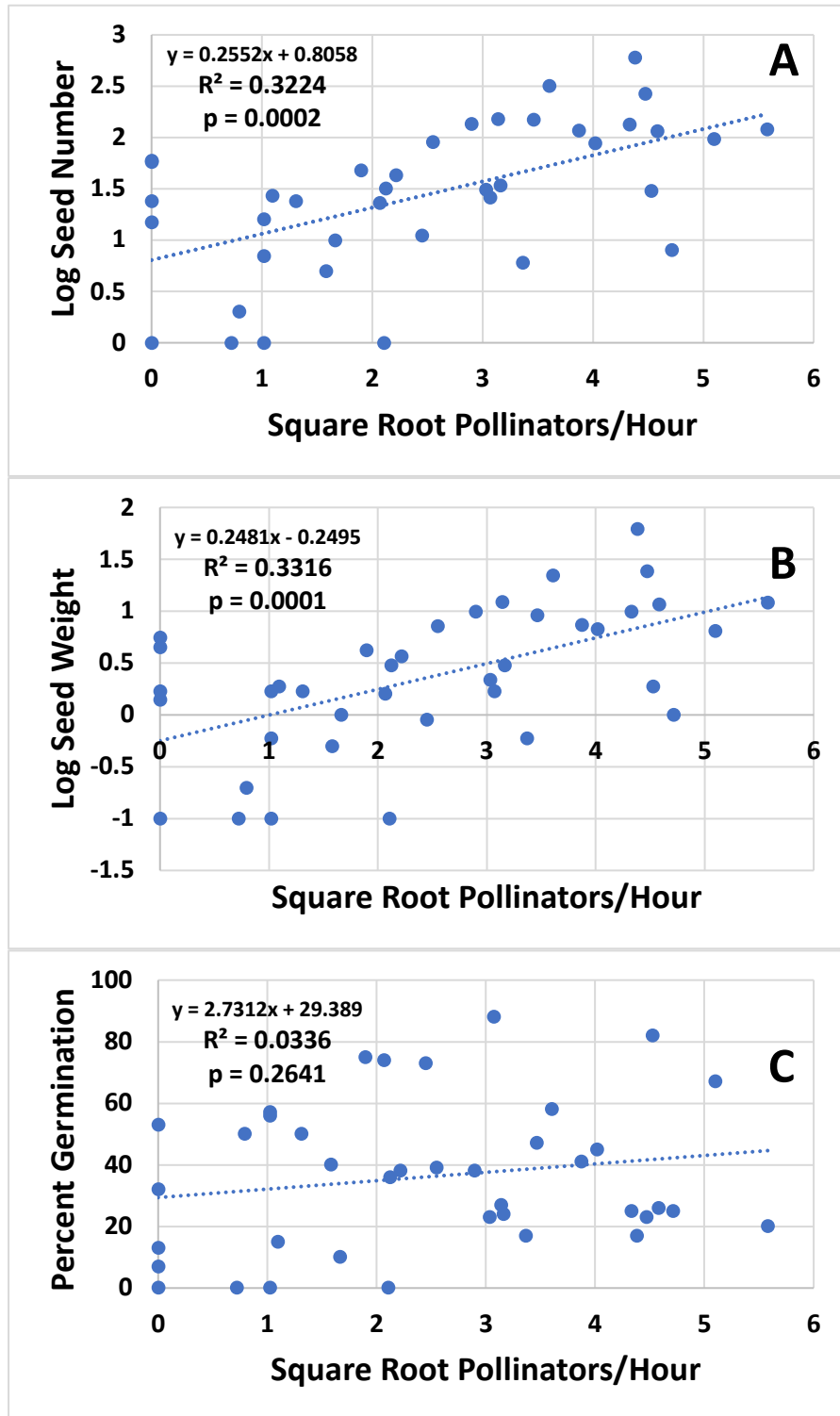


Figure 3.9 Correlations between rate of visits by pollinators per hour and A) seed number, B) seed weight, and C) percent germination of seed collected in buckets under *Rhododendron maximum* L., Great Smoky Mountains National Park, 2018.

Chapter IV

Table 4.1 Taxa observed (n = 711) and collected on *Rhododendron maximum* L. flowers at selected sites in the Great Smoky Mountains National Park, 2018.

Order	Family	Genus	Species	Site ^a and Number of Specimens ^b				Pollinator ^c	Captured ^c
				GC	GH	LC	SF		
Coleoptera	Cantharidae						1	Y	Y
Coleoptera	Cerambycidae				4	3	10	Y	N
Coleoptera	Cerambycidae	<i>Pidonia</i>	<i>aurata</i> (Horn)			1		Y	Y
Coleoptera	Cerambycidae	<i>Strangalepta</i>	<i>abbreviata</i> (Germar)	1	1	1		Y	Y
Coleoptera	Cerambycidae	<i>Typocerus</i>	<i>velutinus</i> (Olivier)				1	Y	Y
Coleoptera	Curculionidae						1	N	Y
Coleoptera	Elateridae			1			1	Y	Y
Coleoptera	Scraptiidae				1			N	Y
Coleoptera	(larva)				1			N	Y
Coleoptera						1	1	N/D	N
Diptera	Bombyliidae				1			Y	N
Diptera	Empididae					1		N	Y
Diptera	Syrphidae			1	1	1	2	Y	N
Diptera	Syrphidae	<i>Mallota</i>	<i>posticata</i> (F.)				2	Y	Y
Diptera	Syrphidae	<i>Toxomerus</i>	<i>germinatus</i> (Say)				2	Y	Y
Diptera							1	N/D	N
Hemiptera	Aphididae						1	N	N
Hemiptera	Aphididae						1	N	Y
Hemiptera	Cicadellidae				14	1		N	N
Hemiptera	Cicadellidae	<i>Graphocephala</i>	<i>fennahi</i> Young		1		2	N	Y
Hemiptera	Cicadellidae	<i>Gyponana</i>			1			N	Y

Table 4.1 (Continued)

Order	Family	Genus	Species	Site ^a and Number of Specimens ^b				Pollinator ^c	Captured ^c
				GC	GH	LC	SF		
Hemiptera	Miridae			1	1			N	Y
Hymenoptera	Andrenidae	<i>Andrena</i>	<i>cornelli</i> Viereck	3	29	12	24	Y	N
Hymenoptera	Andrenidae	<i>Andrena</i>	<i>cornelli</i> Viereck	2	5	4	11	Y	Y
Hymenoptera	Apidae	<i>Anthophora</i>	<i>abrupta</i> Say				1	Y	Y
Hymenoptera	Apidae	<i>Apis</i>	<i>mellifera</i> L.			6		Y	N
Hymenoptera	Apidae	<i>Apis</i>	<i>mellifera</i> L.			2		Y	Y
Hymenoptera	Apidae	<i>Bombus</i>		3	49	88	119	Y	N
Hymenoptera	Apidae	<i>Bombus</i>	<i>bimaculatus</i> Cresson		4		2	Y	Y
Hymenoptera	Apidae	<i>Bombus</i>	<i>impatiens</i> Cresson				2	Y	Y
Hymenoptera	Apidae	<i>Bombus</i>	<i>perplexus</i> Cresson				4	Y	Y
Hymenoptera	Apidae	<i>Bombus</i>	<i>vagans</i> Smith		5	11	13	Y	Y
Hymenoptera	Apidae	<i>Nomada</i>	<i>cressonii</i> Robertson		1			N	Y
Hymenoptera	Formicidae	Formicinae ^d			12			N	N
Hymenoptera	Formicidae	Formicinae ^d			1			N	Y
Hymenoptera	Formicidae	<i>Camponotus</i>	<i>pennsylvanicus</i> (De Geer)		1		11	N	N
Hymenoptera	Formicidae	<i>Camponotus</i>	<i>pennsylvanicus</i> (De Geer)		1		2	N	Y
Hymenoptera	Formicidae	<i>Lasius</i>		17				N	N
Hymenoptera	Formicidae	<i>Lasius</i>		1				N	Y
Hymenoptera	Halictidae			5	30	46	61	Y	N
Hymenoptera	Halictidae	<i>Augochlora</i>	<i>pura</i> (Say)	1	2	3	5	Y	Y
Hymenoptera	Halictidae	<i>Augochloropsis</i>	<i>metallica</i> (F.)	1	2			Y	Y
Hymenoptera	Halictidae	<i>Lasioglossum</i>	(<i>Dialictus</i>) ^e		5	7	4	Y	Y

Table 4.1 (Continued)

Order	Family	Genus	Species	Site ^a and Number of Specimens ^b				Pollinator ^c	Captured ^c
				GC	GH	LC	SF		
Hymenoptera	Ichneumonidae						1	N	Y
Hymenoptera	Vespidae				2	3	2	N	N
Hymenoptera	Vespidae	<i>Vespula</i>	<i>consobrina</i> Saussure			1		N	Y
Hymenoptera	Vespidae	<i>Vespula</i>	<i>maculifrons</i> Buysson		1		1	N	Y
Hymenoptera						2	1	N/D	N
Lepidoptera	Hesperiidae				1	2		Y	N
Lepidoptera	Papilionidae					4		Y	N
Lepidoptera	Papilionidae	<i>Battus</i>	<i>philenor</i> L.			1	2	Y	N
Lepidoptera	Papilionidae	<i>Battus</i>	<i>philenor</i> L.			1		Y	Y
Mecoptera				1				N	N
Neuroptera	Chrysopidae	(larva)			1			N	Y
Orthoptera	Tettigoniidae				1			N	Y
Total				38	179	202	292		

^a GC = Gilliland; GH = Groundhog; LC = Lynn Camp; SF = Spruce Flats.

^b Total number of specimens observed/collected at site.

^c Y = Yes; N = No; N/D = Not Determined (pollinator status determined by species that specialize in eating pollen or nectar).

^d Subfamily.

^e Subgenus.

Total Visitors by Order/Family

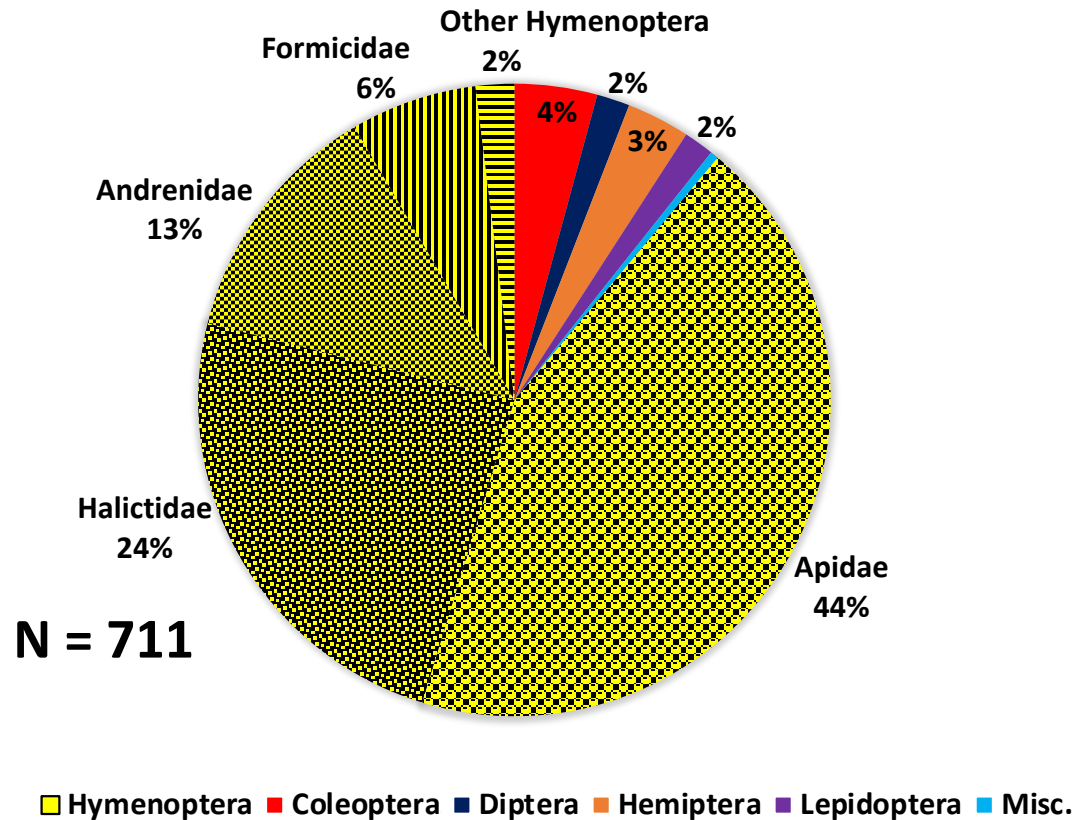


Figure 4.1 Visitors by order and by hymenopteran family observed on *Rhododendron maximum* L. flowers, Great Smoky Mountains National Park, 2018. Yellow and black patterns represent hymenopteran families.

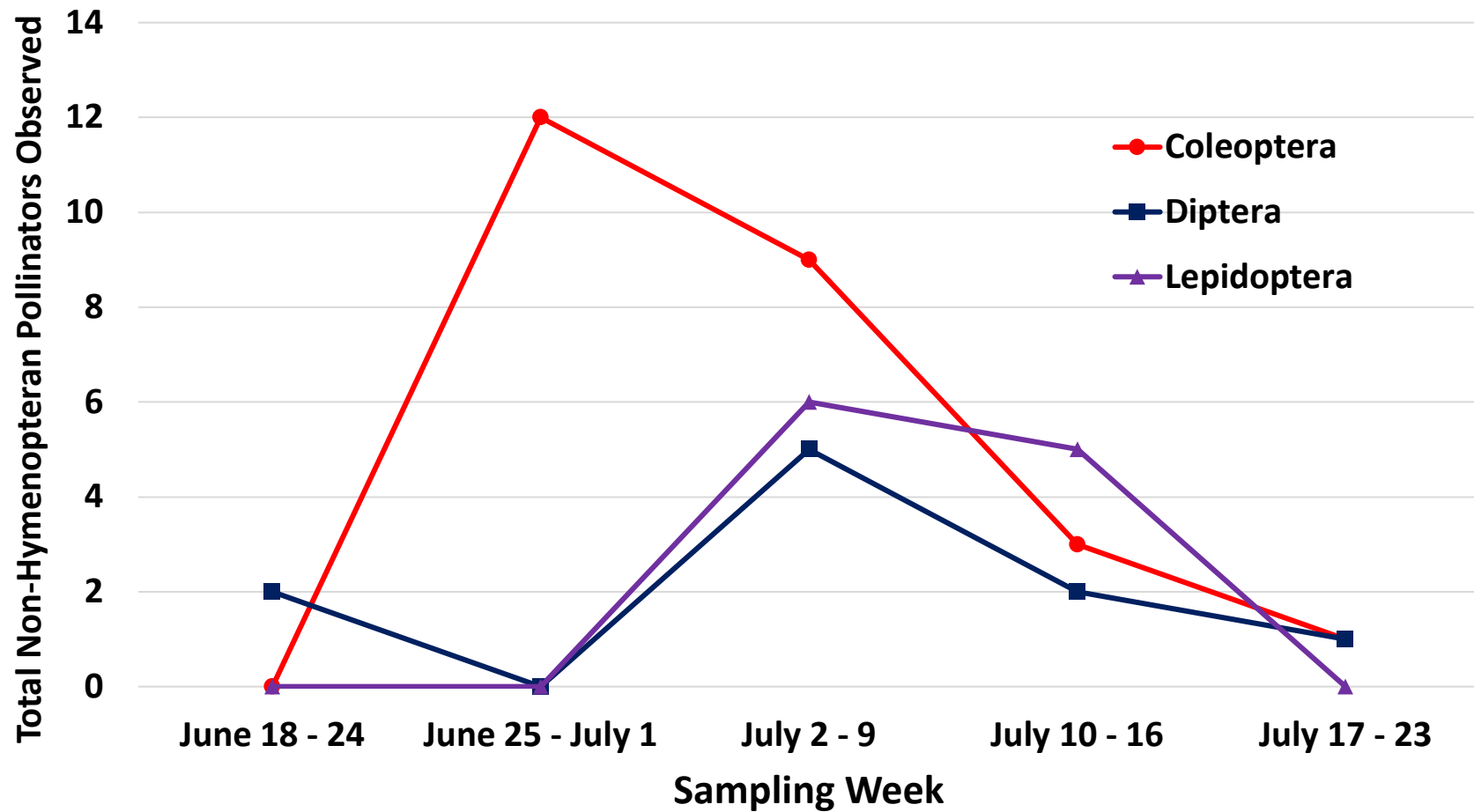


Figure 4.2 Total number of non-hymenopteran pollinators observed by week of the flowering period on *Rhododendron maximum* L. flowers, Great Smoky Mountains National Park, 2018.

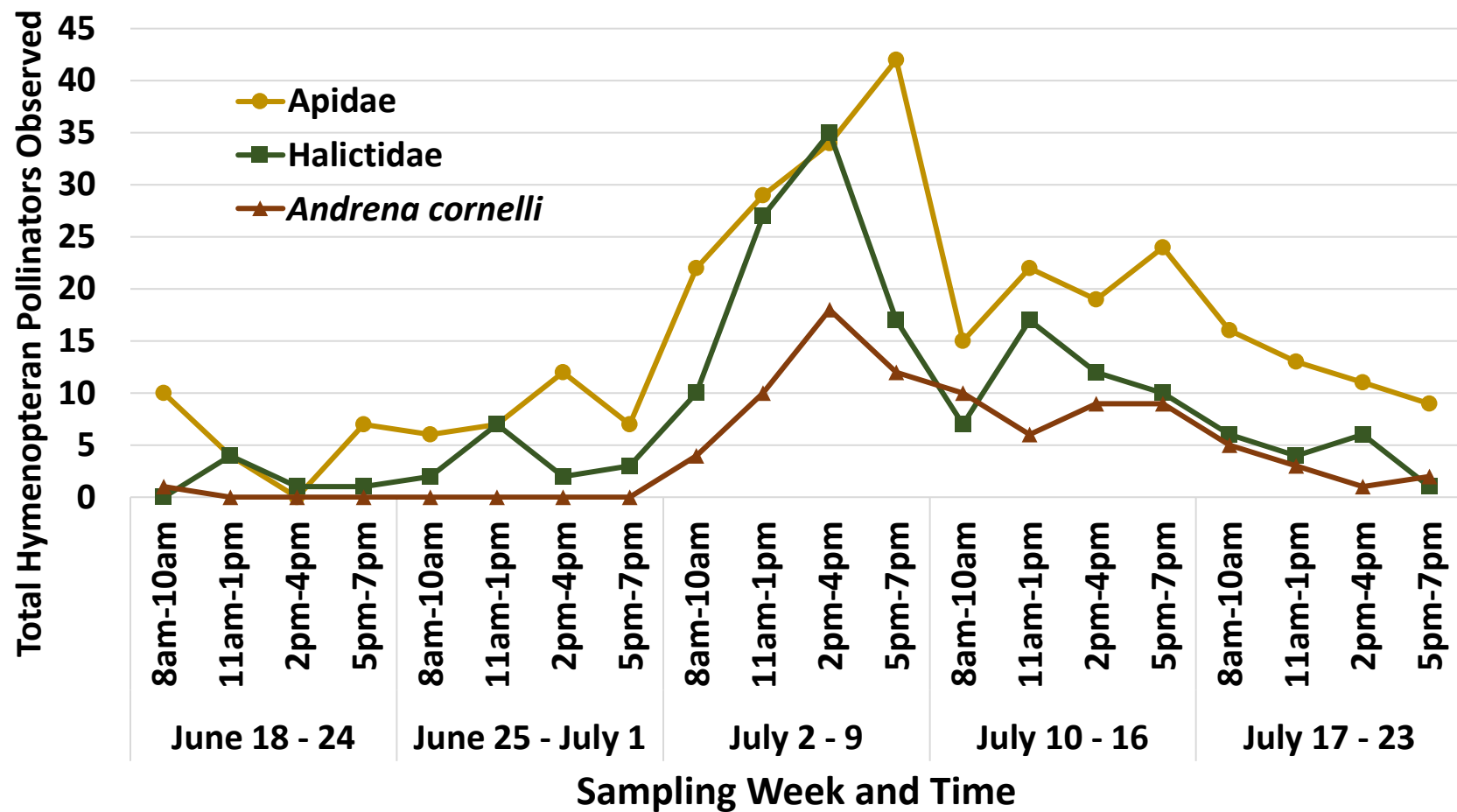


Figure 4.3 Total number of hymenopteran pollinators observed on *Rhododendron maximum* L. flowers by week of the flowering period and time of day, Great Smoky Mountains National Park, 2018.

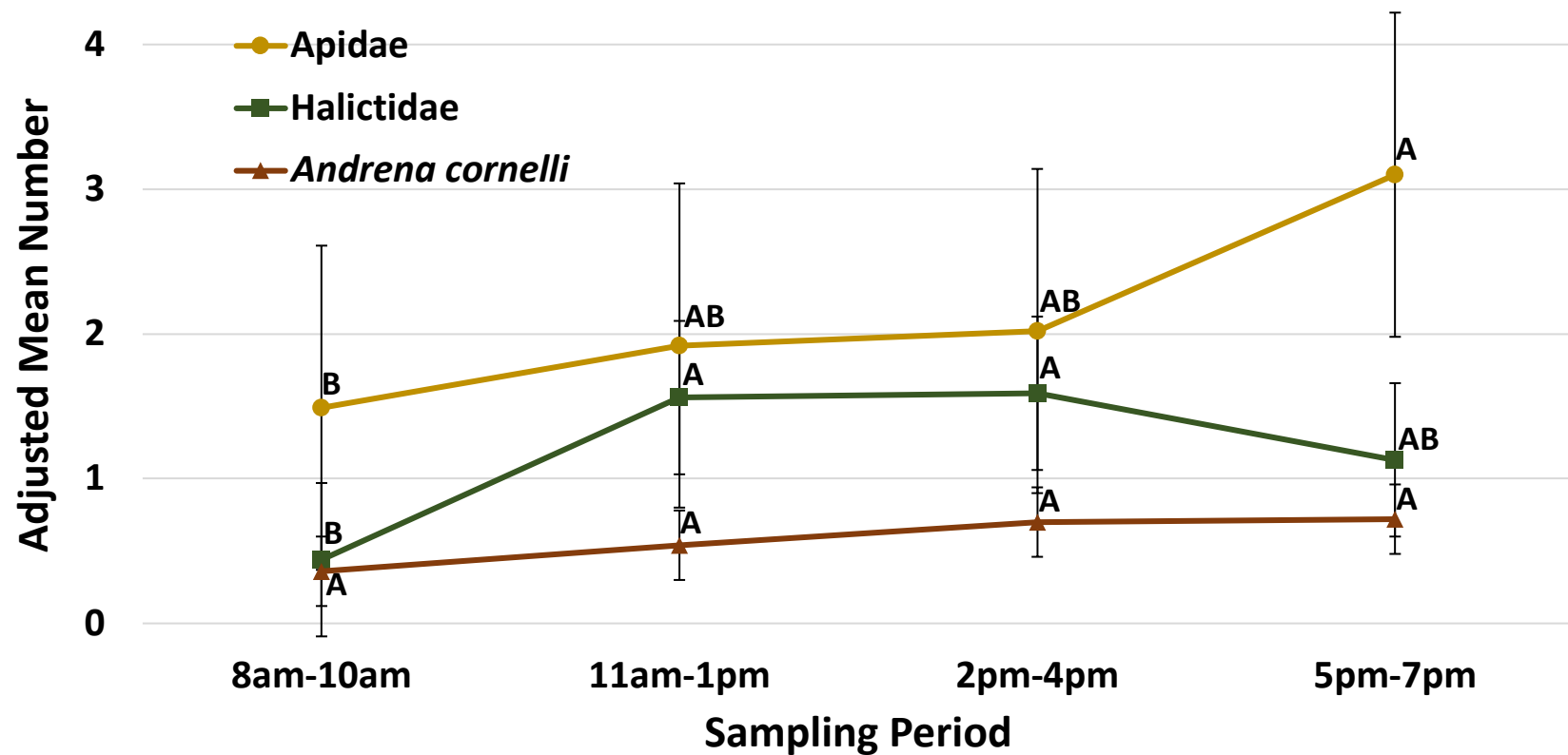


Figure 4.4 Adjusted mean number of hymenopteran pollinators observed at each sampling period (visitation rate), 25 minutes of observation, for Apidae, Halictidae, and *Andrena cornelli*; letter groups indicate significant differences ($p < 0.05$) across sampling time periods for each family or species separately, Great Smoky Mountains National Park, 2018.

Table 4.2 Diversity indices for species of pollinators collected on *Rhododendron maximum* L. flowers by site with time spent observing and cumulative number of flowers observed, Great Smoky Mountains National Park, 2018.

Site	Time Spent Observing (hr:min)	Number of Flowers	Number of Pollinators	Shannon's Diversity Index	Evenness	Species Richness
Gilliland Creek ^t	15:30	3,036	6	1.5607	0.9697	5
Groundhog Creek ^c	19:50	2,405	24	1.9298	0.9281	8
Lynn Camp Prong ^c	14:10	1,888	30	1.9128	0.8307	10
Spruce Flats ^t	15:00	2,853	49	2.2259	0.8434	14

t = Treated site.

c = Control site.

Table 4.3 Diversity indices for species of pollinators collected on *Rhododendron maximum* L. flowers by week with time spent observing and cumulative number of flowers observed, Great Smoky Mountains National Park, 2018.

Week	Time Spent Observing (hr:min)	Number of Flowers	Number of Pollinators	Shannon's Diversity Index	Evenness	Species Richness
June 18 - 24	5:30	739	9	1.6770AB ^a	0.9359	6
June 25 - July 1	18:10	3,636	12	2.0947A	0.9534	9
July 2 - 9	20:10	3,827	49	2.1425A	0.8353	13
July 10 - 16	14:20	1,590	22	2.0983A	0.9113	10
July 17 - 23	6:20	390	17	1.2997B	0.8076	5

^aLetter groups on Shannon's diversity index indicate significant differences ($p < 0.05$).

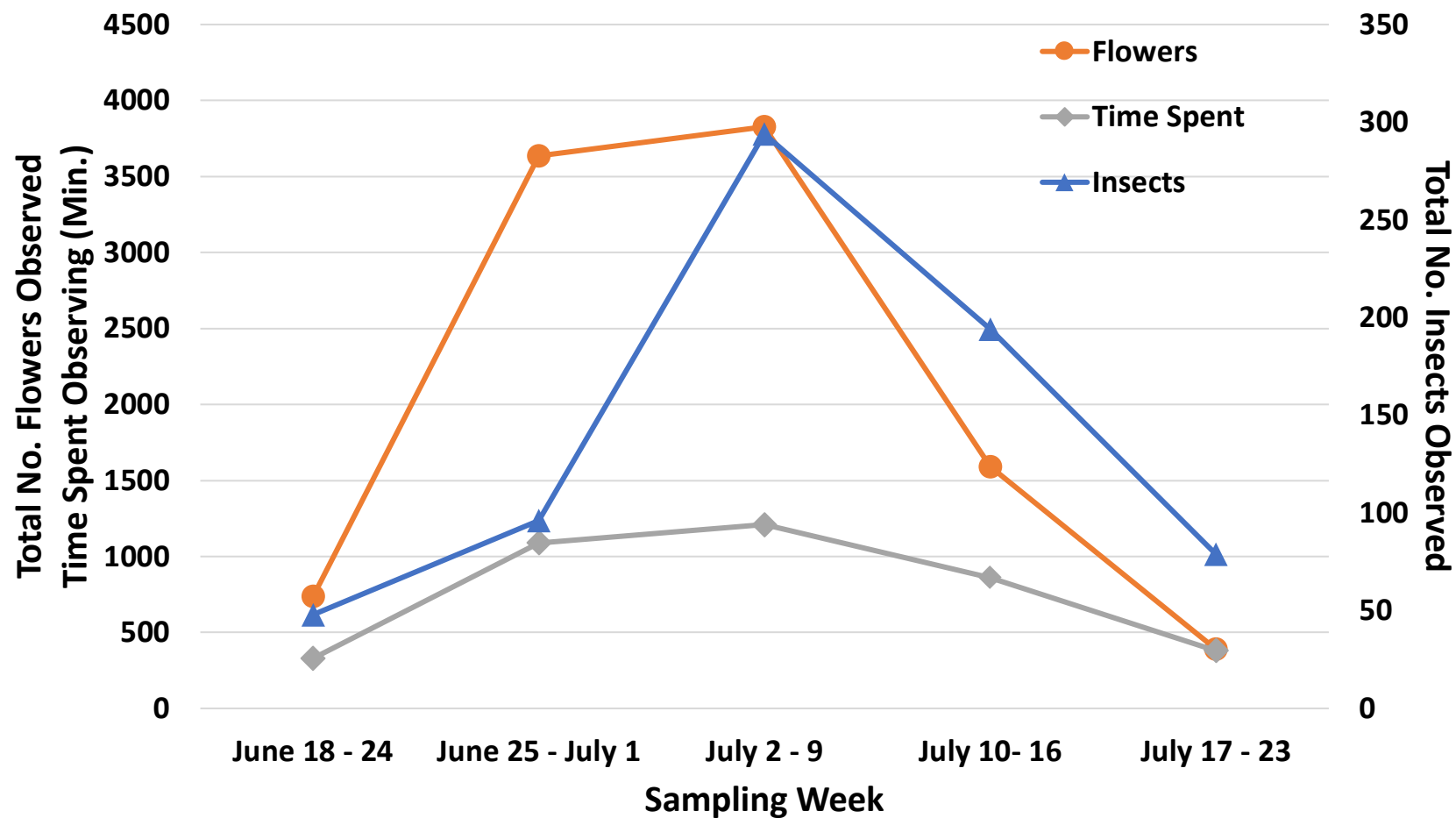


Figure 4.5 Total number of *Rhododendron maximum* L. flowers observed, time spent observing, and total number of insects observed on flowers by week, Great Smoky Mountains National Park, 2018.

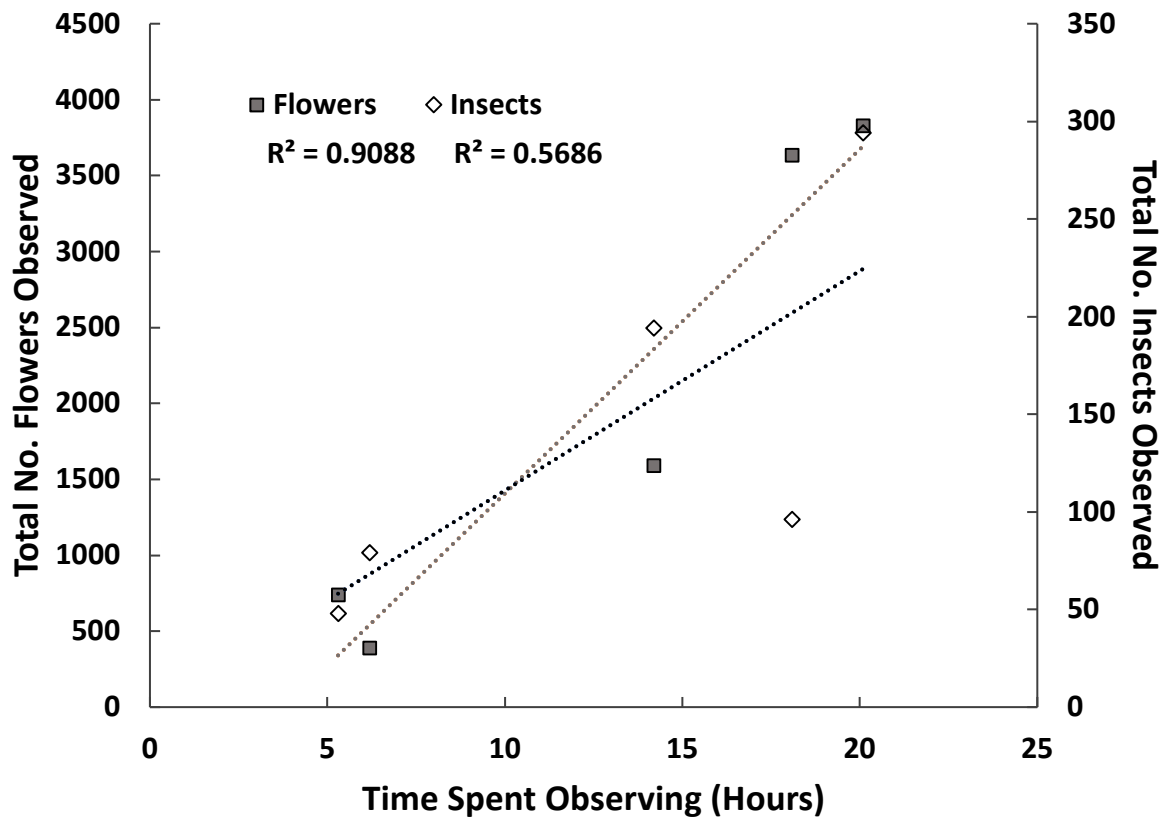


Figure 4.6 Correlations between time spent observing and total number of flowers observed, and time spent observing and total number of insects observed at *Rhododendron maximum* L. flowers by sampling week, Great Smoky Mountains National Park, 2018.

VITA

David Bruce Bechtel was born September 4, 1963 in Williamsport, Pennsylvania to his parents Bruce L. and Martina Martz Bechtel. As a child, he enjoyed sports and fishing. He got hooked on fly fishing at a young age when his dad took him to the mouth of Wallis Run where they stood on huge rocks and he watched his dad catch chubs on dry flies he could not see. He enjoyed tying flies and spending time on Loyalsock Creek, and summer weeklong vacations visiting family on Lake Oneida, developing his interest in the natural world.

In 1981 he graduated from Williamsport Area High School and began attending Carnegie-Mellon University that fall. During college, he played for the Tartan football team two years and was an active member of the Sigma Nu fraternity. Sigma Nu buggy was at its zenith with David reliably getting his team in the lead in every heat with his explosive hill 1, and they captured first place twice during his four years. He graduated spring 1985 with a B.S. in Civil Engineering and moved to Cleveland, OH where he worked as a civil engineer in state and county highway departments, and with a consultant to the USEPA for superfund sites. In 1990 he earned his Professional Engineering License in Ohio and subsequently Pennsylvania and New York.

In November 1991 he entered the Peace Corps and spent two years in Namibia teaching math in a secondary school. During this time, he indulged in the Namibian culture, and exploring and studying nature. On returning in 1994, he entered an organic farm internship on Keith's Farm in Westtown, NY, learning to grow organic produce and marketing in Union Square, New York City. At 30 years old, under Keith Stewart's tutelage, he finally came to understand taxonomy and systematics while working at the farm.

After two more years as a civil engineer in bridge construction and with the Pennsylvania Emergency Management Agency, he became a fulltime dad. For two decades he was a fulltime stay-at-home dad teaching his two children about nature and all good things. He volunteered at their schools, churches, sports teams, boy scouts, and local organizations dedicated to improving the environment. This volunteer work included performing maintenance and small construction projects for Conservation Fisheries, Inc., and official positions as church treasurer for two years and treasurer on the board of directors for the Little River Watershed Association for two years.

With an interest in eventually returning to employment in biological sciences, he began to study formally at University of Tennessee. Having had no classes in biological sciences since his sophomore year of high school in 1979, he enrolled in fall 2010 in General Botany and followed up in the next four semesters with a second semester of Botany, General Ecology, Aquatic Insects, and Ichthyology. After exploring potential fields of study, he latched onto entomology like a tick and took Insect Morphology and Insect Physiology spring 2017 while auditing Economic Entomology and Molecular Biology to hone his knowledge. Fall semester 2017, he officially began a Master of Science program in the Department of Entomology and Plant Pathology at the University of Tennessee and graduated May 2020. David is a member of the Tennessee Entomological Society and the Entomological Society of America.