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RISK ASSESSMENT OF SPOTTED LANTERNFLY (*LYCORMA DELICATULA*) NEONICOTINOID CONTROL TACTICS ON NON-TARGET INSECT FLORAL VISITORS OF RED MAPLE (*ACER RUBRUM*) AND TREE-OF-HEAVEN (*AILANTHUS ALTISSIMA*)

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ABSTRACT

Systemic neonicotinoid insecticides are used in the US for management of spotted lanternfly (*Lycorma delicatula*; SLF), a phloem-feeding planthopper and recent invasive pest of economic importance. Tree-of-heaven (*Ailanthus altissima*), a highly preferred host of spotted lanternfly and a widespread invasive tree in the US, is treated with the neonicotinoid dinotefuran by professional applicators under the direction of the US Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and several state agricultural departments. Application of the neonicotinoids imidacloprid or dinotefuran may be recommended by extension services for the protection of ornamental trees against SLF, such as red maple (*Acer rubrum*), a common eastern US native and important nutritional resource for early spring emerging bees. Since the introduction of neonicotinoids, much evidence has been gathered showing their potential for harm to non-target beneficial invertebrates, particularly bee pollinators, which can be exposed via contaminated floral resources in treated trees. But potential impacts of systemic neonicotinoid applications to trees for SLF control to non-target floral visitors has been largely unexplored. Therefore, I sought to determine the levels of neonicotinoid residues in flowers of tree-of-heaven and red maple treated with neonicotinoids for SLF control and identify the floral visitors to these trees to provide an assessment of their potential risk.

To accomplish this, neonicotinoid residue concentrations were determined in whole flowers of tree-of-heaven and red maple following post-bloom applications of dinotefuran or imidacloprid which differed in timing and method of application. Application timing, but not method of application, significantly influenced neonicotinoid residue concentrations in flowers of treated red maple, with dinotefuran residues significantly higher from red maple treated in fall than summer and imidacloprid residues significantly higher from red maple treated in spring or summer than fall. In the tree-of-

heaven we sampled, neonicotinoid residues were only found in flowers of one tree, at 2.75 ppb. Acute risk quotients were calculated from mean (+ 95% confidence interval) neonicotinoid residue concentrations determined in flower samples for each relevant treatment group. Dinotefuran and imidacloprid LC_{50}s for honey bees (*Apis mellifera*) and Japanese orchard bees (*Osmia cornifrons*) were determined via 48-hour ingestion bioassays and I compared these risk quotients to a specified level of concern, which is a method similar to those used by North American regulatory agencies for assessing pesticide risk to bees. No risk quotients calculated for honey bees for any relevant treatment group exceeded the level of concern, indicating minimal acute risk of mortality to honey bees, which is a surrogate for social bee species, from exposure to neonicotinoid residues at levels found in the whole flowers sampled. In contrast, several risk quotients calculated for *O. cornifrons* exceeded the level of concern, indicating potential for acute risk to *O. cornifrons*, a surrogate for solitary bee species, from exposure to neonicotinoid residues at levels found in whole flowers sampled from several treatment groups.

To determine the floral visitors of red maple and tree-of-heaven that could be exposed to neonicotinoid residues in flowers of these trees treated for SLF control in Pennsylvania, observations of insect visits to flowers and collections and identifications of insect visitors to flowers of these trees were conducted. The dominant floral visitor of tree-of-heaven in southeastern Pennsylvania was the margined leatherwing beetle (*Chauliognathus marginatus*), followed in abundance mostly by bee hymenopterans, especially the small bee genera *Halictus* and *Lasioglossum*, in addition to flies. The most abundant floral visitors to red maple flowers were bee hymenopterans, mostly honey bees and *Andrena* spp., and flies. These floral visitor groups are the most likely insects to be exposed to neonicotinoid residues in flowers of red maple and tree-of-heaven treated for SLF control in Pennsylvania.

The results of this work suggest that some bee floral visitors of red maple may be at risk of mortality from acute exposure to neonicotinoid residues found in flowers of this tree species treated with post-bloom applications of imidacloprid or dinotefuran for SLF control and that this risk may be impacted by several factors, particularly application timing. More, well-replicated studies are recommended to provide more comprehensive risk assessments to non-target beneficial insects from neonicotinoid use in SLF management and explore reduced-risk alternatives for SLF control.

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Chapter 1

Introduction

Pollination by animals, accomplished mostly by bees and other insect groups, is important for ecosystem health and agricultural production (Ollerton 2017). Pollen transfer can be facilitated by abiotic factors, such as wind and water; however, animal-mediated pollination provides a more direct and reliable vector for pollen transfer between conspecific plants. An estimated 87.5% of flowering plant species are pollinated by animals (Ollerton et al. 2011), indicating the importance of animal pollination in sustaining ecosystems that rely on flowering plants as a resource. Approximately 35% of global crop production by volume is dependent, to some degree, on animal-mediated pollination, with 87 of the leading global food crops having some reliance on animal pollinators, particularly by managed and wild bees (Klein et al. 2007). In the United States, the economic contribution of honeybees and wild bees to 7 pollinator-dependent crops (pumpkin, watermelon, tart cherry, apple, sweet cherry, blueberry, and almond) was recently valuated at $6.4 billion and $1.5 billion, respectively (Reilly et al. 2020). In 2012, the total estimated economic contribution of insect-mediated pollination, directly and indirectly, to U.S. pollinator-dependent crops was $34 billion (Jordan et al. 2021). While 60% of global crop production (e.g., wind- or self-pollinating grasses) does not rely on pollinators (Klein et al. 2007), pollinator-dependent crop production has grown disproportionately in recent decades, following diversification of the human diet and expansion of trade, which has increased reliance on pollination services in agriculture (Pelto and Pelto 1983; Aizen et al. 2009).

There is compelling evidence of widespread pollinator declines, and of insects in general, worldwide (Sánchez-Bayo and Wyckhuys 2019). Declines in specialist wild bees have been reported in the United Kingdom and the Netherlands post-1980, along with declines in plant species dependent on bees for pollination (Biesmeijer et al. 2006). Out of 14 bumblebee species collected from Southern Ontario between 1971-1973, seven were found either absent or in declining relative abundance in surveys performed in 2004-2006, with only four species showing increasing abundance (Colla and Packer 2008). Insect samples collected from malaise traps across 63 protected natural areas of Germany have shown a decrease of over 75% in insect biomass over 27 years (Hallmann et al. 2017). While there is reason to take claims of global pollinator and insect decline with caution (e.g., extrapolation from North American and European studies, interpreting extirpations and faunal turnover as extinctions, bias against insignificant results, and lack of long-term population data on species that are rare or difficult to study) (Wagner 2019), there is a growing body of evidence from sources around the world which suggest an overall trend in decline of biodiversity due to a multitude of factors (Sánchez-Bayo and Wyckhuys 2021; Wagner et al. 2021). A complete lack of animal pollination, while an unlikely scenario, could result in a direct reduction in global crop production by mass of up to 5% in the developed world and 8% in the developing world, where total agricultural production is greater (Aizen et al. 2009). Prior estimates of pollinator-dependent crop production have been higher than those reported by Aizen et al. (2009) and Klein et al. (2007); however, those estimates did not consider the partial dependence among these crops. Researchers have suggested declines of pollinators, and the broader entomofauna, to be primarily driven by land-use change, pathogens, introduced invasive species, climate change, and pollution from agrochemicals, such as systemic insecticides (Potts et al. 2010; Sánchez-Bayo and Wyckhuys 2019).

Neonicotinoid insecticides are a source of chemical pollution of particular concern to insect pollinators. Neonicotinoids, globally recognized and widely used for pest management, function by binding to arthropod nicotinic acetylcholine receptors, which can lead to paralysis and death in target pests (Simon-Delso et al. 2015). These insecticides are preferred for their persistent nature, selective toxicity to arthropods, diversity of application methods, and ability to be absorbed systemically by plants due to moderate-to-high water solubility (Ney 1995; Simon-Delso et al. 2015). The neonicotinoid class includes imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran, and nitenpyram (Simon-Delso et al. 2015). While these chemicals share the same mode of action, they differ in characteristics such as persistence, ability to translocate in target plants, and toxicity (Hopwood et al. 2016).

Starting with the introduction of imidacloprid in the early 1990s (Tomizawa and Casida 2011), neonicotinoids began replacing use of the former leading insecticide classes, such as organophosphates and carbamates, which were much more relatively toxic to humans and other vertebrates and faced issues of increasing resistance from insect pests (Casida and Quistad 1998; Roberts and Reigart 2013). Using neonicotinoids as alternatives may also reduce contact exposure to non-targets, given that they can be applied directly to plants with systemic applications and that foliar sprays of neonicotinoids can be readily absorbed into target plants under certain formulations (Hopwood et al. 2012; Jeschke et al. 2011). Neonicotinoids have been particularly helpful in U.S. apple production, which involves complex integrated pest management approaches to combat more than two dozen major pests, by serving as reduced-risk alternatives to organophosphates and pyrethroids while being capable of maintaining crucially important biological control (Agnello et al. 2009; Hopwood et al. 2016).

Neonicotinoids are now one of the leading insecticide classes, accounting for over 25% of insecticide sales worldwide in 2014 (Bass et al. 2015). They are most often used in seed treatments for staple crops such as corn and soy, but can also be used for the protection of vegetable and fruit crops (such as pome fruits, citrus, and potatoes), ornamentals, turfgrass, and in veterinary medicine as ectoparasiticides (Jeschke et al. 2011; Douglas and Tooker 2015). Chemicals within the neonicotinoid class vary in their registered uses. For example, while imidacloprid is registered as a seed treatment in the U.S., dinotefuran is not registered for this purpose and is instead limited to use in indoor settings, and on leafy vegetables, turfgrass, and ornamentals (USEPA 2004; Hopwood et al. 2016). While the systemic and persistent qualities of neonicotinoid insecticides can facilitate long-lasting protection from insect pests via target plants, there are multiple routes of neonicotinoid exposure to non-target organisms, including floral resources such as nectar, which is derived from the plant's vascular system (De la Barrera and Nobel 2004; Bonmatin et al. 2015).

Since their introduction, much evidence has been gathered regarding the potential harmful effects of neonicotinoids on non-target arthropods (Pisa et al. 2015). This is not surprising, as neonicotinoids are applied with the intention of targeting insects. While neonicotinoid applications can be more selective as systemics to target insects feeding directly on the target plant, non-target insects may be exposed at the time of application, especially with foliar sprays and soil drenches, or exposed to persistent residues (Biddinger and Rajotte 2015; Bonmatin et al. 2015; Simon-Delso et al. 2015). A recent meta-analysis of 44 studies showed that neonicotinoids significantly reduce multiple performance metrics of terrestrial arthropods, including behavior, survival, reproductive success, condition, and abundance (Main et al. 2018). Harm to non-target organisms from neonicotinoid exposure may threaten important ecosystem services, such as nutrient cycling, biocontrol, and pollination (Chagnon et al. 2015). Given their importance as pollinators and reported trends of bee declines, much effort has been directed towards understanding the impacts of neonicotinoids on bees (Blacquière et al. 2012).

Bees can also be important as bioindicators. They collect resources throughout the environment which may contain environmental pollutants, such as neonicotinoid residues. Measurements of bee health or pollutant levels in collected resources may provide insights into ecosystem health (Celli and Maccagnani 2003; Zioga et al. 2020).

Laboratory bioassays have shown that neonicotinoids vary greatly by compound in toxicity to honey bees (Hopwood et al. 2016). The United States Environmental Protection Agency (USEPA) categorizes chemicals by toxicity to bees using LD_{50} values, with LD_{50}s less than 2 µg considered highly toxic, LD_{50}s between 2 and 10.99 µg considered moderately toxic, LD_{50}s between 11 and 100 µg considered slightly toxic, and LD_{50}s over 100 µg considered practically nontoxic (Hopwood et al. 2016). Iwasa et al. (2004) investigated the acute contact toxicity of the 7 neonicotinoids to honey bees and found the nitro-substituted neonicotinoids to be highly toxic (LD_{50}s: 18 ng/bee for imidacloprid, 22 ng/bee for clothianidin, 30 ng/bee for thiamethoxam, 75 ng/bee for dinotefuran, and 138 ng/bee for nitenpyram), whereas the cyano-substituted neonicotinoids were slightly to moderately toxic (LD_{50}s: 7.1 µg/bee for acetamiprid and 14.6 µg/bee for thiacloprid). Neonicotinoids have been shown to be more toxic to bee species when ingested rather than exposed via contact (Suchail et al. 2000; Nauen et al. 2001; Schmuck et al. 2001).

While research on neonicotinoids and pollinators has been heavily biased towards honey bees, recent studies have provided insights into the effects of neonicotinoids on other bee species, with most attention to species managed by humans for pollination services, such as *Bombus* spp. (bumble bees) and *Osmia* spp. (Lundin et al. 2015). Recent bioassays have shown imidacloprid to be highly toxic to the buff-tailed bumble bee (*Bombus terrestris*) when exposed via contact (48-hr LD_{50}: 0.38 µg/bee), with acetamiprid and thiacloprid being practically non-toxic (48-hr LD_{50}: >100 µg/bee) (Reid et al. 2020). In acute contact bioassays with *Osmia bicornis*, imidacloprid has been shown to be highly toxic (48-hr LD_{50}: 0.046 µg/bee), while thiacloprid has low toxicity (48-hr LD_{50}: >100 µg/bee) (Beadle et al. 2019). These findings are similar to the pattern of relative toxicity between the nitro- and cyano-substituted neonicotinoid groups shown in honey bees (Iwasa et al. 2004). In acute contact bioassays with honey bees and *Osmia* *cornifrons*, imidacloprid and acetamiprid were found to be highly toxic (48-hr LD_{50}: 0.2 µg/bee) and slightly toxic (48-hr LD_{50}: 64.6 µg/bee,), respectively, to honey bees, while in *O. cornifrons*, both of these were only moderately toxic (48-hr LD_{50}: 3.8 and 4.0 µg/bee, respectively) (Biddinger et al. 2013). In recent ingestion bioassays, *O. cornifrons* were found to be 2,378, 43.32, and 2,684 times more sensitive than honey bees to acetamiprid, imidacloprid, and thiamethoxam, respectively (Phan 2021). These results highlight the importance of species-specific and chemical-specific context when considering the extent of harm from neonicotinoid use.

While exposure to neonicotinoids in real-world settings is likely lower than doses given to bees in laboratory experiments (Godfray et al. 2014; Biddinger and Rajotte 2015), field-realistic exposure may lead to sublethal behavioral, developmental, and reproductive effects even when exposure is not great enough to directly kill bees. Neonicotinoids have been shown to produce sublethal effects in honey bees, including delayed development and impairments to olfactory and visual learning and mobility, which may negatively impact long-term colony health (Blacquière et al. 2012). Sub-lethal neonicotinoid exposure can also impair learning and memory and reduce sonication in bumble bees which are important qualities for foraging success (Stanley et al. 2015; Switzer and Combes 2016). A recent meta-analysis on the impacts of field-realistic exposure to neonicotinoids on non-*Apis* bees indicated that neonicotinoids reduce the reproductive output of bumble bees and *Osmia* spp. bees and impair development in bumble bees (Siviter et al. 2021). The current data regarding the effects of neonicotinoids on bees is biased towards managed bees and certain neonicotinoids, with imidacloprid being the most well studied compound, followed by thiamethoxam and clothianidin (Lundin et al. 2015). While differences in sensitivity to neonicotinoids across bee species studied so far show that it can be inappropriate to use data from a select few species to predict neonicotinoid toxicity to bees as a group (Manjon et al. 2018), there is clear evidence that neonicotinoids can and do harm several bee species. Therefore, it is important to use neonicotinoids judiciously, in an integrated pest management approach that considers pollinator health, to take advantage of the benefits of neonicotinoids as alternatives to earlier insecticide classes while reducing the potential for harm (Hopwood et al. 2012; Biddinger and Rajotte 2015; Hopwood et al. 2016).

Evidence of harm to pollinators from neonicotinoids has motivated governments to regulate their use. Since 2013, the United States Environmental Protection Agency has required pollinator protection labels for products containing the neonicotinoids clothianidin, dinotefuran, imidacloprid, or thiamethoxam for outdoor foliar applications with the intent of minimizing exposure of these neonicotinoids to pollinators (USEPA 2013). In 2013, the European Union placed significant restrictions on the usage of the neonicotinoids clothianidin, imidacloprid, and thiamethoxam, and, in 2018, completely banned their use in outdoor settings to protect honeybees (EU 2013; EU 2018a; EU 2018b; EU 2018c). In 2020, the E.U. rejected the renewal of approval for thiacloprid, further restricting the use of neonicotinoids in member states (EU 2020). A study by Woodcock et al. (2018) showed a decline in neonicotinoid residue detections in U.K. honey samples from about 50% of samples tested prior to the E.U. moratorium to 20% of samples in the year following implementation of the 2013 moratorium. Another U.K. study assessed the presence of neonicotinoids in bumblebee-collected nectar and pollen from 2013 to 2015, which indicated that neonicotinoid exposure decreased for rural bees but remained similar across years for bumblebees in peri-urban habitats (Nicholls et al. 2018). In western France, imidacloprid, clothianidin, and thiamethoxam were detected in the nectar of oilseed rape crops in at least one year between 2014 and 2018 following the 2013 E.U. moratorium, with imidacloprid being found each year with no clear trend of declining concentrations across years (Wintermantel et al. 2020). These studies suggest that there may have been a reduction in neonicotinoid exposure to bees following the

E.U. moratorium; however, evidence is limited, and it is unclear whether the restrictions have made any positive impacts on pollinator health. Still, neonicotinoids continue to be widely used in insect pest management, from pre-emptive use in U.S. field crop protection as seed treatments, to facilitating reduced-risk pest suppression in apple and peach production, to managing newly invasive species, such as the spotted lanternfly (*Lycorma delicatula*) (Agnello et al. 2009; Douglas and Tooker 2015; Leach et al. 2019; Lee et al. 2019).

The spotted lanternfly (hereafter, SLF) is a pestiferous, phloem-feeding planthopper native to China, Taiwan, India, and Vietnam (Lee et al. 2019). It was introduced to South Korea, Japan, and most recently, to the U.S. (Han et al. 2008; Tomisawa et al. 2013; Dara et al. 2015). First reported in the U.S. in Berks County, Pennsylvania in 2014, SLF has since expanded its range to include New York, Maryland, Delaware, New Jersey, Connecticut, Virginia, West Virginia, Ohio, Indiana, and Massachusetts (Barringer et al. 2015; NYSIPM 2022). The continuing spread of SLF is concerning, given its potential to cause significant economic harm. In China, SLF is a pest of fruit crops, such as grapes (*Vitis* spp.) and apples (*Malus* spp.), as well as, hardwoods, such as willows (*Salix* spp.) and black locust (*Robinia pseudoacacia*) (Dara et al. 2015). In South Korea, SLF is considered a pest of grapes, apples, and stone fruits, and is a nuisance in urban areas (Han et al. 2008; Park et al. 2009).

SLF feeding can weaken host plants directly, by consuming phloem and creating wounds at feeding sites, and indirectly, by limiting the host plant's photosynthesis via honeydew deposition, which can foster the growth of sooty mold (Dara et al. 2015). SLF has been reported to feed on 103 different plant taxa worldwide, with 56 of these taxa present in North America, including those of importance to agriculture and forestry, such as grapes, hops (*Humulus lupulus*), walnuts (*Juglans* spp.), and maples (*Acer* spp.) (Barringer and Ciafré 2020). While the immediate impacts of SLF in the U.S. remain to be quantified, in Pennsylvania, SLF can reduce grape yields and lead to increased grapevine mortality, with one vineyard reporting 90% yield loss (Urban 2020). SLF has been found feeding in large numbers on fruit trees, such as apple and peaches (*Prunus* spp.); however, short-term damage has not yet been observed in orchards (Urban 2020). Researchers have estimated the expected direct economic impact of SLF to Pennsylvania agriculture to be $42.6 million per year, provided SLF's range expands throughout the entirety of the state, with nursery operators, fruit growers (especially grape growers), timber producers, and Christmas tree growers particularly affected. The estimated overall economic damage by SLF to Pennsylvania forestry has been estimated at $152.6 million, with damage especially impactful to maple, oak, and black walnut production (Harper et al. 2019).

To manage SLF, the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) and several state agricultural departments have coordinated a control program involving multiple pest management tools, such as monitoring, physical traps, and pesticide applications (USDA-APHIS 2021). Soon after SLF's detection in Berks County, Pennsylvania, a quarantine zone, which regulates the transportation of SLF and articles that may harbor SLF, was established across 6 counties, all within eastern PA (Urban et al. 2021). The range of SLF has since expanded, now including 45 counties in PA and 11 total states (NYSIPM 2022). Given SLF's strong preference for tree-of-heaven (*Ailanthus altissima*) as a feeding host (Han et al. 2008; Liu 2019; Derstine et al. 2020), this invasive tree has been targeted for removal by the Pennsylvania Department of Agriculture and other plant protection agencies, which involves application of herbicides to mature trees and developing shoots (USDA-APHIS 2021). Tree-of-heaven is a flowering, deciduous tree native to China, which was introduced to the U.S. from England in 1784 as a popular ornamental, where it has since widely expanded its range, especially in areas with high disturbance (Hu 1979; Miller

1990). Tree-of-heaven was previously considered an essential host for SLF; however, recent evidence showed that SLF can complete its life cycle and reproduce without ever feeding on it (Uyi et al. 2020 and 2021). Still, tree-of-heaven is considered a highly preferred host of SLF and is thus the primary target for SLF control programs by government agencies.

Neonicotinoids are also used in the SLF control program as part of a trap tree tactic, in which mature tree-of-heaven are treated with trunk spray applications of dinotefuran to attract and kill SLF (USDA-APHIS 2021). While currently listed as an option under the SLF control program, imidacloprid trunk injections were previously reserved for special circumstances and neonicotinoid applications to trap trees applied under USDA for SLF control now only use dinotefuran as the active ingredient, because imidacloprid is less effective (Leach et al. 2019; USDA-APHIS 2021). For homeowners and parks in Pennsylvania, landscape professionals often recommend the neonicotinoids imidacloprid or dinotefuran, among several other insecticide classes, as options for chemical control of SLF for protection of ornamental trees (Korman et al. 2021a; Korman et al. 2021b), such as red maple (*Acer rubrum*).

Red maple is one of the most widespread native trees in the eastern U.S. and an important food resource for bees and other pollinating insects that emerge in early spring (Walters and Yawney 1990; Batra 1985). Ornamental flowering trees can attract and provide nutritional resources to bee pollinators in urban areas (Hausmann et al. 2016; Somme et al. 2016; Mach and Potter 2018), so there is potential for systemic insecticide applications to harm pollinators.

Given that neonicotinoids are systemic nature with the potential for long-term persistence, neonicotinoid treatments used in the management of SLF have the capacity to be harmful to non-target beneficial insect groups, particularly to pollinating insects visiting flowers of treated plants. It is therefore important to assess the risk of neonicotinoid use in SLF management to determine the likelihood for toxicity to non-target arthropods. Neonicotinoids have been shown to differ in uptake and persistence in woody landscape plants when applied via soil injection (Mach et al. 2018) and have also shown differential toxicity to bees (Hopwood et al. 2016). There are multiple methods of application for neonicotinoids, including trunk injections, trunk sprays, foliar sprays, and soil drenches (Herms et al. 2014; Korman et al. 2021b). Trunk injections allow for more accurate dosing with less insecticide needed, as little is lost to the environment, and can move more quickly through the tree relative to other methods (Korman et al. 2021b; Herms et al. 2014). Trunk sprays and foliar sprays are conducted by directly spraying the tree with insecticide, whereafter the insecticide penetrates and is absorbed into the vascular system. Trunk sprays target the base of the tree, while foliar sprays target the tree's leaves and branches. Trunk sprays are relatively easy to perform and, when performed correctly, should not lose insecticide to nearby soil and the surrounding environment. Pesticides applied via foliar spray, however, are likely to drift through the air to non-target plants and the surrounding environment (Herms et al. 2014). Contact exposure of neonicotinoids to non-target arthropods from foliar spray residues may be minimal due to quick absorption of the insecticide into plant vegetation, as shown with the neonicotinoid dinotefuran, and the ability for non-targets to move throughout the environment to non-contaminated surfaces, particularly for flying insects such as honey bees (Durkin 2009). Soil drenches are applied by pouring diluted insecticide onto the soil surrounding the base of the target tree, after which it is absorbed by the tree's roots. Since the insecticide is applied to the soil rather than the tree itself, soil drenches can easily contaminate the surrounding environment (Herms et al. 2014). All four of these application methods (trunk injection, trunk spray, foliar spray, and soil drench) are currently listed for neonicotinoid treatment for SLF control by Pennsylvania State University Extension; however, application by a licensed professional is recommended for trunk injections due to the use of specialized equipment, while foliar sprays are recommended only in limited circumstances, such as for controlling SLF nymphs (Korman et al. 2021a). While neonicotinoids are recommended to be applied after bloom to prevent exposure to pollinators (Korman et al. 2021a), differences in timing of post-bloom application have been shown to result in different levels of neonicotinoid residues at the time of the next flower bloom (Mach et al. 2018). All these factors (e.g., persistence, toxicity to beneficial insects, application method, and timing of application) should be considered in the development of pest control programs to reduce harm to pollinators and other beneficial insects.

The primary goal of this research project was to determine the potential risk of neonicotinoid use on non-target pollinators within the context of SLF management. Our objectives were to: (1) Determine the levels of neonicotinoid residues in whole flowers of tree-of-heaven and red maple following summer and fall applications of dinotefuran or imidacloprid at different application timings and methods of application; and (2) Identify and quantify insect visitors to tree-of-heaven and red maple flowers to assess which pollinators may be at risk from neonicotinoid usage in SLF control programs.

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Chapter 2

Neonicotinoid residues in flowers of red maple (*Acer rubrum*) and tree-of-heaven (*Ailanthus altissima*) following post-bloom insecticide applications for control of the spotted lanternfly, *Lycorma delicatula*

INTRODUCTION

Insect pollinators, and the broader entomofauna, are experiencing declines worldwide (Sánchez-Bayo and Wyckhuys 2019 and 2021; Wagner et al. 2021), and chemical pollution, particularly from agrochemicals such as neonicotinoid insecticides, is considered a primary driver of these declines (Potts et al. 2010; Sánchez-Bayo and Wyckhuys 2019). Neonicotinoids were first introduced into the market in the 1990's in response to increasing resistance in insect pests to the leading insecticide classes; they were safer alternatives to humans and other vertebrates given their higher selectivity for invertebrates (Casida and Quistad 1998; Roberts and Reigart 2013; Simon-Delso et al. 2015). They work by binding to nicotinic acetylcholine receptors, causing continuous muscle contraction which can lead to paralysis and death (Simon-Delso et al. 2015). Neonicotinoids are now the most widely used of the insecticide classes, highly preferred for several characteristics, including high selectivity for invertebrates, persistence, multiple methods of application, and moderate-to-high water solubility, which allow them to be transported systemically via the plant's vascular system (Ney 1995; Simon-Delso et al. 2015). While neonicotinoids are mostly used for seed treatments in staple crops such as corn and soy, they can also be used for the protection of vegetable and fruit crops and ornamentals (Jeschke et al. 2011; Douglas and Tooker 2015).

Neonicotinoids are currently being used in control efforts against the spotted lanternfly (*Lycorma delicatula* (White) [Hemiptera: Fulgoridae]; hereafter SLF), a phloem-feeding, pestiferous planthopper from southeast Asia recently introduced to the United States (Lee et al. 2019; Dara et al. 2015; USDA-APHIS 2021). First detected in Berks County, Pennsylvania in 2014 (Barringer et al. 2015), SLF's range in N. America has since expanded, with established populations in New York, Maryland, Delaware, New Jersey, Connecticut, Virginia, West Virginia, Ohio, Indiana, and Massachusetts in addition to Pennsylvania (NYSIPM 2022).

Feeding by SLF can damage host plants directly, by consuming phloem and creating leaking wounds in the plant, and indirectly, by secreting honeydew which can facilitate the growth of sooty mold that covers and blackens plants, reducing their ability to photosynthesize (Dara et al. 2015). It has been proposed that SLF, if spread statewide, could cost the Pennsylvania economy up to $42.6 million in direct economic damage per year (Harper et al. 2019). Much effort has been made to slow the spread and reduce populations of SLF (Urban et al. 2021). These efforts include the application of systemic neonicotinoids, namely imidacloprid and dinotefuran, to host plants to manage SLF (USDA-APHIS 2021; Korman et al. 2021b).

Neonicotinoids have been shown to produce lethal and sublethal effects in bees, many of which are important insect pollinators (Hopwood et al. 2016; Siviter et al. 2021). Neonicotinoids can move into the flowers of target plants, providing a route of exposure to bees and other insects that visit flowers for pollen, nectar, and other resources (Bonmatin et al. 2015; Mach et al. 2018; Heller et al. 2020; Zioga et al. 2020). Given the potential for harm to non-target pollinators from exposure to pesticides, researchers have argued that pollinator health should be considered in the development of integrated pest management strategies (Biddinger and Rajotte 2015). Therefore, it is important to determine if, and when, neonicotinoid applications for SLF control present a potential risk to beneficial insects.

In this study, we aimed to determine the concentrations of neonicotinoid residues in whole flowers of red maple (*Acer rubrum* L. [Sapindales: Sapindaceae]) and tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle [Sapindales: Simaroubaceae]) that had been treated post-bloom with imidacloprid or dinotefuran for SLF control in the year before flower collection. Due to the difficulty of collecting enough pollen and nectar for residue analyses from these flowers, we analyzed whole flowers to provide a proxy for the residue concentrations in these floral resources. Tree-of-heaven, native to China and invasive in the United States (Hu 1979; Miller 1990), is considered a highly preferred host of SLF (Han et al. 2008; Liu 2019; Derstine et al. 2020), and so is targeted for removal by herbicides or treated with systemic insecticides to create trap trees by applications of dinotefuran by regulatory agencies (USDA-APHIS 2021). Tree-of-heaven is often found along transportation corridors and disturbed areas and occurs less frequently in managed landscapes, such as neighborhoods, where ornamental trees are abundant (Hu 1979; Kowarik and Säumel 2007; Mason et al. 2020). Red maple is one of the most widespread native trees in the eastern United States and a popular ornamental (Walters and Yawney 1990). Its flowers provide an important food resource for early spring-emerging bees and other pollinating insects when few other floral resources are available (Batra 1985). All life stages of SLF can utilize red maple as a host and adults are often found feeding on red maples in high abundance, primarily in the late fall just prior to egg deposition when adult SLF congregate in high numbers from the surrounding landscape and tree-of-heaven has senesced and is unattractive to SLF (Kowarik and Säumel 2007; Barringer and Ciafré 2020; Mason et al. 2020). Therefore, commercial applicators generally apply dinotefuran or imidacloprid to red maple for SLF control in the fall (Urban 2020; Korman et al. 2021a).

Our approach was to determine how application method and timing of application may affect residue concentrations of dinotefuran and imidacloprid in whole flowers the following bloom period the year after application. Neonicotinoids can be applied using multiple methods, including trunk injection, trunk sprays, foliar sprays, and soil drenches, which can differ in their ability to provide accurate insecticide dosage and their likelihood for environmental contamination (Herms et al. 2014; Korman et al. 2021b). Neonicotinoid residue levels in woody plants can also differ when applied at different times of the year and can differ depending on which neonicotinoid is applied given differences in uptake, translocation, and degradation between neonicotinoids, which can be explained by differences in water solubility, soil sorption, and half-life (Bonmatin et al. 2015; Simon-Delso et al. 2015; Mach et al. 2018). Understanding how application method and timing affect neonicotinoid residue levels may help inform management practices to limit exposure to non-target pollinators.

To assess risk to non-target bees similar to those performed by North American regulatory agencies for assessing pesticide risk to bees (OPP, PMRA and CALDPR 2014), we calculated risk quotients using the mean and 95% confidence intervals (CI) for residue concentrations in whole flower samples for each treatment group in our study and LC_{50} values obtained from acute oral bioassays involving honey bees (*Apis mellifera* L. [Hymenoptera: Apidae]) and the mason bee *O. cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae), comparing these risk quotients to a specific level of concern (LoC).

MATERIALS AND METHODS

Insecticide applications

Dinotefuran or imidacloprid treatments to red maples were applied by commercial applicators and university researchers primarily for studies investigating insecticide efficacy against SLF, while applications of dinotefuran to tree-of-heaven were made by contractors under the direction of the Pennsylvania Department of Agriculture as part of their SLF control program (Table 2-S1). No tree-of-heaven sampled were treated with imidacloprid; tree-of-heaven sampled for this study were only treated with dinotefuran using trunk sprays. None of the tree-of-heaven or red maple sampled in our study were treated by foliar application (Herms et al. 2014). Untreated red maple controls were sampled from the same sites as trees treated with neonicotinoids, whereas untreated tree-of-heaven controls were sampled from multiple sites across Southeastern PA at sites distinctly separate from where dinotefuran-treated trees were sampled since untreated tree-of-heaven were not available at the same sites as treated trees.

Insecticide treatments applied to sampled trees were ostensibly applied at the maximum label rate; in practice, tree diameter at breast height (DBH) is used to calculate the amount of active ingredient to use. Known exceptions were the imidacloprid trunk injections applied in May 2018, which were done at double the maximum label rate, and imidacloprid soil drenches applied in October 2018 at half the maximum label rate (Table 2-S1). All insecticide applications were conducted post-bloom to reduce the risk of exposure to pollinators.

Flower Collections

Whole flowers of red maple were collected from untreated red maples and red maples treated by trunk injection, soil drench, or trunk spray of imidacloprid or dinotefuran. Blooming branches were pruned, and flowers were removed from branches by hand then placed into sterile 50 mL plastic centrifuge tubes. Collectors wore nitrile gloves while removing flowers, and gloves were changed between each tree sampling to prevent cross-contamination. Pruner blades were cleaned using 70% ethanol between each tree sampling to prevent potential sample contamination or the spread of *Verticillium* wilt between trees. Red maple sampling occurred on multiple dates in late-winter/early-spring of 2019, 2020, and 2021 due to differences in timing of the bloom period between trees (Table 2-S1). Collections began in the morning and continued until no more trees could be sampled on each respective date. All flower samples were stored in ice-filled chests while in the field and transferred to -80 °C until they were processed, except for samples collected in 2019 that were not kept in cold storage while in the field and were stored at -20 °C for several days before transfer to -80 °C.

Whole tree-of-heaven flower samples were collected from non-treated tree-of-heaven and tree-of-heaven treated with trunk sprays of dinotefuran (Table 2-S1). Collection methods for tree-of-heaven samples were conducted as described above. Tree-of-heaven collections occurred on multiple dates during June of 2021 due to differences in timing of the bloom period between trees.

Neonicotinoid Residue Determination

Pesticide residue extractions from whole flower samples were performed using a modified QuEChERS method (Lehotay 2006). Samples were ground and homogenized with liquid nitrogen (LN_2) using a mortar and pestle. Ten grams of each sample were transferred to a sterile 50 mL centrifuge tube, into which 10 mL of 1% acetic acid in acetonitrile (MeCN), an internal standard of chlorpropamide (200 ng/g), 4 g of anhydrous magnesium sulfate ($MgSO_4$) and 1 g of sodium acetate (NaOAc) were added. The contents were then vortexed for 1 minute. Samples were centrifuged at 3,000-4,000 relative centrifugal force (RCF) for 5-10 minutes. One mL of each sample's supernatant was pipetted into 2 mL dispersive solid-phase extraction (d-SPE) clean up tubes (catalog #26219, RESTEK, Bellefonte, PA) and shaken for 1 minute. Samples were centrifuged again at 3,000-4,000 RCF for 5-10 minutes. The resulting supernatant (0.3 mL) from each sample was then transferred to 1.5 mL microcentrifuge tubes (for samples collected in 2020 and 2021, 0.4 mL of supernatant was transferred into 2.0 mL microcentrifuge tubes) and evaporated for approximately 45 min using a SpeedVac vacuum concentrator

(ThermoFisher Scientific, Waltham, MA). Evaporated samples were then re-suspended in 0.3 mL of 3% MeCN. Processed samples were stored at -80 °C until submission to Penn State's Metabolomics Core Facility, where liquid chromatography-mass spectrometry (LC–MS/MS) was performed. For all whole flower samples collected in 2020 and 2021, changes to the extraction methods were made to account for different LC/MS-MS instruments used between samples collected in 2019 and those collected in 2020 and 2021, as the pesticide residue analysis was performed on a more recently acquired instrument due to a change in personnel at Penn State's Metabolomics Core Facility: 1) evaporated samples were re-suspended in 0.1 mL of 50% MeCN and 2) the internal standard used for 2019 samples, chlorpropamide (200 ng/g), was replaced by 1 μM D_4-imidacloprid, added at the re-suspension step.

For whole red maple flower samples collected only in 2019, the pesticide residue analysis was performed using a Prominence 20 UFLCXR system (Shimadzu, Columbia, MD) coupled with a 5600 (QTOF) TripleTOF using a Duospray™ ion source (Sciex, Framingham, MA) with a Waters (Milford, MA) C18 BEH UPLC column (2.1 x 100 mm, 1.7 μm) maintained at 55 °C with a flow rate of 250 μL/min. Solvent A consisted of HPLC grade water with 0.1% formic acid and Solvent B was HPLC grade MeCN with 0.1% formic acid. The initial conditions were 97% Solvent A and 3% Solvent B, increasing to 45% Solvent B at 10 minutes, then 75% Solvent B at 12 minutes, where it was held at 75% Solvent B until 17.5 minutes before returning to the initial conditions. The injection volume was 10 μL. The capillary voltage was set at 5.5 kV in positive ion mode with a de-clustering potential of 80 V. Product ion mass spectra were obtained for the parent ions of imidacloprid (256 m/z) and dinotefuran (203 m/z) using a collision energy of 25V. Limits of detection (LoD) and quantification (LoQ) of the 5600 TripleTOF for imidacloprid (LoD: 0.3 ppb; LoQ: 1 ppb) and dinotefuran (LoD: 0.8 ppb; LoQ: 3 ppb) were obtained by analyzing signal-to-noise ratios (Dong et al. 2020).

For all other whole flower samples (collected in 2020 and 2021), samples were run on an Acquity UPLC system coupled with a Waters Xevo TQ-S mass spectrometer with a Waters Acquity C18 BEH UPLC column (2.1 x 100 mm, 1.7 µm) maintained at 40 °C at a flow rate of 300 µL/min. Solvent A was a mixture of 2:98 (by volume) methanol/HPLC grade water with 5mM ammonium formate and 0.1% formic acid, while Solvent B was a mixture of 98:2 methanol/HPLC grade water with 5mM ammonium formate and 0.1% formic acid. The initial conditions were 80% Solvent A and 20% Solvent B, increasing to 90% Solvent B at 3.5 minutes, where it was held at 90% Solvent B until 8 minutes before returning to the initial conditions. The injection volume was 5 µL. The capillary voltage was set at 5.5 kV in positive ion mode with a de-clustering potential of 80 V. Product ion mass spectra were obtained for the parent ions of imidacloprid (256 m/z), D_4-imidacloprid (260 m/z), and dinotefuran (203 m/z) using collision energies of 12 V, 20 V, and 14 V, respectively to produce standard curves for calculating residue concentrations for all treatments. Limits of detection (LoD) and quantification (LoQ) of the TQ-S for imidacloprid (LoD: 0.2 ppb; LoQ: 0.7 ppb) and dinotefuran (LoD: 3 ppb; LoQ: 11 ppb) were obtained by analyzing signal-to-noise ratios (Dong et al. 2020).

Data Analysis

Data analyses were performed separately for each active ingredient of interest (dinotefuran or imidacloprid). Analysis of samples treated in 2018 were separated from those treated in 2019 and 2020 due to differences in sample extraction and LC/MS-MS methods. To reduce rightward skew in the distribution of the dependent variable (imidacloprid or dinotefuran residue concentrations) to better fit the normal distribution as assumed in our statistical tests, the following data transformations were performed: for red maples treated with dinotefuran in 2018, we used a square root transformation; for red maples treated with dinotefuran or imidacloprid in 2019 or 2020, we used a cube root transformation.

For each subset of data for which meaningful analyses could be performed (i.e., for data that had treatments with detectable neonicotinoid residue levels which were not rare or low and data which had at least one shared relevant predictor level), we began with a full linear model to determine which candidate predictors (application method, treatment season, treatment year, and site location) significantly influenced neonicotinoid residue concentrations in whole flower samples. Red maple and tree-of-heaven were analyzed separately, as were the imidacloprid and dinotefuran treatments. For treatment season, we assigned treatments to the appropriate season such that applications performed in May were assigned to spring, treatments performed in July or August to summer, and treatments in September or October to fall. While tree diameter at breast height (DBH) was recorded for most (151/167) trees, this factor was not considered as a candidate predictor, as it is intrinsically correlated with the application rates for each application method, which were given in grams of active ingredient per inch DBH (Table 2-S1). The candidate predictor site location was not included in the model development for red maples treated in 2018, due to unequal sampling between sites among treatment groups. Beginning with a full linear model containing the above listed candidate predictors, we used backwards Akaike information criterion (AIC) selection to arrive at reduced models containing only the predictors that were statistically significant at $p < 0.05$ (except data subsets for which no predictors were significant). Data were analyzed separately by treatment year where this variable significantly affected residue concentrations; data for red maple treatments in 2019 and 2020 were pooled since year was not statistically significant. The best fit reduced models contained only treatment season. Untreated controls were not included in the above analyses, as the controls did not have enough variation (i.e., no differences in application method or treatment season) to make meaningful comparisons, and their inclusion heavily skewed the data. Dunnett's tests were performed instead to compare untreated controls to each treatment where appropriate. Data analyses were conducted in RStudio (version 1.4.1717, "Juliet Rose").

Risk Quotients

Risk quotients were calculated using the following formula: risk quotient = exposure/toxicity (Phan 2021), where exposure was the mean concentration (95% CI) (in ppb) of whole flower samples for each treatment group of relevance in this study; toxicity was the LC_{50} (in ppb) obtained from acute oral bioassays with imidacloprid or dinotefuran on honey bees or *O. cornifrons* using methods reported in Phan et al. (2020). In short, oral bioassays were conducted using bees in containers that were fed 50:50 (by weight) sucrose solutions spiked with either dinotefuran or imidacloprid using formulated commercial products (Scorpion 35SL and Admire Pro, respectively) at different field-relevant concentrations based on those previously determined in apple nectar and pollen (Heller et al. 2020). Mortality was assessed at 12-hour intervals for 48 hours, after which mortality was assessed every 24 hours for 8 days using 15 bees per dose, and the bioassay was replicated 3 times. Concentration-mortality curves (Fig. 2-1; Fig. 2-2; Fig. 2-3; Fig. 2-4) were produced using POLOPlus 2.0 (LeOra Software 2005). While honey bees are often used as surrogates for other bee species, this does not take into account differences in pesticide metabolism and natural history between bee groups. Thus, we included *O. cornifrons* in our risk assessment as a representative of solitary bee species since solitary bees make up the majority of bee species for which far less is known about risks from insecticides than for honey bees (Lundin et al. 2015; Manjon et al. 2018; Danforth et al. 2019). Risk quotients were calculated using concentrations at the mean and lower and upper limits of the 95% CIs (RQ_{mean}, RQ_{lower}, RQ_{upper}), then were compared to the level of concern (LoC) value for acute exposure of 0.4, with quotients above the LoC indicating potential risk as described previously by OPP, PMRA and CALDPR (2014). This LoC value is considered highly conservative and is based on an effect level consistent with control mortality from laboratory studies. We included risk quotients calculated using concentrations at the upper and lower limits of the 95% confidence intervals because the population (or "true") means may be different than our sample means.

RESULTS

Dinotefuran Residues

For red maples treated with dinotefuran in 2018 and sampled in April of 2019, comparison of flower residue concentrations by application method and treatment season was not significant (F = 3.39, df = 2, 14, $p = 0.063$). Comparison by treatment season only showed dinotefuran treatments applied in fall had significantly higher residue concentrations in whole flowers than treatments applied in summer (F = 7.13, df = 1, 15, $p = 0.018$) (Table 2-1).

For red maples treated in 2019 or 2020 and sampled the following March of each respective year, we compared residue concentrations by application method, treatment season, treatment year, and site location. Residue concentrations from trees treated in 2019 were significantly higher than those treated in 2020 ($p < 0.001$) (Table 2-1), so we separated the subsequent analyses by treatment year. For red maples treated in 2019 and sampled in March of 2020, comparison by application method, treatment season, and site location was overall insignificant (F = 1.77, df = 4, 26, $p = 0.166$). In the best fit reduced model, which compared means by treatment season only, residue concentrations from treatments applied in fall were significantly higher than those from treatments applied in summer (F = 4.37, df = 1, 29, $p = 0.046$) (Table 2-1). Residue concentrations in whole flowers from each treatment season were significantly higher than those from flowers of untreated controls that were collected in the same year (all $p < 0.001$).

For red maples treated in 2020 and sampled in March of 2021, comparison by application method, treatment season, and site location was not significant (F = 2.05, df = 4, 18, p = 0.131), and there was no significant difference in mean residue concentrations between treatments applied in summer compared with fall (F = 3.64, df = 1, 21, p = 0.070; Table 2-1). However, residue concentrations in flowers from dinotefuran treatments applied in 2020 were significantly higher than those from flowers of untreated controls collected in the same year ($p < 0.001$). For tree-of-heaven treated in summer of 2020 and sampled in June of 2021 (n = 28), dinotefuran residues were only detected in flowers of one tree (2.75 ppb), which was treated with a trunk spray in July.

For honey bees, none of the risk quotients calculated using mean (95% CI) concentrations and LC_{50}s for dinotefuran (Fig. 2-1) exceeded the LoC value of 0.4 (Table 2-1). For *O. cornifrons*, only dinotefuran treatments applied to red maples in fall 2018 had RQ_{mean}, RQ_{lower}, and RQ_{upper} values that exceeded the LoC (Table 2-1; Fig. 2-2). No other dinotefuran treatment group for red maple or tree-of-heaven had RQ values for *O. cornifrons* that exceeded the LoC.

Imidacloprid Residues

For red maples treated in 2019 and 2020, application method, treatment year, treatment season, and site location did not significantly affect mean residues (F = 1.89, df = 6, 33, p = 0.112). However, with backward selection, the best fit reduced model found that residues from treatments applied in summer were significantly higher than residues from treatments applied in fall (F = 11.88, df = 1, 38, p = 0.001; Table 2-1). Residue concentrations in whole flowers from the summer treatments were significantly higher than those from flowers of untreated controls collected in 2020 and 2021 (all $p < 0.05$), while residues in whole flowers from the fall treatments did not differ from untreated controls.

None of the risk quotients calculated using mean (95% CI) concentrations and LC_{50}s for imidacloprid in honey bees (Fig. 2-3) exceeded the LoC value of 0.4 (Table 2-1). For *O. cornifrons*, however, imidacloprid treatments applied in spring 2018 (at double the maximum label rate) had RQ_{mean}, RQ_{lower}, and RQ_{upper} values that exceeded the LoC (Fig. 2-4). Imidacloprid treatments applied in summer of 2019 or 2020 had RQ_{mean} and RQ_{upper} values for *O. cornifrons* that exceeded the LoC, whereas only the RQ_{upper} value for imidacloprid treatments applied in fall of 2019 or 2020 were above the LoC.

DISCUSSION

Extensive research has been conducted to understand the lethal and sublethal impacts of neonicotinoids on bee pollinators, with imidacloprid being the most studied of these compounds (Lundin et al. 2015). The acute oral LC_{50} for imidacloprid in honey bees has been previously reported as low as 185 ppb (Schmuck et al. 2001), while the chronic oral LC_{50} has been reported at 20 ppb (Schmuck 2004); this acute oral LC_{50} is well below the acute oral LC_{50} of imidacloprid for honey bees reported herein at 877 ppb from ingestion bioassays using diluted commercial products (Fig. 2-3). However, the mean residue concentration of the spring 2018 imidacloprid treatment approached the LC_{50} reported by Schmuck et al. (2001) with no other mean residue concentrations from the imidacloprid treatment groups exceeding 20 ppb (Table 2-1). It should be emphasized that the spring 2018 imidacloprid treatment was applied at double the maximum label rate, so real-world implications for results from this treatment should be interpreted with caution.

In honey bees, acute exposure to imidacloprid at 75 ppb can impair navigation and at 100 ppb can temporarily reduce motor activity (Medrzycki et al. 2003; Fischer et al. 2014). Only the mean residue concentration from the spring 2018 treatment (applied at double the maximum label rate) exceeded the above sublethal concentrations, so the real-world risk of sublethal effects to honey bees from acute exposure to residue concentrations from the imidacloprid treatments groups may be low. In buff-tailed bumblebees (*Bombus terrestris*), the chronic oral LC_{50} for imidacloprid was reported at 59 ppb when provided treated sugar water in an artificial nest box, and even lower at 20 ppb in a separate test where bees were required to leave the nest box to forage (Mommaerts et al. 2010). Again, only residue concentrations from the spring 2018 imidacloprid treatments (applied at double the maximum label rate) exceeded these concentrations, so the real-world risk of sublethal effects in bumblebees from chronic exposure to residues from the imidacloprid treatment groups may be low. Another study reported that *B. terrestris* fed exclusively pollen and sugar water containing 6 and 0.7 ppb of imidacloprid, respectively, were significantly less likely than controls to bring back pollen and returned with less pollen per hour when allowed to forage (Feltham et al. 2014). Considering these concentrations are within the 95% CIs for our summer and fall treatments in 2019 and 2020 and the spring 2018 treatment, there is potential for sublethal effects to occur in bumblebees exposed to flowers containing imidacloprid residues at the concentrations found in these imidacloprid treatment groups.

While little is known about the impacts of neonicotinoids on solitary bees, it has been shown that blue orchard bee (*Osmia lignaria*) larvae chronically exposed to 30 ppb of imidacloprid via pollen had prolonged development (Abbott et al. 2008; Lundin et al. 2015). Only the mean imidacloprid residue concentrations from the spring 2018 treatment (applied at double the maximum label rate) exceeded this sublethal concentration. More research should be undertaken to understand the impacts of neonicotinoids on solitary bees, which comprise the overwhelming majority of bee species (Danforth et al. 2019). Comparisons of RQ values for honey bees and *O. cornifrons* to a LoC suggest differences in risk between these bee species from imidacloprid residues in flowers from treatments to red maple. This was expected given the differences in insecticide metabolism previously shown between bee groups (Manjon et al. 2018). Since none of the RQ values for honey bees for any imidacloprid or dinotefuran treatment groups exceeded the LoC, it appears that the acute risk to honey bees from these treatments may be minimal.

However, some treatment groups showed residue concentrations with potential for acute risk to *O. cornifrons* (Table 2-1). Flower samples from imidacloprid treatments applied to red maple in spring of 2018 (applied at double the maximum label rate) and summer of 2019 or 2020 each had RQ_{mean} and RQ_{upper} values that exceeded the LoC, and the RQ_{lower} value for the spring 2018 treatment group exceeded the LoC. While the group of imidacloprid treatments applied to red maple in fall of 2019 or 2020 did not have RQ_{lower} or RQ_{mean} values exceeding the LoC, the RQ_{upper} value for this group did exceed the LoC. These RQs suggest potential for acute risk to some solitary bee species from imidacloprid treatments, particularly applied in spring or summer rather than fall.

The impacts of dinotefuran exposure to bees have received less attention than most other neonicotinoids (Lundin et al. 2015). The acute oral LC_{50} for dinotefuran in honey bees has been reported as low as 380 ppb (Hopwood et al. 2016), which is much lower than the LC_{50} we obtained (LC_{50} = 9,823 ppb; Fig. 2-1). Since the mean residue concentration from dinotefuran applied to red maple in fall 2018 exceeded the previously reported LC_{50} of 380 ppb (Table 2-1), there may be potential for acute risk to honey bees from these treatments. Concentrations of racemic dinotefuran as low as 130 ppb can impair honey bee short- and long-term memory performance and responsiveness to sucrose (Liu et al. 2019). Dinotefuran treatments applied in fall 2018 and fall 2019 had residue concentrations within their respective 95% CIs that exceeded this lower threshold (Table 2-1), suggesting potential for sublethal effects to honey bees from these treatments. To our knowledge, dose-mortality studies of dinotefuran have seldom been done for solitary bees. A recent meta-analysis found no data on the sublethal effects of field-realistic exposure to dinotefuran on non-*Apis* bees (Siviter et al. 2021).

In this study, we reported the first comparison of relative toxicity of dinotefuran between *O. cornifrons* and honey bees using formulated product (Scorpion 35SL) (Fig. 2-1; Fig. 2-2), from which we obtained LC_{50}s for risk quotient calculations (Table 2-1). The only dinotefuran treatment group that had RQ values for *O. cornifrons* that exceeded the LoC were those applied to red maple in fall 2018. Systemic neonicotinoid applications to trees by USDA-APHIS for SLF control are only applied to tree-of-heaven (USDA-APHIS 2021), in which we detected residues in the flowers of only one sample, and these residues were at a very low concentration (2.75 ppb), with RQ values for honey bees and *O. cornifrons* that did not exceed the LoC. This suggests that there may be minimal acute risk of direct mortality to non-target bee pollinators from tree-of-heaven trap trees made via neonicotinoid application under the USDA-APHIS SLF control program in the context of our results.

Residue concentrations in red maple flowers sampled in the spring after post-bloom treatments the year before were significantly different by treatment year for dinotefuran treatments, but not for imidacloprid treatments (Table 2-1). Differences in residue concentrations between years may be explained by several abiotic factors, including average yearly rainfall or temperature (Bonmatin et al. 2015). Residue concentrations in red maple flowers did not differ significantly by method of application, which was unexpected since different methods of application determine how quickly neonicotinoids are absorbed and translocated (Herms et al. 2014), resulting in different residue concentrations in pollen and nectar among application methods (Cowles and Eitzer 2017). Dinotefuran residue concentrations in red maple flowers trended significantly higher for fall than summer applications for treatment years 2018 and 2019, but not for treatment year 2020 (Table 2-1). In contrast, imidacloprid residue concentrations in red maple flowers trended significantly higher for spring and summer than fall applications. These contrasting findings may be due to the lower water solubility of imidacloprid (610 mg/L) compared to dinotefuran (39,830 mg/L), which could limit imidacloprid's ability to travel through the plant's vascular system before it begins to enter winter dormancy, or due to differences in half-life between these neonicotinoids, which influences persistence in target plants (Bonmatin et al. 2015; Hopwood et al. 2016; Mach et al. 2018; Fadón et al. 2020).

Our limited ability to detect dinotefuran residues in tree-of-heaven nearly one year following treatment is consistent with a report that showed dinotefuran trunk sprays yielded residues in foliage that decreased greatly throughout the year following the initial application (Lewis 2017). The lack of dinotefuran residues in flowers of tree-of-heaven compared to red maple may be explained by potential differences in metabolism between the two species (Manjon et al. 2018). However, given that we found dinotefuran residues in flowers to be highly variable between application years in red maples and we only have one year of residue data from tree-of-heaven, the lack of dinotefuran residues in tree-of-heaven flowers should be interpreted with caution, as further sampling across years may yield different results.

There have been few surveys performed to ascertain the assemblage of floral visitors to red maple or tree-of-heaven, but what has been reported thus far can provide insights into the floral visitors that may be exposed to neonicotinoid residues from treatments for SLF control. Research on floral visitors to red maple conducted at the Beltsville Agricultural Research Center in Maryland found red maple to be important for early-spring emerging pollinators, with species from the bee families Colletidae, Andrenidae, Halictidae, Megachilidae, and Apidae found foraging in red maple flowers along with species from other insect groups, such as flies, wasps, and beetles (Batra 1985). This survey, combined with our results, suggests that a diversity of early spring-emerging bees and other pollinator groups may be negatively impacted by neonicotinoid treatments to red maple for SLF control, especially if trees are treated with dinotefuran in the fall or with imidacloprid in the spring or summer.

Surveys of floral visitors to tree-of-heaven conducted in Virginia and West Virginia were dominated by the margined soldier beetle, *Chauliognathus marginatus*, along with large numbers of ants (Thompson 2008), while surveys in Chicago showed most visits were made by bees and flies (Aldrich et al. 2008). However, we did not detect dinotefuran residues in most flowers of treated tree-of-heaven, suggesting that floral visitors to this tree species may face minimal risk. There are other tree species besides red maple and tree-of-heaven being treated with neonicotinoids for SLF control by homeowners and landscape professionals, so the list of beneficial non-target insect species potentially impacted by exposure to neonicotinoids from residues in flowers of treated trees is likely larger than that composed from these surveys.

There are several other factors that should be taken into consideration when interpreting our results. The real-world concentrations that non-target floral visitors may be exposed to when foraging is likely different than the residue concentrations obtained from our whole flower samples, considering that residue concentrations have previously been shown to be different between whole flowers, pollen, and nectar (Heller et al. 2020; Zioga et al. 2020; Phan 2021). Individual trees may have variable residue concentrations due to differences in age, health, and local environmental factors (soil type, rainfall, temperature) (Bonmatin et al. 2015). There is also potential for neonicotinoid residue levels to increase in flowers of treated plants across years following initial applications of soil drenches (Hopwood et al. 2016), particularly for imidacloprid due to its affinity to bind to soil, persistence, and lower water solubility (Gervais et al. 2010; Bonmatin et al. 2015; Mach et al. 2018). Repeated applications of neonicotinoids to the same target plants may lead to accumulation of residues in plant parts (Hopwood et al. 2016). The different life histories of floral visitor groups should also be considered, as this may impact the extent to which a species is directly exposed to residues. For example, there is variation among solitary bee species in the kinds of floral resources they collect (Danforth et al. 2019), and these resources in treated plants likely contain different levels of neonicotinoid residues (Mach et al. 2018; Heller et al. 2020). Our whole flower samples contained vegetative plant parts that are unlikely to be consumed by pollinators visiting flowers for pollen and nectar (Bonmatin et al. 2015). Also, our risk assessment comparisons primarily focused on acute exposure; floral visitors may be exposed chronically, which should be considered when interpreting our results. While adult bees could be at acute risk of exposure, immature bee larvae are likely to be exposed chronically, as they are fed pollen and nectar resources throughout their development (Danforth et al. 2019). Foraging adult bees may reduce their exposure to contaminated nectar as they empty their crop when they return to the nest (Carreck and Ratnieks 2014). Generalist floral visitors of tree-of-heaven may have many options of other flowering plant species to forage upon during the late-spring/early-summer period when tree-of-heaven blooms, while availability of alternative forage to red maple floral visitors is much more limited during early spring when few other flowering plants are in bloom. Accessibility to alternative untreated forage may help to mitigate the effects of neonicotinoid exposure from treated plants, as has been suggested in previous work with treated oilseed rape crops (Carreck and Ratnieks 2014; Balfour et al. 2017).

In conclusion, our results suggest that there is potential for risk to bees and other floral visitors to trees treated with neonicotinoids for SLF control, however the degree of risk is dependent on multiple factors, such as application timing, type of neonicotinoid, the species of tree being treated, and bee sensitivity. While we found that whole flower residue concentrations did not significantly differ by application method, the season when neonicotinoids are applied can significantly influence residue concentrations in red maple flowers the following spring, with higher dinotefuran residues found in fall treatments relative to summer and higher imidacloprid residues found in summer treatments relative to fall. Our acute risk assessments using RQs suggest minimal acute risk to honey bees from the residue concentrations we observed in flowers from red maple treated with imidacloprid or dinotefuran and tree-of-heaven treated with dinotefuran, while there is potential for acute risk to solitary bee species from some of the red maple treatment groups in our study. Further studies are recommended to better understand how variability in many biotic and abiotic factors may impact risk of toxicity of neonicotinoids used for SLF control to beneficial insects. To provide more comprehensive risk assessments to beneficial non-targets and explore reduced-risk alternatives within the context of SLF management, future studies should consider greater replication, a greater diversity of plants likely to be treated for SLF control (e.g., other maple species, willows, walnuts), multiple samplings within and across years following initial applications, the effects of multiple neonicotinoid applications across time, sampling specific plant resources beyond whole flowers (e.g., leaves, pollen, nectar), effects on beneficial organisms beyond bee pollinators (e.g., beetles and fly pollinators, biocontrol agents), alternative treatment timings (i.e., pre-bloom applications) and methods (e.g., foliar applications), and more active ingredients (e.g., alternative neonicotinoids, pyrethroids).

TABLES AND FIGURES

Table **2-1**: Mean (95% CI) neonicotinoid residue concentrations in whole flower samples collected during bloom for red maple (March or April) and tree-of-heaven (June) treated with dinotefuran or imidacloprid the year before. Included are risk quotients for *Apis mellifera* and *Osmia cornifrons*.

Tree Species	**Active Ingredient**	**Treatment Year**	**Treatment Season**	**Mean** (95% CI) Residue Conc. (ppb)**	***Apis mellifera***			***Osmia cornifrons***		
					RQ_{mean}	**RQ_{lower}**	**RQ_{upper}**	**RQ_{mean}**	**RQ_{lower}**	**RQ_{upper}**
Acer rubrum	Dinotefuran	2018	Summer	0a	0	NA	NA	0	NA	NA
			Fall	441.1b (247.9 –634.2)	0.04	0.03	0.06	1.09*	0.61*	1.57*
		2019	Summer	71.1a (37.9 – 104.2)	0.01	< 0.01	0.01	0.18	0.09	0.26
			Fall	122.0b (83.0 – 161.0)	0.01	0.01	0.02	0.30	0.20	0.40
		2020	Summer + fall	11.1 (6.7 – 15.5)	< 0.01	< 0.01	< 0.01	0.03	0.02	0.04
	Imidacloprid	2018	Spring	106.3(NA) (46.2 – 166.4)	0.12	0.05	0.19	5.31*	2.31*	8.32*
			Fall	0(NA)	0	NA	NA	0	NA	NA
		2019, 2020	Summer	12.3a (7.0 – 17.6)	0.01	0.01	0.02	0.62*	0.35	0.88*
			Fall	4.7b (0.0 – 11.2)	0.01	< 0.01	0.01	0.24	0	0.56*
	Control	NA	NA	0	0	NA	NA	0	NA	NA
Ailanthus altissima	Dinotefuran	2020	Summer	0.2 (0.0 – 0.6)	< 0.01	0	< 0.01	< 0.01	0	< 0.01
	Control	NA	NA	0	0	NA	NA	0	NA	NA

*Exceeds the level of concern (LoC) of 0.4, indicating potential acute risk to *Apis mellifera*, a surrogate for social bees, or *Osmia cornifrons*, a surrogate for solitary bees.

**Means followed by different letters within treatment year groups are significantly different at $p < 0.05$.

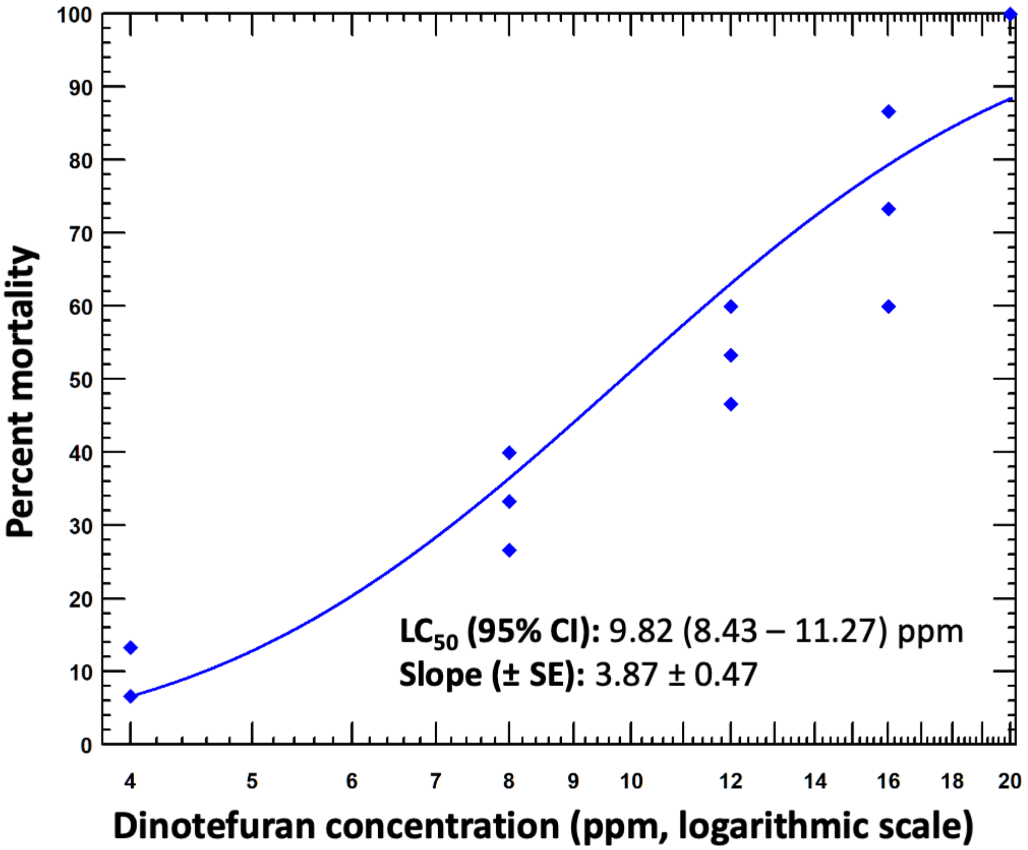

Figure **2-1**: Concentration-mortality curve derived from acute oral ingestion bioassays of dinotefuran in *Apis mellifera*. Each blue diamond indicates a replicate group of 15 bees used to generate the curve and the experiment was replicated three times. No mean (95% CI) residue concentrations from whole flower samples of any dinotefuran treatment in this study were high enough to exceed the lower concentration threshold of the curve.

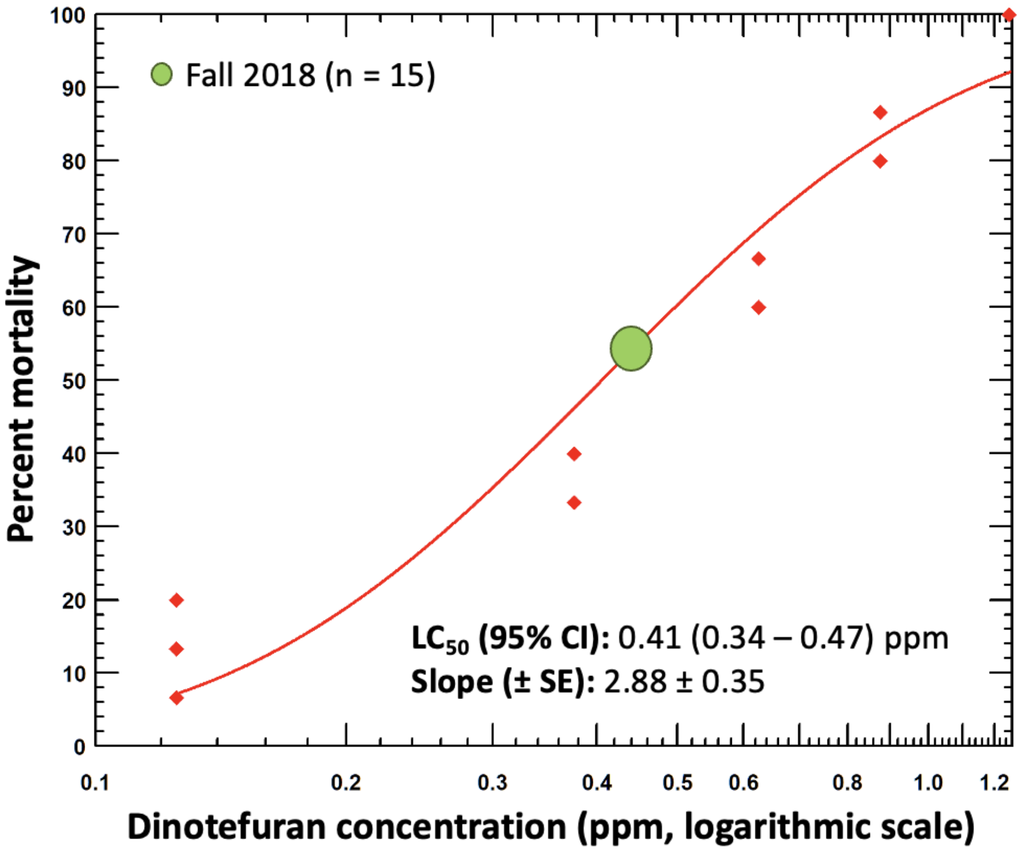

Figure **2-2**: Concentration-mortality curve derived from acute oral ingestion bioassays of dinotefuran in *Osmia cornifrons*. Each red diamond indicates a replicate group of 15 bees used to generate the curve and the experiment was replicated three times. Fall 2018 applications of dinotefuran to red maple had a mean residue concentration of 0.441 ppm in the flowers the following spring, shown on the curve. While the mean residue concentration for dinotefuran treatments applied in fall 2019 did not exceed the lower threshold of the curve, these treatments did have a 95% confidence interval upper limit that exceeded the lower threshold of the curve at 0.161 ppm.

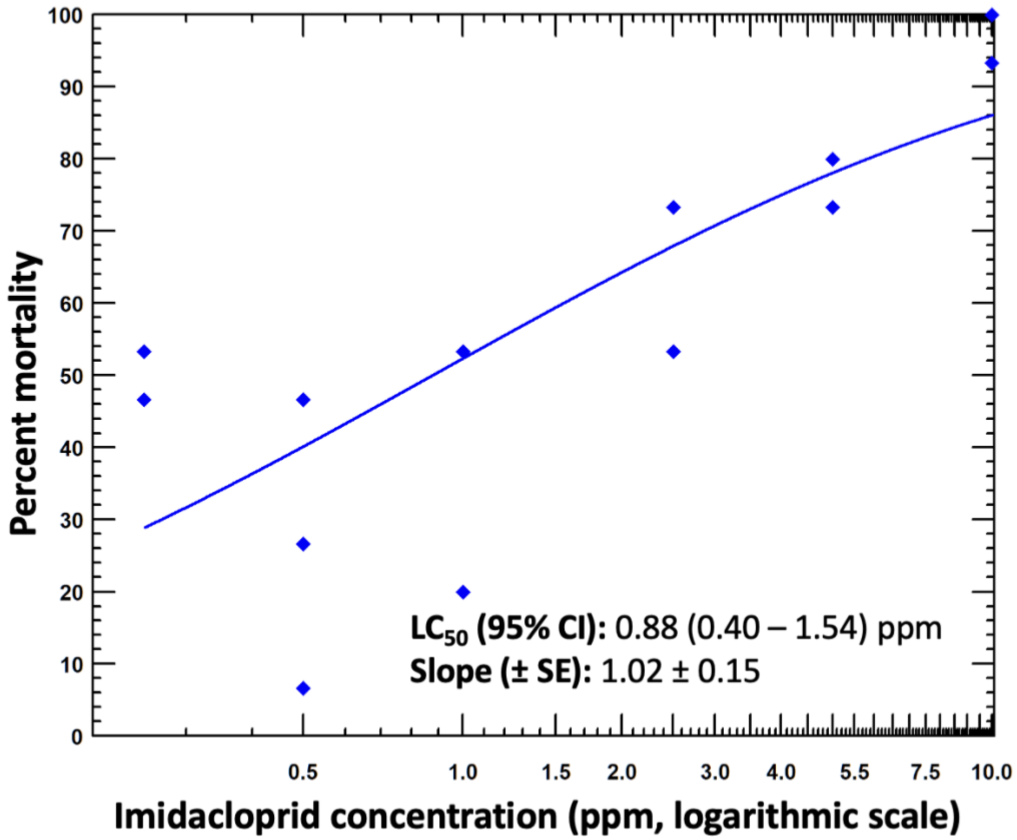

Figure **2-3**: Concentration-mortality curve derived from acute oral ingestion bioassays of imidacloprid in *Apis mellifera*. Each blue diamond indicates a replicate group of 15 bees used to generate the curve and the experiment was replicated three times. No mean (95% CI) residue concentrations from whole red maple flower samples of any imidacloprid treatment were high enough to exceed the lower concentration threshold of the curve during bloom the following year.

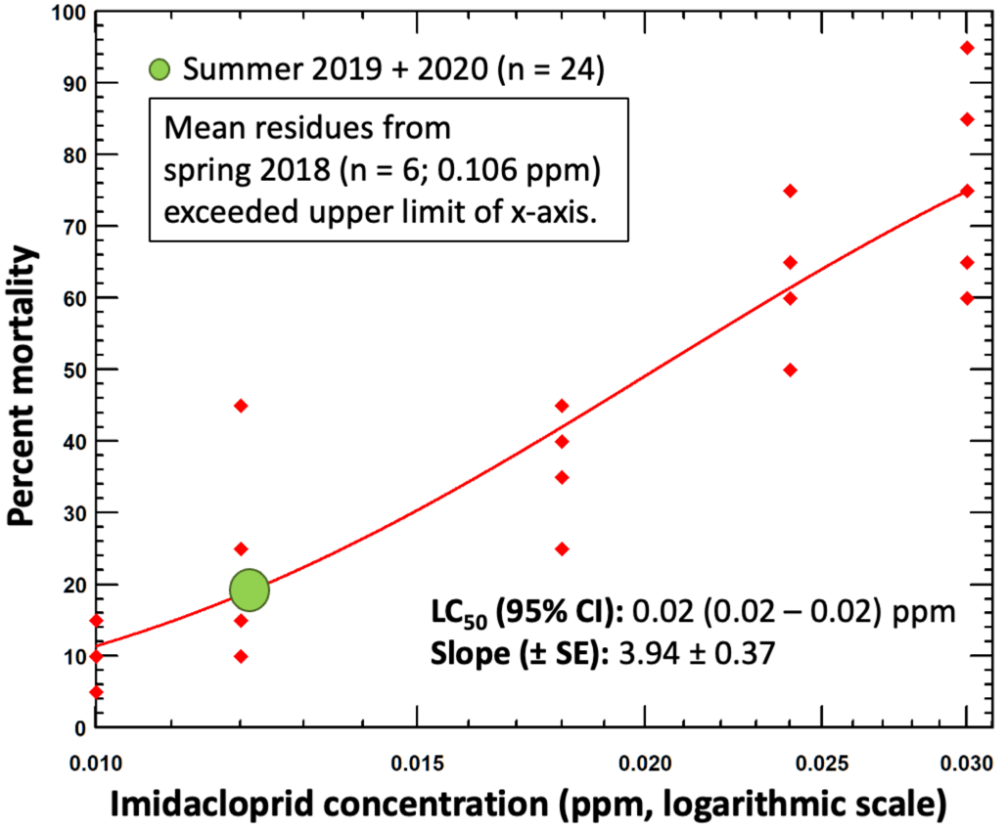

Figure **2-4**: Concentration-mortality curve derived from acute oral ingestion bioassays of imidacloprid in *Osmia cornifrons*. Each red diamond indicates a replicate group of 15 bees used to generate the curve and the experiment was replicated three times. Imidacloprid treatments applied in summer 2019 or 2020 with a mean residue concentration within the upper and lower concentration thresholds of the curve in red maple flowers were placed along the curve at 0.012 ppm. The mean residue concentration for imidacloprid treatments applied in spring 2018 exceeded the upper limit of the x-axis at 0.106 ppm. Mean residue concentration for imidacloprid treatments applied in fall 2019 or 2020 did not exceed the lower threshold of the x-axis, but these treatments did have a 95% confidence interval upper limit that exceeded the lower threshold of the x-axis at 0.011 ppm.

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Chapter 3

Insect floral visitors of red maple (*Acer rubrum*) and tree-of-heaven (*Ailanthus altissima*) at potential risk of neonicotinoid residue exposure from spotted lanternfly (*Lycorma delicatula*) control

INTRODUCTION

The spotted lanternfly (*Lycorma delicatula* (White) [Hemiptera: Fulgoridae]; hereafter SLF) is a pestiferous planthopper which has recently invaded the United States (Dara et al. 2015). First reported in Berks County, Pennsylvania in 2014 (Barringer et al. 2015), SLF has since expanded its range to include 11 US states, mostly in the mid-Atlantic, where it threatens grape, timber, and other industries, as well as the health of ornamental trees (Urban 2020; NYSIPM 2022). SLF damages host plants directly by feeding on the plant's phloem, creating wounds where feeding has taken place, and indirectly by secreting honeydew, which facilitates the growth of sooty mold that covers leaves and hinders the plant's photosynthetic capabilities (Dara et al. 2015).

Efforts to manage this pest in the US include applications of systemic neonicotinoid insecticides (IRAC code 4A) to SLF host plants (IRAC 2021; Korman et al. 2021b; USDA-APHIS 2021). Neonicotinoids control pests by binding to nicotinic acetylcholine receptors, which can cause continuous muscle excitement, impaired motor function, and death, among other sublethal effects such as reductions in reproductive output and impairments to memory (Blacquière et al. 2012; Simon-Delso et al. 2015; Siviter et al. 2021). They are the most widely used class of insecticides and are preferred for several characteristics, including persistence, selectivity for invertebrates, and moderate-to-high water solubility, which allows for movement throughout target plants via the vascular system (Ney 1995; Jeschke et al. 2011; Simon-Delso et al. 2015).

Neonicotinoid applications to tree-of-heaven (*Ailanthus altissima*) are conducted by the US Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) and state agricultural departments, such as the Pennsylvania Department of Agriculture, under the SLF control program (USDA-APHIS 2021). Tree-of-heaven is a deciduous tree, native to China, that was introduced to the US in 1784 as an ornamental and is now a widespread invasive in the United States (Hu 1979; Miller 1990). Tree-of-heaven is a highly preferred host of SLF and so is targeted for removal by herbicides or treated with neonicotinoids as trap trees to attract and kill SLF (Liu 2019; Derstine et al. 2020; USDA-APHIS 2021).

Homeowners and landscape professionals treat ornamental trees, such as red maple (*Acer rubrum*), with neonicotinoids to reduce populations of SLF (Korman et al. 2021a; Korman et al. 2021b). Red maple is a deciduous hardwood tree native to and commonly found in the eastern United States (Walters and Yawney 1990); it can be used for lumber and syrup production and is a popular landscape ornamental. Red maple can be a host for all life stages of SLF, while it is primarily used by the adults in late fall when tree-of-heaven begins to senesce (Barringer and Ciafré 2020; Mason et al. 2020).

Since the introduction of neonicotinoids, much evidence has been gathered of their potential for harm to non-target invertebrates that provide important ecosystem services, such as pollination (Blacquière et al. 2012; Chagnon et al. 2015; Pisa et al. 2015; Main et al. 2018; Siviter et al. 2021). The persistent and systemic nature of neonicotinoids can facilitate the accumulation of residues in floral resources (pollen, nectar) of treated plants, collected as nutritional resources by bees and other insect pollinators (Bonmatin et al. 2015). Researchers have proposed the inclusion of pollinator health in the development of pest management programs (Biddinger and Rajotte 2015).

Currently, there is little knowledge of the identities of insects that visit flowers of red maple or tree-of-heaven, which could be at risk of exposure to neonicotinoid residues in flowers of trees treated post-bloom the year before for SLF control. Red maples are polygamodioecious (individual trees can have entirely male flowers, entirely female flowers, or both male and female flowers on the same tree) with small, often red, structurally perfect flowers that bloom before leafing (Walters and Yawney 1990). Previous surveys of the floral visitors of red maple in Maryland reported visits by early spring emerging bees, flies, wasps, and beetles, suggesting that while considered to be wind-pollinated, this tree is an important nutritional resource in the early spring when few other flowering plants are in bloom (Batra 1985).

Tree-of-heaven are dioecious (individual trees have either entirely male or female flowers) with small, yellowish-green flowers arranged in panicles (Hu 1979; Miller 1990). Honey bees have been reported to forage on the floral resources of tree-of-heaven, based on identification of tree-of-heaven pollen grains in honey samples and by observation (Melville, 1944; Elton, 1945). Collections of insect visitors to tree-of-heaven flowers in Virginia and West Virginia were dominated by the margined soldier beetle (*Chauliognathus marginatus*), with other visitors including ants, bees, and flies (Thompson 2008), while most insects collected from tree-of-heaven flowers in surveys conducted in Chicago were flies and bees (Aldrich et al. 2008).

To better understand which insects may be at risk of exposure to neonicotinoid residues from flowers of trees treated for SLF control, we performed observations and collections of floral visitors to red maple and tree-of-heaven in Pennsylvania. With these observations and collections, we sought to determine which insect taxa were the most abundant floral visitors of each tree species and which taxa performed the most visits to flowers of each tree species.

MATERIALS AND METHODS

Survey Sites

Surveys of insect visitors to red maple flowers were performed at multiple sites across Pennsylvania in March of 2021 and 2022. In 2021, surveys were performed at six sites; four of these sites were at Penn State University's main campus in Centre County, PA, and the remaining two sites were located at Penn State's Fruit Research and Extension Center (FREC) in Adams County, PA and a neighborhood in Berks County, PA. In 2022, these surveys were performed across seven sites; five were at Penn State University's main campus, with three out of these five sites previously surveyed in 2021, and the remaining two sites were located at the FREC, with one of these two sites previously surveyed in 2021. Each site contained five mature red maple trees, which were all surveyed except for when weather conditions and daylight availability were not conducive to insect flight.

Surveys of insect visitors to tree-of-heaven flowers were performed at multiple sites across Berks County, PA during the summers of 2020 and 2021. In June and July 2020, surveys were performed at three sites located at the Penn State's Berks campus (Reading, PA), Blue Marsh Lake Recreation Area (Bernville, PA), and near the Reading-Muhlenberg Career and Technology Center (Reading, PA). Surveys were performed on one mature tree-of-heaven at each of the sites in 2020. In June 2021, surveys were performed at six sites; two of these sites were previously surveyed in 2020 (at Berks campus and Blue Marsh), three of the sites added in 2021 were at Blue Marsh, and one site added in 2021 was located between Berks campus and Blue Marsh, along a highway exit. Surveys were performed at two mature tree-of-heaven at each of the sites in 2021.

Floral Visitor Observations

To determine the frequency of insect visitations to red maple and tree-of-heaven flowers, insects were observed approaching flowers and their visits were tallied. A visit was tallied when an insect contacted a blooming flower and appeared to actively forage for nectar or pollen. Each distinct visit was tallied, even when performed by the same visitor. Visit tallies were categorized into broad morpho-groups for ease of identification by observers.

For each red maple observation period, two observers were positioned on opposite sides of a single red maple tree. Each observer selected a flowering branch and tallied visits for 5 minutes from where the base of the branch was at least 1-inch in diameter to the terminal end of the branch.

For each tree-of-heaven observation period, each observer selected a flowering panicle and tallied visits to flowers along the entire panicle. In 2020, one observer performed surveys for 30 minutes at a single tree per site (except for the first day of surveys which were performed for 15 minutes each period). In 2021, two observers performed surveys for 10 minutes, with each observer at a single tree per site. The observation period times between 2020 and 2021 were decreased from 30 minutes to 10 minutes to allow for more sites to be surveyed each day.

Floral Visitor Collections

To taxonomically identify insect visitors to red maple and tree-of-heaven flowers, insects were observed approaching flowers and collected upon each visit. Collections were conducted immediately following each observation period at the same tree from the prior observation period and with the same criteria for ‘visit’ for observations that were used for collections. For red maple collections, each observer maintained their positions on opposite sides of the tree and collected insect visitors with nets across their respective halves of the tree within reach for 5 minutes. For tree-of-heaven floral visitor collections, each observer collected insect visitors with jars at the same panicle from the prior observation period. After collections at a single panicle, the observer collected insect visitors along the entire tree within reach of their net. Panicle and whole-tree collections were each performed for 30 minutes in 2020 (except for the first day of surveys which were performed for 15 minutes each period) and 10 minutes in 2021. During 2020 net collections, flowers were periodically swept when visitors were not visible, which allowed for capture of small insects that could not easily be seen by collectors. This tactic was abandoned for the 2021 collections to ensure visitors could be confirmed to forage from flowers by sight. Insects were captured in jars, with ethyl acetate as a killing agent, stored in ice chests while in field, and stored at -20 °C until identification by David Biddinger via reference specimens from various bee specialists including Jason Gibbs, Robert Jean, Karen Wright, and Sam Droege and the bee identification keys of Discover Life (Ascher and Pickering, 2010).

Data Analysis

Chi-square tests were performed separately by tree species to determine if significant differences existed in total visits between morpho-groups and total collected specimens between taxonomic groups. Sites were pooled because we wanted to identify the diversity of floral visitors of red maple and tree-of-heaven, not to compare floral visitors between sites. For collected specimens, total insects were compared by order, bees collected at red maple and tree-of-heaven flowers were compared by family, and beetles collected at tree-of-heaven flowers were compared by family because these were the most abundant orders. All statistical analyses were performed in RStudio (version 4.1.0, “Camp Pontanezen”).

RESULTS

Floral Visitor Observations

For red maple, most floral visits were made by bees (407/561), particularly honey bees (n = 62) and other small bee species (n = 340), followed by flies (n = 137) (Fig. 3-1). Only 17 visits to red maple flowers were made by other insect groups. Observations at red maple flowers were performed for a total of 17.17 hours.

Most floral visits to tree-of-heaven were made by beetles (796/1498), followed by bees (n = 464), flies (n = 133), other unspecified groups (n = 86), and wasps (n = 19) (Fig. 3-2). Visits by bees were mostly made by small bees (n = 245), followed by bumblebees (n = 153), other large bees (n = 34), and honey bees (n = 32). Observations at tree-of-heaven flowers were performed for a total of 15.5 hours.

Floral Visitor Collections

For red maple, most collected floral insect visitors belonged to the order Hymenoptera (114/150), followed by Diptera (n = 34), with a single specimen each collected from the orders Hemiptera and Plecoptera (Table 3-1; Fig. 3-3). Most collected bee visitors were from the Apidae family (56/102), followed by Andrenidae (n = 32) and Megachilidae (n = 14) (Fig. 3-S1). Specimens from the family Apidae were mostly honey bees (55/56) with one bumblebee (*Bombus vagans*) collected (Table 3-1). All andrenids captured were from a single genus, *Andrena*, with seven species collected, including *A. carlini* (n = 2), *A. forbesii* (n = 1), *A. imitatrix* (n = 24), *A. miserabilis* (n = 1), *A. nivalis* (n = 2), *A. pruni* (n = 1), and *A. tridens* (n = 1). Most Megachilidae captured were *Osmia cornifrons* (13/14) along with a single captured *Osmia lignaria*. Visitor collections at red maple flowers were performed for a total of 17 hours.

For tree-of-heaven, most collected floral insect visitors belonged to the order Coleoptera (193/429), followed by Hymenoptera (n = 156), Diptera (n = 70), and

Hemiptera (n = 8), with a single specimen each collected from the orders Lepidoptera and Orthoptera (Table 3-2; Fig. 3-4). Most collected beetle visitors were from the Cantharidae family (182/193), with only 11 visits to tree-of-heaven flowers made by other beetle families (Fig. 3-S2). All cantharid specimens collected were of a single species, *Chauliognathus marginatus*. Most bee visitors were from the Halictidae family (61/127), followed by Apidae (n = 52) and Andrenidae (n = 13), with a single specimen collected from the Colletidae family (Fig. 3-S3). The halictid genera captured were *Halictus* spp., including *H. confusus* (n = 2) and *H. rubicundus* (n = 4), as well as *Lasioglossum* spp., including *L. coeruleum* (n = 1), *L. gotham* (n = 2), *L. imitatum* (n = 13), *L. paradmirandum* (n = 15), *L. pectorale* (n = 18), and *L. pilosum* (n = 15) (Table 3-2). The Apidae collected were honey bees (n = 18) and three *Bombus* spp., mostly *B. impatiens* (n = 27), followed in abundance by *B. bimaculatus* (n = 4) and *B. vagans* (n = 2). All andrenid specimens captured were of the genera *Andrena*, including *A. forbesii* (n = 2), *A. hippotes* (n = 3), *A. rugosa* (n = 3), *A. vicina* (n = 2) and *A. wilkella* (n = 3). The single colletid specimen collected was *Hylaeus modestus*. Visitor collections at tree-of-heaven flowers were performed for a total of 30.17 hours.

DISCUSSION

From our surveys of insect visitors to red maple flowers, it appears that hymenopterans, mostly honey bees and other early spring emerging small bee species, particularly from the genus *Andrena*, and dipterans are the most likely insect groups that could be exposed to neonicotinoid residues via flowers of red maples treated for SLF control in central and southeastern Pennsylvania. Of the bees we collected at red maple flowers, the western honey bee, *Apis mellifera*, is an introduced, managed, social bee in the US native to Europe, the Middle East, and Africa (Mortensen et al. 2021). In the US, this species is widely used for crop pollination and the production of hive products, including honey and beeswax, and is a generalist pollinator that can be found visiting a large variety of native and introduced flowering plants (Wilson and Carril 2015; Mortensen et al. 2021). Bees from the genera *Andrena* are solitary (some communally nest), ground-nesting, and can be native to the US (Wilson and Carril 2015; Danforth et al. 2019). Some andrenids are generalist pollinators, while others are specialists of plants including maples and willows, and they are among the first bee species to emerge in spring (Wilson and Carril 2015). The two *Osmia* species we collected, *O. lignaria* and *O. cornifrons*, are solitary mason bees managed in the US for fruit tree and almond pollination, the former being native to the US and the latter being an introduced species native to Japan (Biddinger 2018). Previous surveys of the insect visitors to red maple flowers in Maryland by Batra (1985) reported several insect families which were not found in our surveys, such as braconid and vespid wasps, colletid and halictid bees, scathophagid and tachinid flies, and coccinellid beetles. In contrast, we collected several insect families not previously reported by Batra (1985), including anthomyiid and muscid flies, Formicidae (ants), ichneumonid wasps, and single specimens from orders Hemiptera and Plecoptera (Table 3-1).

From our surveys of insect visitors to tree-of-heaven flowers, the cantharid beetle, *Chauliognathus marginatus*, was the most abundant and is the most likely insect species that could be exposed to neonicotinoid residues via flowers of tree-of-heaven treated for SLF control in southeastern Pennsylvania. *C. marginatus* is considered a beneficial insect (Catron et al. 2019); adults can be found in abundance during spring and summer in the eastern US visiting a variety of wild and cultivated flowers for pollen and nectar, making them likely pollinators of a diversity of plants. Their larvae can prey on soft-bodied insect pests, such as aphids, moth larvae, and thrips. Outside of this single coleopteran species, it appears the most likely insect groups that could be at risk from neonicotinoid treatments via contaminated tree-of-heaven flowers are hymenopterans (especially bees) and dipterans. Bees from the genus *Lasioglossum*, also known as sweat bees, were the most abundant bee species visiting tree-of-heaven flowers in our study and are some of the most abundant bee species native to North America. *Lasioglossum* species vary in their sociality; most nest in the ground and most are generalist pollinators (Wilson and Carril 2015). The bumblebee *Bombus impatiens* was also relatively well represented in our bee specimens. *B. impatiens* is a social, ground-nesting species, native and commonly found in the eastern US, where it is a generalist pollinator and is also managed for the pollination of crops, such as blueberries and tomatoes (Mallinger 2018). While previous surveys of floral visitors to tree-of-heaven in Virginia and West Virginia by Thompson (2008) also reported *C. marginatus* as the dominant visitor, those surveys had a much greater representation of ant visitors and fewer bee and fly visitors than reported here (Table 3-2). In contrast, previous surveys of the floral visitors of tree-of-heaven in Illinois by Aldrich et al. (2008) reported flies as the most common floral visitor of tree of heaven, followed by bees, with very few collections of beetles.

The survey results reported here, along with results from previous surveys of the same tree species, highlight that the insect assemblages that visits red maple or tree-of-heaven are more diverse than reported previously (Batra 1985; Thompson 2008; Aldrich et al. 2008). Our surveys of red maple were markedly limited by availability of weather conditions conducive to insect activity, so it is even likely that further surveys in the areas of central and southeastern Pennsylvania could find an assemblage of floral visitors different or more diverse than our findings.

Homeowners and landscape professionals may use neonicotinoid applications for SLF control in trees other than red maple and tree-of-heaven, such as silver maples and willows (Korman et al. 2021a; Korman et al. 2021b), so the floral visitors of these tree species may also be at risk of exposure to neonicotinoid residues. Non-target insect pollinators may also be at risk of exposure to neonicotinoid residues from systemic treatments to SLF host plants via exposure other to honeydew, which is heavily secreted by SLF feeding on host plants. Non-target beneficial insects, such as bees and parasitic wasps, can forage on honeydew, especially when nectar is scarce (Calvo-Agudo et al. 2022). While we did not observe or collect insects foraging on SLF honeydew, observations of honey bees, bumblebees, flies, ants, and wasps ingesting SLF-generated honeydew secreted by adult SLF feeding on tree-of-heaven trap trees have been reported previously by Underwood et al. (2019). More studies are recommended to build a more comprehensive list of non-target insects that could be impacted from pesticide use for SLF control.

TABLES AND FIGURES

Table **3-1**: Floral visitors of red maple collected in Berks County, PA in 2021 and Adams and Centre Counties in 2021 and 2022.

Order	**Family**	**Genus**	**Species**	**Total**
Diptera	Total			34
	Anthomyiidae			10
	Calliphoridae			1
	Muscidae			13
	Syrphidae	*Eupeodes*	*pomus* (Curran)	8
		Syrphus	*ribesii* (L.)	1
		Other		1
Hemiptera	Total			1
	Miridae	*Lygus*	*lineolaris* (Palisot de Beauvois)	1
Hymenoptera	Total			114
	Andrenidae	*Andrena*	*carlini* Cockerell	2
			forbesii Robertson	1
			imitatrix Cresson	24
			miserabilis Cresson	1
			nivalis Smith	2
			pruni Robertson	1
			tridens Robertson	1
	Apidae	*Apis*	*mellifera* L.	55
		Bombus	*vagans* Smith	1
	Formicidae			5
	Ichneumonidae			3
	Megachilidae	*Osmia*	*cornifrons* (Radoszkowski)	13
			lignaria Say	1
	Tenthredinidae			4
Plecoptera	Total			1
TOTAL				156

Table **3-2**: Floral visitors of tree-of-heaven collected in Berks County, PA in 2020 and 2021.

Order	**Family**	**Genus**	**Species**	**Total**
Coleoptera	Total			193
	Cantharidae	*Chauliognathus*	*marginatus* (F.)	182
	Chrysomelidae	*Diabrotica*	*undecimpunctata* Mannerheim	1
	Coccinellidae	*Coccinella*	*septumpunctata* (L.)	1
		Cycloneda	*munda* (Say)	1
		Harmonia	*axyridis* (Pallas)	2
		Propylea	*quatorodecimpunctata* (L.)	1
	Curculionidae			1
	Dermestidae			1
	Nitidulidae			2
	Scarabaeidae	*Popillia*	*japonica* Newman	1
Diptera	Total			70
	Anthomyiidae			4
	Calliphoridae			4
	Dolichopodidae			9
	Muscidae			17
	Platystomatidae	*Rivellia*	*quadrifasciata* (Macquart)	3
	Sarcophagidae			4
	Stratiomyidae	*Myxosargus*	*nigricormis* Greene	3
	Syrphidae	*Allograpta*	*obliqua* (Say)	1
		Sphaerophoria	*asymmetrica* Knutson	1
		Syritta	*pipiens* (L.)	3
		Syrphus	*ribesii* Robertson	1
		Toxomerus	*geminatus* (Say)	8
			marginatus (Say)	7
	Tachinidae			3
	Other			2
Hemiptera	Total			8
	Cynidae			1
	Cercopidae			1
	Fulgoridae	*Lycorma*	*delicatula* (White)	1
	Miridae	*Lygus*	*lineolaris* (Palisot de Beauvois)	3
		Other		2
Hymenoptera	Total			156
	Andrenidae	*Andrena*	*forbesii* Robertson	2
			hippotes Robertson	3
			rugosa Robertson	3
			vicina Smith	2
			wilkella (Kirby)	3

Order	**Family**	**Genus**	**Species**	**Total**
	Apidae	*Apis*	*mellifera* L.	18
		Bombus	*bimaculatus* Cresson	4
			impatiens Cresson	27
			vagans Smith	2
		Ceratina	*calcarata* Robertson	1
	Chrysididae	*Elampus*	*viridicyaneus* Norton	1
		Hedychrum	*confusum* du Buysson	1
	Colletidae	*Hylaeus*	*modestus* Say	1
	Crabronidae			1
	Formicidae			11
	Gasteruptiidae			1
	Halictidae	*Halictus*	*confusus* Smith	2
			rubicundus (Christ)	4
		Lasioglossum	*coeruleum* (Robertson)	1
			gotham Gibbs	2
			imitatum (Smith)	13
			paradmirandum (Knerer and Atwood)	15
			pectorale (Smith)	18
			pilosum (Smith)	5
			Other	1
	Ichneumonidae			1
	Sphecidae	*Isodontia*	*mexicana* (Saussure)	1
	Vespidae	*Ancistrocerus*	*antilope* Panzer	1
		Monobia	*quadridens* (L.)	2
		Parancistrocerus	*bircornis* (Robertson)	1
			pensylvanicus (de Saussure)	1
			perennis (de Saussure)	4
		Pseudodynerus	*quadrisectus* Say	1
		Zethus	*spinipes* Say	1
	Other			1
Lepidoptera	Total			1
	Lycaenidae	*Celastrina*	*neglecta* (W. H. Edwards)	1
Orthoptera	Total			1
	Other			1
TOTAL				431
*Criteria “Other” indicates specimens not identified to taxonomic level of the column the criterion is listed under (and more specific taxonomic levels, where available).				

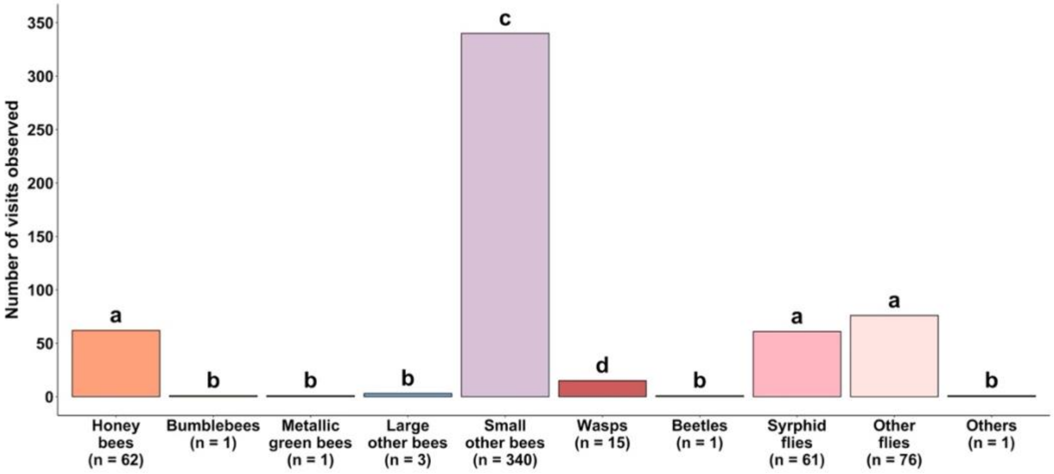

Figure **3-1**: Total number of visits to red maple flowers by morpho-groups observed over 17.2 hours in Berks County, PA in 2021 and Adams and Centre Counties in 2021 and 2022. Morpho-groups that do not share any letters above the bars were significantly different in total number of visits observed ($p < 0.05$).

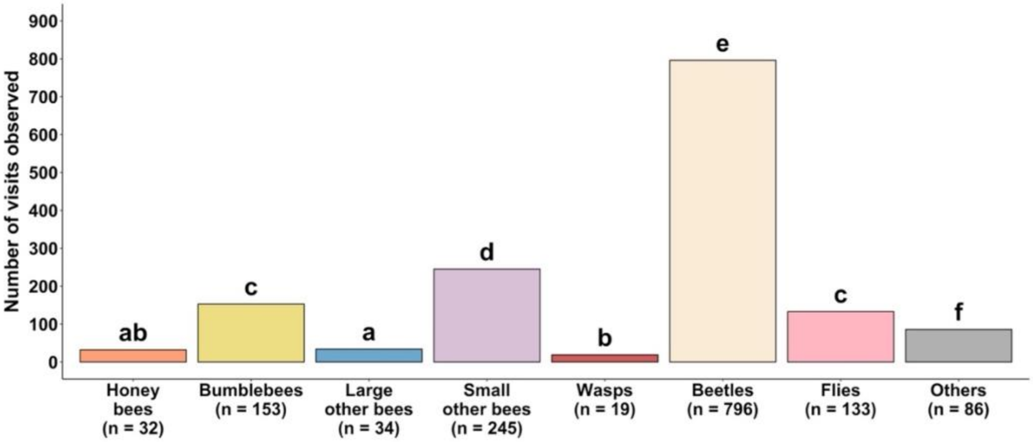

Figure **3-2**: Total number of visits to tree-of-heaven flowers by morpho-groups observed over 15.5 hours in Berks County, PA in 2021 and 2022. Morpho-groups that do not share any letters above the bars were significantly different in total number of visits observed ($p < 0.05$).

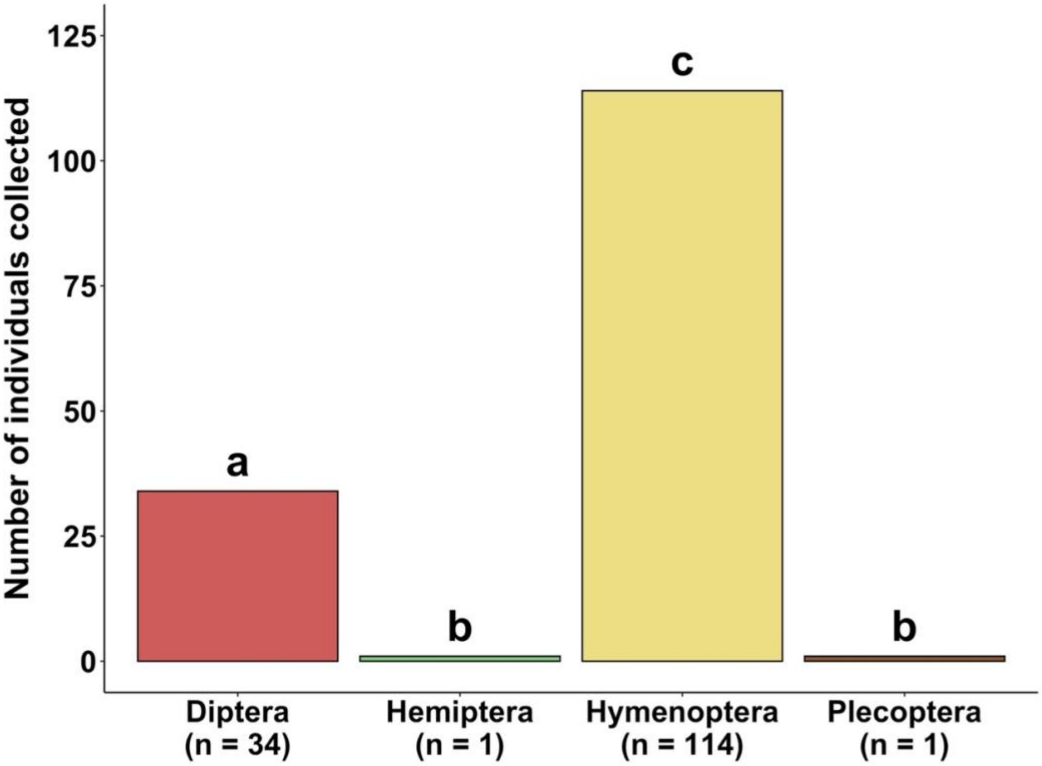

Figure **3-3**: Total number of visitors to red maple flowers by order collected over 17 hours in Berks County, PA in 2021 and Adams and Centre Counties in 2021 and 2022. Orders that do not share any letters above the bars were significantly different in total number of individuals collected ($p < 0.05$).

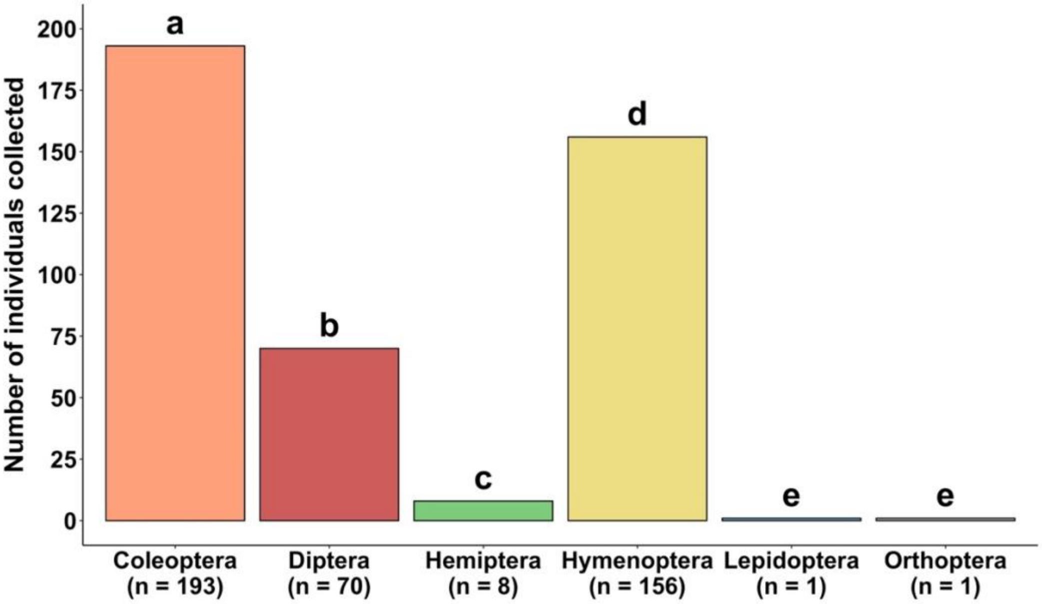

Figure **3-4**: Total number of visitors to tree-of-heaven flowers by order collected over 30.2 hours in Berks County, PA in 2020 and 2021. Orders that do not share any letters above the bars were significantly different in total number of individuals collected ($p < 0.05$).

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Appendix A

Supplementary material for Chapter 2

Table **2-S1**: Red maple and tree-of-heaven treatments. Flowers from these trees were collected the following spring for red maple and June for tree-of-heaven.

Tree Species	**App. Rate (g AI/ inch DBH)**	**App. Method**	**App. Year**	**App. Date(s)**	**Site(s)**	**No. of Trees**
Dinotefuran						
Acer rubrum	0.26	trunk injection	2018	July 27, August 30	Limerick	2
	0.26	trunk injection	2018	October 1, 9	Greenville, Limerick, Pennsburg	12
	2.53	trunk spray	2018	October 1	Pennsburg	3
	0.26	trunk injection	2019	August 14, 19	Colony, Wethersfield	6
	0.26	trunk injection	2019	September 9	Colony	5
	2.09	trunk spray	2019	August 14, 20	Colony, Wethersfield	6
	2.09	trunk spray	2019	September 10	Colony	5
	2.53	soil drench	2019	August 14, 20, 21	Colony, Wethersfield	6
	2.53	soil drench	2019	September 10	Colony	3
	0.26	trunk injection	2020	July 27, 28	Meadowood, Topton	5
	2.09	trunk spray	2020	July 27, 28	Meadowood, Topton	5
	2.09	trunk spray	2020	September 17	Topton	4
	2.53	soil drench	2020	July 27, 28	Meadowood, Topton	5
	2.53	soil drench	2020	September 17, 21	Meadowood, Topton	5
Ailanthus altissima	2.04	trunk spray	2020	July 7, 15, 20, 21	Angelica Park, Minnich Tract, Pendora Park, Wenger’s	10
	2.04	trunk spray	2020	August 28, 20	Hanover Park	4
Imidacloprid						
Acer rubrum	0.44	trunk injection	2018	May 1	Limerick	6
	1.42	soil drench	2018	October 26	Bartlett	3
	0.22, 0.33*	trunk injection	2019	August 16, 19	Colony, Wethersfield	6
	0.22, 0.33*	trunk injection	2019	September 9	Colony	4
	1.42	soil drench	2019	August 15, 19, 20	Colony, Wethersfield	6
	1.42	soil drench	2019	September 10	Colony	2
	0.45, 0.68*	trunk injection	2020	July 27, 28	Meadowood, Topton	5
	0.45, 0.68*	trunk injection	2020	September 17	Topton	5
	1.42	soil drench	2020	July 27, 28, 29	Meadowood, Topton	5
	1.42	soil drench w/ adjuvant	2020	July 28	Topton	2
	1.42	soil drench w/ adjuvant	2020	September 21	Meadowood	5
Control						
Acer rubrum	NA	NA	NA	NA	Colony (2020)	3
	NA	NA	NA	NA	Meadowood (2021)	5
Ailanthus altissima	NA	NA	NA	NA	Blue Marsh, Exit, PSU Berks, RMCTC, Sinking Spring (2021)	14
*Note: Per label, IMA-jet and IMA-jet 10 application rates increased for trees with 12-23" DBH.						

Appendix B

Supplementary material for Chapter 3

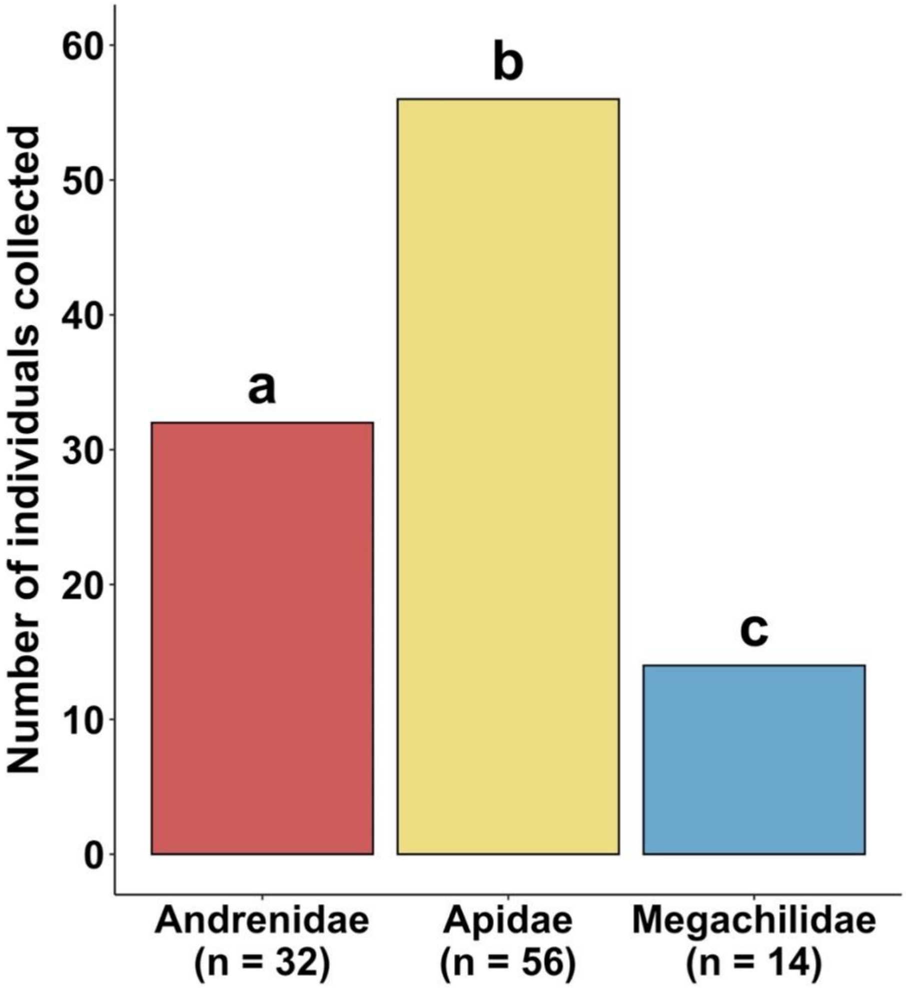

Figure **3-S1**: Total number of bee visitors to red maple flowers by family collected over 17 hours in Berks County, PA in 2021 and Adams and Centre Counties in 2021 and 2022. Families that do not share any letters above the bars were significantly different in total number of individuals collected ($p < 0.05$).

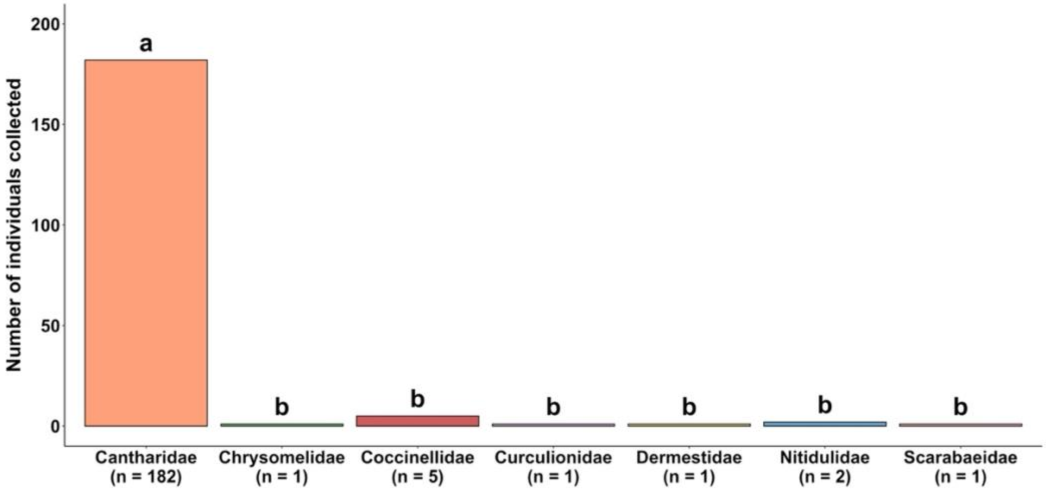

Figure **3-S2**: Total number of beetle visitors to tree-of-heaven flowers by family collected over 30.2 hours in Berks County, PA in 2020 and 2021. Families that do not share any letters above the bars were significantly different in total number of individuals collected ($p < 0.05$).

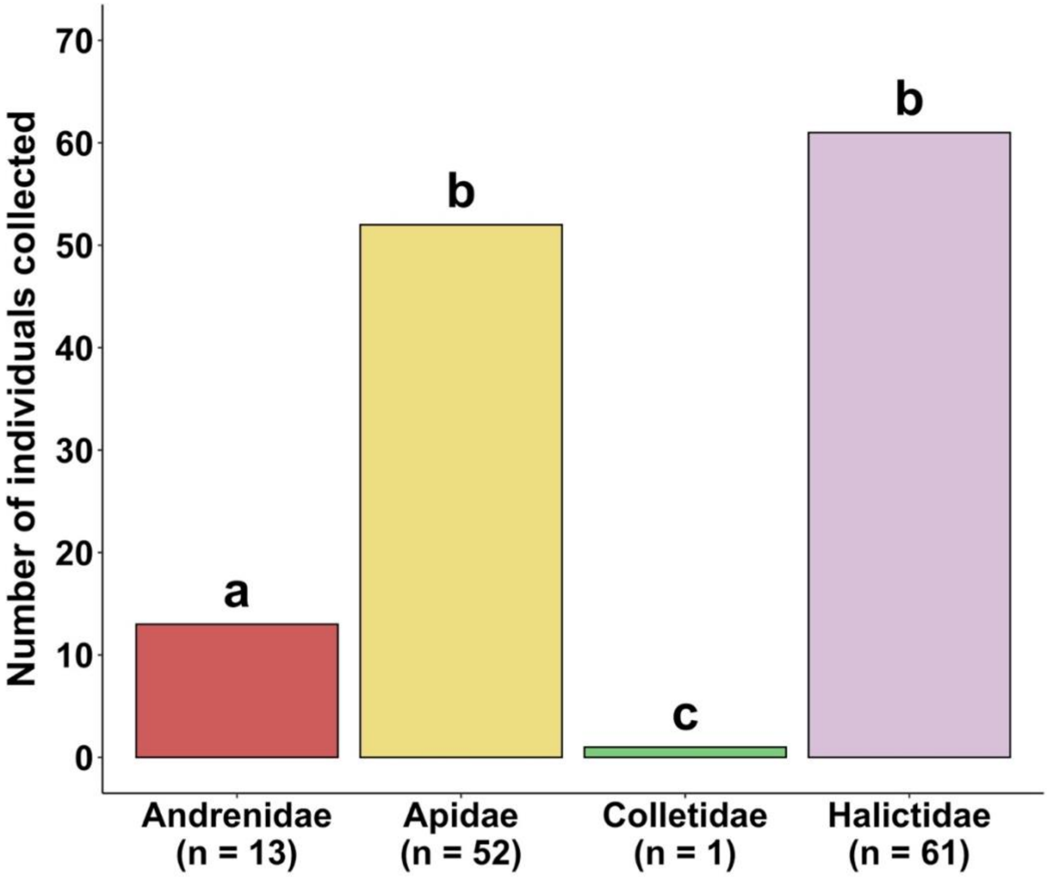

Figure 3-S3: Total number of bee visitors to tree-of-heaven flowers by family collected over 30.2 hours in Berks County, PA in 2020 and 2021. Families that do not share any letters above the bars were significantly different in total number of individuals collected ($p < 0.05$).