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Impacts of insecticide treatments on *Platytetranychus multidigituli* (Acari:Tetranychidae) and predatory mites on *Gleditsia triacanthos* var. *inermis* (honeylocust)

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Impacts of insecticide treatments on *Platytetranychus multidigituli* (Acari:Tetranychidae) and
predatory mites on *Gleditsia triacanthos* var. *inermis* (honeylocust)

For the degree of Master of Science



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11/21/2013

Date

IMPACTS OF INSECTICIDE TREATMENTS ON *PLATYTETRANYCHUS*
MULTIDIGITULI (ACARI: TETRANYCHIDAE) AND PREDATORY MITES ON
GLEDITSIA TRIACANTHOS VAR. *INERMIS* (HONEYLOCUST).

A Thesis

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of

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Adam Robert Witte

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of

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ABSTRACT

Witte, Adam R. M.S., Purdue University, December 2013. Impacts of insecticide treatments on *Platytetranychus multidigituli* (Acari: Tetranychidae) and predatory mites on *Gleditsia triacanthos* var. *inermis* (honeylocust). Major Professor: Clifford S. Sadof.

Spider mite outbreaks can occur in urban forests after insecticides are applied to control early season pests. We used thornless honeylocust (*Gleditsia triacanthos* var. *inermis*) and honeylocust spider mite (*Platytetranychus multidigituli*) as a model to investigate this phenomenon by applying both topical and systemic insecticides to treat early season pests of honeylocust. Impacts of these applications on populations of *P. multidigituli* and its associated predatory mites were assessed. Early season applications of bifenthrin caused outbreaks of honeylocust spider mite and drastically reduced phytoseiids but not the stigmaeid mite, *Zetzellia mali*. In contrast later season applications suppressed populations of *P. multidigituli*. Pyriproxyfen, an insect growth regulator, did not affect predatory mite populations, nor did they stimulate outbreaks of spider mites. Soil applications of imidacloprid and dinotefuran were not regularly associated with spider mite outbreaks. Due to irregular irrigation and inconsistent performance against calico scale, it is difficult to determine whether the absence of outbreaks after neonicotinoid applications was due to lack of product uptake or compound activity. Historically extreme drought and high temperatures of 2012 in Indiana drastically

reduced populations of phytoseiid mites but not the stigmatid mite, *Z. mali*. Overall, *Z. mali*, did not seem to impact *P. multidigituli* densities as much as the phytoseiid mites. Densities of *Z. mali* typically followed the abundance of their spider mite prey. We conclude that outbreaks of *P. multidigituli* could be attributed to conditions that reduced the abundance of phytoseiid mites of honeylocust trees.

CHAPTER 1. BIOLOGY OF SPIDER MITES AND THEIR ROLES AS SECONDARY PESTS IN URBAN LANDSCAPES

1.1 Introduction

Urban forest trees are often prone to arthropod pests that require intervention with insecticides to protect their health and value (Raupp and Herms 2010). Many of the more serious pests have the potential to decimate urban forests. (Poland and McCullough 2006, Vitullo and Sadof 2007, Rhainds and Sadof 2009, McClure 1992). The invasive borer emerald ash borer (*Agrilus planipennis*) alone threatens billions of ash trees (Oleaceae: Fraxinus) in North America and insecticidal treatment is the only demonstrated method to protect individual trees. The pesticide market has become a multi-billion dollar industry as a result of high demand for preservation of plants' health and appearance.

Although treatments may reduce injury from the targeted pests, leaf discoloration and premature leaf drop may follow due to secondary pest outbreaks like spider mites. For this reason, many studies have been conducted examining the effects of insecticides on spider mites and their natural enemies (Hill and Foster 1998, Szczepaniec et al. 2011, Sclar et al. 1998, Sato et al. 2001, Penman and Chapman et al. 1988). Although spider mite levels are often observed to be higher when host plants are treated with neonicotinoid insecticides (Sclar et al. 1998, Szczepaniec et al. 2011), it is difficult to establish a direct link between spider mite outbreaks and insecticide exposure due to variation in biology across spider mite taxa, host plant effects, and the ever changing

chemistry of available insecticides (Szczepaniec et al. 2013). For instance, in Autumn Blaze freeman maples, bifenthrin (a broad-spectrum pyrethroid insecticide) negatively affects predatory mite populations and spider mite outbreaks are often observed. However, when the same mite species are exposed to the same treatment in Red Sunset red maples no outbreaks occur, possibly due to the capacity of domatia to serve as refugia for predatory mites during insecticide applications (Prado 2013).

Predicting effects of insecticide applications on spider mite outbreaks requires understanding how these applications affect interactions between communities of spider mites and their predators on their host plants. My objective was to determine whether insecticides commonly used to control early season pests of honeylocust result in outbreaks of the resident populations of honeylocust spider mite, *Platytetranychus multidigituli*, and to investigate their effects on phytoseiid and stigmatid mites.

1.2 Overview of Herbivore Outbreaks on Urban Trees

High densities of herbivorous arthropods on trees in urbanized spaces have been well documented over recent decades (Dreistadt et al. 1990, Frankie and Ehler 1978, Hanks and Denno 1993, Stoetzel and Davidson 1971, Tooker and Hanks 2000, Watson et al. 1994). Possible mechanisms behind this phenomenon are forest fragmentation; heat islands and air pollution; periodic inputs of fertilizer and pesticides; reduction in plant diversity, richness, and density; and overall stress of trees (Raupp et al. 2010, Dreistadt et al. 1990).

Fragmentation of the urban forest can inhibit arthropod movement between hosts. This inherently results in lower arthropod diversity which aids in outbreaks of

herbivorous arthropods as immigration of natural enemies may be impeded. Flanders (1986) suggested that spatial and temporal isolation of hosts and natural enemies can create an enemy-free space that favors herbivore populations. Fragmentation and modern urbanization results in higher temperatures in urban areas due to the abundance of buildings and pavement with little room for vegetation (Akbari et al. 2001). This can further exacerbate herbivorous arthropod outbreaks.

A recent study by Meineke et al. (2013) demonstrates heat can dramatically increase populations of oak lecanium scale, *Parthenolecanium quercifex*. Multiple locations with varying temperatures were assessed in North Carolina for their abundance of *P. quercifex* and the researchers were able to correlate areas with higher temperatures to have significantly higher population levels of the soft scale. Spider mite outbreaks have also been associated with high temperatures. Spider mite levels are found to be higher in honeylocust trees surrounded by hardscapes (Sperry et al. 2001) which are often warmer areas than those dominated by vegetation (Landsberg, 1981). Hot temperatures can have negative effects on phytoseiid mites. *Euseius finlandicus*, (Acari: Phytoseiidae) is a common predatory mite on the European red spider mite, *Panonychus ulmi* Koch. In the Netherlands, Broufas and Koveos (2001) constructed a life table of *E. finlandicus* which shows survivorship and longevity to drastically decline at 34°C. A review by Holtzer et al. (1988) reports that humidity is another regulator of spider mite and phytoseiid populations. They note phytoseiid mites are more capable of withstanding higher levels of humidity than spider mites, but drier conditions favor spider mites over phytoseiids. Other environmental factors, such as rainfall, have been reported to have

negative effects on spider mites (Putman 1970, Yaninek and Animashaun 1987).

Therefore, it is intuitive to assume hot, dry conditions favor outbreaks of spider mites.

Effects of air pollution on herbivorous arthropods are not well known. A meta-analysis conducted by Zvereva and Kozlov (2009) found air pollution to have a positive effect on herbivorous arthropod populations. They note many experimental design flaws, however, attesting the findings to a reduction in natural enemies, specifically parasitoids. Parasitoids play a significant role in regulating populations of aphids and scales (McClure 1986, Olkowski et al. 1982) and a significant decline in individuals could alleviate pests of top-down pressure.

Areas of low tree diversity are more prone to herbivore arthropod pests than more diverse stands of trees (Dreistadt et al. 1990). Short travel distances to hosts, greater quantity of food, and fewer natural enemies all contribute to outbreaks of herbivores. Pimentel (1961) used *Brassica oleracea* L. to demonstrate this phenomenon. He found more insect diversity in mixed-planting stands than in single-planted fields. The greater insect diversity leads to the ability of top-down regulation of potential outbreak pests. Multiple examples of areas with low plant diversity resulting in outbreaks of pests have occurred across many plant species exists. The most notorious example being American elm, *Ulmus americana* L., decline in the 1970s due to Dutch elm disease (DED), *Ceratocystis ulmi*. This species of tree was very prevalent in United States (*U. americana* made up approximately 45% of Chicago's street trees in 1971) (Schmid 1975) and was decimated by the DED fungus. The close proximity of trees and lack of overall tree species diversity made it easy for the spread of fungal spores via bark beetles.

Ash trees may reach the same fate as elm trees as a result of emerald ash borer. Tens of millions of trees have died as of 2013 and billions (Griffith et al. 1993) of ash trees are threatened across the United States. Raupp et al. (2006) surveyed 12 large eastern cities in North America to assess the potential risk associated with the invasive borers emerald ash borer and Asian longhorned beetle. They found almost 50% of the trees to be at risk, indicating an overall lack of tree species diversity.

The plant-vigor hypothesis is the idea arthropod herbivores perform best on the most vigorously growing plants in a population of plants (Price 1991). Fertilizers are used to enhance plant growth, vigor, and health by providing an excellent, albeit sometimes excessive, source of nitrogen. According to the plant-vigor hypothesis, herbivorous arthropods should perform better on plants receiving adequate fertilizer. Prado (2013) correlated significantly higher number of maple spider mites, *O. aceris* (Acari: Tetranychidae) with higher nitrogen concentration in leaves of two cultivars of maple tree. Another example of nitrogen fertilization having a positive effect on an herbivore is the aphid *Aphis gossypii* on cotton plants (Nevo and Coll 2001).

Populations of some herbivorous arthropods can increase after pesticide use. Multiple mechanisms have been proposed or observed to describe this phenomenon. Negative effects on natural enemies can create enemy-free space for herbivores. Many instances in which the use of common pesticides resulting in outbreaks of an herbivorous pest are well documented in the literature (Luck and Dahlsten 1975, DeBach and Rose 1977, Ehler and Endicott 1984). Many arthropod pests are r-selection strategists with a high reproductive rate (Matthews and Kitching 1987). When a community of arthropods is exposed to lethal doses of pesticides, populations of r-selection strategists can resurge

at a fast rate due to their high fecundity and short developmental time in the absence of natural enemies. Acquisition of pesticide resistance is another advantage of many pests which fall into the r-selection spectrum as they have the capacity to develop large populations with a medley of potential resistant genes. The absence of top-down regulation of herbivores has historically been the proposed mechanism for pest resurgence. However, physiological effects on the host or herbivore from insecticides may be the tipping point, inducing explosive population growth, especially in systems where spider mites are present.

Szczepaniec et al. (2011) demonstrate that when *Tetranychus shoenei* ingest elm leaves treated with imidacloprid their fecundity increases by almost 40% in laboratory conditions. An unrelated study found imidacloprid increased spider mite populations and damage on honeylocust and marigold (Sclar et al. 1998). The increase in fecundity and damage suggests that the populations are healthy and are positively affected by imidacloprid applications. Proposed mechanisms by Szczepaniec et al. (2013) include an alteration of gene expression in plant defenses that reduces the amount of secondary metabolites expressed when spider mites feed. A higher concentration of the secondary metabolites phenylalanine ammonia lyase, chitinase, co-enzyme A ligase, and trypsin protease inhibitor is found in leaves that have been induced by spider mite feeding. Neonicotinoids suppressing two or more of these natural defense compounds occurred in cotton, corn, and tomato crops.

1.3 *G. triacanthos* var. *inermis* as a Model System

Honeylocust (*Gleditsia triacanthos* var. *inermis*) is a commonly used street tree found throughout the US. Its popularity grew after Dutch elm disease wiped out elm trees across the country. Honeylocust trees generally live to 125 years old and reach a height of 21-24m (Sullivan 1994). Many small hairs (trichomes) grow on the surface of the compound leaves and create domatia for small invertebrates.

Honeylocust is an adaptable tree for urban conditions. Soil types for this tree range from acidic to alkaline and they tolerate low nitrogen more than many other species of urban tree (Sullivan 1994). According to the National Tree Benefit Calculator (2013), an 18” caliper honeylocust tree adds \$303 to a residential home in Lafayette, IN per year, which is higher than both white ash and oak of equivalent caliper (\$235 and \$181 respectively). The home receives benefits such as increased property value, collection of stormwater runoff, improvement of air quality, CO₂ uptake, and energy conservation from having a honeylocust tree. Property value and stormwater collection provide the highest value of these benefits (\$160 and \$60 respectively). When considering street trees, stormwater interception is an important quality as stormwater can contain salt, oil, pesticides, fertilizer and other chemicals that could end up in bodies of water (Peper et al. 2009). In the residential setting, an 18” caliper honeylocust tree prevents the runoff of over 5600 gallons of stormwater (white ash intercepts 4300 gallons and silver maple 4200 gallons).

With these characteristics it is not surprising that honeylocust has become one of the most popular urban trees throughout eastern North America. Raupp et al. (2006) conducted a tree survey in 12 cities in eastern North America to demonstrate the amount

of trees vulnerable to attack by current invasive borers. As it turns out, *Gleditsia* is one of the most commonly planted genera along with *Acer* (maple), *Quercus* (oak), *Ulmus* (elm), and *Fraxinus* (ash). In Chicago, *Gleditsia* accounts for 22% of the urban trees (second only to *Acer* at 37%) and makes the top-ten list of most abundant trees in 8 of the 12 cities surveyed (Table 1.1).

Honeylocust trees host a number of important insect pests. Honeylocust plant bug, *Diaphnicoris chlorionis* (Say), is an early season pest that causes distortion of leaves due to feeding by nymphs and adults in late spring (Sperry et al. 2001, Krischik and Davidson 2004). Honeylocust plant bug tends to be a perennial pest that has the potential to build up to high populations in urban areas (Wheeler and Henry 1976). Typically, trees are treated during the early summer to control this pest.

Another perennial pest of honeylocust is mimosa webworm, *Homadaula anisocentra*. The larva of this lepidopteran pest damages both honeylocust and mimosa trees, *Albizia julibrissin* (Fabaceae), and usually has two generations per year in the Midwest (Miller and Hart 1987, Sadof and Snyder 2005), but typically tree injury is more noticeable in the second generation. Pre-emptive treatments are applied in the early summer, whereas rescue treatments targeting the second generation are made later in the season.

Calico scale is an invasive pest with a wide range of hosts and has the potential to reach high population levels on honeylocust trees (Quesada 2013). Overwintering second instar crawlers emerge in the spring and mate after molting to adults. Early season pesticide applications can be made to target overwintering scales or later in the summer months when eggs hatch and new crawlers move to leaves to feed.

These pests of honeylocust are often treated with pesticides to preserve health and vigor of the tree. However, many pesticides used for these instances have the potential to stimulate secondary outbreaks of honeylocust spider mite, *Platytetranychus multidigituli* (Sclar et al. 1998, preliminary data 2011). *P. multidigituli* is typically not a problem for urban landscape managers. Yet, when the ecosystem is disturbed with inputs of some insecticides, damaging levels of spider mite populations can occur which may require additional use of acaricides.

1.4 Biology of *P. multidigituli* and its Natural Enemies

Honeylocust spider mite. *P. multidigituli*, honeylocust spider mite, is not very representative of its genus as it is the only member of its genus to feed on a deciduous tree (honeylocust) rather than a coniferous species (Jeppson et al. 1975). *P. multidigituli* injures honeylocust trees by piercing plant cells with their stylets and depleting chlorophyll concentration (Jeppson et al. 1975). This type of feeding causes leaves to become bronze in color, often referred to as stippling. Canopy bronzing, stunted growth, and premature leaf drop can occur in trees with high populations of *P. multidigituli*.

Overwintering adults leave tree bark crevices and bud scales in the spring to begin feeding on the underside of leaves. In just over ten days, a female can lay up to 70 small, white eggs that hatch in 4 to 9 days (Jeppson et al. 1975). Eggs are laid in leaf domatia (dense trichomes along leaf veins and crotches of leaf veins) to reduce contact with natural enemies. Young stages of honeylocust spider mites will also utilize the densely hair-covered surface of honeylocust leaflets as refuge from large predators (personal observations).

Newly emerged larvae have 6 legs and are somewhat transparent until feeding begins and they then assume a darker green color. After the initial molt, spider mites form the last pair of legs and more closely resemble the adult stage. The five distinct life stages of *P. multidigituli* are egg, 6-legged larvae, 8-legged protonymph, deutonymph, and adult. The developmental time from larvae to adult is roughly 4-11 days (Jeppson et al. 1975). This large variation is influenced by many environmental factors such as weather, humidity, food availability and quality as well as the number of feeding bouts and stress levels. *P. multidigituli* does not produce dense webbing that is associated with other spider mites such as *Tetranychus urticae*. Populations grow rather quickly and have the potential to reach extremely high levels late in the summer months. Dispersal is achieved by riding wind currents during periods of overcrowding. When the cooler season approaches, female mites move to the overwintering locations and turn orange as they become inactive until spring (Jeppson et al. 1975).

Phytoseiid mites. Predatory mites, lacewing larvae, minute pirate bugs, spiders, predatory thrips, predatory midges, and some coccinellid species all feed on *P. multidigituli* (Dreistadt et al. 2004, Flint 1998). However, phytoseiid mites and *Zetzellia mali* (Acari: Stigmaeidae) are the primary predators of *P. multidigituli* (McMurty and Croft 1997, personal observations).

Phytoseiidae is a family of predatory mites that are very effective at controlling spider mites (Kain and Nyrop 1995). These species have significant impact as biological control agents against spider mites, thrips, scales, and whiteflies in agricultural and horticultural arenas due to their wide range of predatory characteristics. McMurty and Croft (1997) assembled a categorical system of feeding behaviors. Type I phytoseiid

mites are specialist predators within the genus *Tetranychus*. Type II phytoseiids feed on a broader host range, but limit themselves within the family Tetranychidae. Type III phytoseiids are more generalized predators feeding on multiple insect and mite species as well as pollen. Type IV phytoseiids are specialized pollen feeders and generalist predators, only represented by the genus *Euseiulus*. Type III and IV phytoseiids are more adapted to periods of low spider mite populations due to their capacity to supplement their diet with pollen. The ability of a predator to capture prey increases with size of the predatory mite. Smaller stages are less mobile than larger stages and require more time to capture prey (Jeppson et al. 1975). Although phytoseiid mites will feed on eriophyoid rust mites in the absence of spider mites, some of the generalist phytoseiids are capable of reproducing solely on eriophyoid rust mites (Jeppson et al. 1975).

Phytoseiid mites are teardrop-shaped, with fewer hairs relative to their tetranychid prey. Phytoseiid mites have shorter life cycles than most spider mites. Adult females overwinter in bark crevices and other protective areas in trees, emerge in early spring, and typically begin laying eggs in 3-5 days (Jeppson et al. 1975). Eggs tend to be laid on the underside of the leaf near the midrib vein which increases interaction of young phytoseiid mites and spider mites due to the tendency for spider mites to be present near the midrib (Jeppson et al. 1975). Phytoseiids lay approximately 30-60 eggs in their lifetime (Jeppson et al. 1975). The capacity to increase egg production under heavy spider mite infestations is an important quality these mites possess (Jeppson et al. 1975).

Random walking patterns have been observed in phytoseiid predators when populations of spider mite prey are high. However, when populations are low predators search near leaf edges which allows for a greater chance to move between leaves.

Chemical cues of prey silk and prey feces help phytoseiids hone in on spider mites as does their own chemical marker that tells them where they have previously been (Hull and Horsburgh 1995). These predators are highly active which leads to frequent interaction with prey in high to moderate populations. When prey populations are limited, phytoseiids will posture their body at the edge of a leaf or bark chip in a way to allow them to be carried by wind (Johnson and Croft 1976). Phytoseiid mites are commonly used in orchards as biological control agents and strains have developed resistance to certain pesticides as a result of exposure to repeated applications (Hull and Horsburgh 1995).

Zetzellia mali. *Zetzellia mali* adults are orange with a broader posterior than anterior. Egg and nymphal stages are commonly found on the underside of the leaf near the mid-vein. *Z. mali* typically are not effective at controlling spider mites alone, but have an additive effect on reducing spider mites when combined with phytoseiid mites (Kain and Nyrop 1995, Prado 2013).

Not a strict predator, *Z. mali* feeds on apple pollen (White and Laing 1977), yeast and grape leaf tissue (Santos unpublished data 1985), possibly apple leaf tissue (Santos 1982), as well as phytoseiid eggs (Santos 1982, Prado 2013), pyralid moth and coccid eggs (Delattre 1971), and multiple spider mite species (Santos 1991, Prado 2013, personal observations). Santos (1991) observed no preferential feeding by *Z. mali* when given multiple common spider mites, *Tetranychus urticae*, *Aculus schlechtendali*, and *Panonychus ulmi*. Recent laboratory work carried out by Prado (2013) found that *Z. mali* will readily consume all stages of *Oligonychus aceris* as well as eggs and nymphs of

Amblyseius fallacis. The low dispersal rate and minimal migratory efforts of *Z. mali* (Slone and Croft 1998) may reflect the broad polyphagous behaviors of *Z. mali*.

Multiple generations (3-4) occur in one year (Hull and Horsburgh 1995). Overwintered females become active in early spring. Populations of *Z. mali* typically peak in August and tend to have a positive correlation with spider mite population abundance (Hull and Horsburgh 1995). Egg, protonymph, deutonymph, and adult stages are present at all times in a population. Mated adult females overwinter in aggregation under bark and in crevices (Hull and Horsburgh 1995, Kain and Nyrop 1995).

1.5 Insecticides in this Project

Bifenthrin is a third generation pyrethroid insecticide. Its frequent use by pest managers is attributed to quick knockdown effects on insects, low odor, and low mammalian toxicity. Bifenthrin is efficient at killing a broad spectrum of arthropods including ants, aphids, beetles, ticks, thrips, caterpillars, whiteflies, scales, and spiders. It is also an effective miticide (Gough 1990). Due to its popularity as an effective insecticide, populations of spider mites have developed a resistance to bifenthrin as a result of sustained and repeated applications (Herron and Wilson 2001, Ay and Gürkan 2005).

Pyrethroid insecticides kill by disrupting the sodium ion-channel gate in the axons of the nervous system. Essentially, the ion-regulating gate is left open allowing constant inflow of ions. This results in overstimulation as the nerve is not able to return to a neutral state. Resistance mechanisms in spider mite strains have been identified as

increased activities of esterase and P450 monooxygenase activities (Van Leeuwen and Van Pottelberge 2005).

Pyriproxyfen is an insect growth regulator introduced to the United States in 1996. The original introduction was used to treat whiteflies on cotton, but it has since been used to treat for multiple arthropod pests. The most important characteristic of pyriproxyfen (and all insect growth regulators) attributed to its use is safety. Mammals do not have insect hormones and, therefore, are not susceptible to pyriproxyfen. Pyriproxyfen acts on the insect hormone system by mimicking juvenile hormone (Ishaaya and Horowitz 1995). This causes immature stages of arthropods to develop abnormally, resulting in death.

Neonicotinoids (also referred to as chloronicotinylns) make up the highest portion of the world insecticide market share with 24% (Jeschke et al. 2011). This is not surprising due to its ease of use, low mammalian toxicity, and low applicator exposure. Neonicotinoids are systemic insecticides that move their way up the xylem system of plants and accumulate in the leaves. When arthropods feed on leaves, they receive the active ingredient which acts on the synapses of the nervous system. The chloronicotinyln molecules mimic acetylcholine, but are not effectively degraded by acetylcholine esterase. Continual firing of the nerves results in overstimulation, resulting in paralysis and death of the arthropod. Recent findings of potentially high exposure and toxicity to bees have resulted in the ban of neonicotinoids in Europe (Gross 2013).

Imidacloprid is the earliest neonicotinoid produced in the United States. Bayer CropScience successfully registered the insecticide in 1994 for turfgrass and ornamental plants. Many instances of spider mite outbreaks have been associated with its use (Sclar et al. 1998, Raupp et al. 2004, Szczepanec et al. 2011).

Dinotefuran is a popular neonicotinoid insecticide used to treat for a wide range of plant pests. Unlike imidacloprid, recent research demonstrated outbreaks of spider mites do not occur in elms when applied with dinotefuran (Szczepaniec et al. 2013). Recently, Oregon has restricted the use of dinotefuran as a large bee kill is being investigated in which dinotefuran was used to treat many flowering linden trees in violation of the label.

Chlorantraniliprole is a new insecticide developed by DuPont and marketed as Acelepryn. It is an anthranilic diamide which acts on the ryanodine receptors in muscle tissue. The result is an overstimulation of muscle fibers and paralysis. Chlorantraniliprole is very effective at killing larvae of lepidopteran pests (Pluciennik 2013, Lahm et al. 2007) and has translaminar properties making it effective against leaf miners. Due to its selectivity to arthropods, chlorantraniliprole has been classified as reduced-risk by the EPA (EPA Pesticide Fast Sheet 2008).

1.6 Objectives

This project aims to evaluate the use of select pesticides and their effects on *P. multigituli* and predatory mites by manipulating the parameters of application timing and presence and absence of calico scale infestations. The common pesticides imidacloprid, dinotefuran, bifenthrin, pyriproxyfen, and chlorantraniliprole were chosen for their potential to control common pests of honeylocust (Hubbard and Potter 2006, Krischik and Davidson 2004). My central hypothesis is: insecticides with broad spectrum selectivity will result in higher populations of *P. multigituli* than the control; conversely,

insecticides with narrower selectivity will not have an effect on *P. multidigituli* and their natural enemies.

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Table 1.1 Most common tree genera in 12 eastern U.S. cities

Genus	Frequency of Occurrence in Top Ten Genera in North America	Average	Minimum	Maximum	Mite Pest Species
<i>Acer</i>	100	32.7	15	57	<i>Oligonychus aceris</i>
<i>Fraxinus</i>	67	10.6	4	19	<i>Tetranychus homorus</i>
<i>Ginkgo</i>	42	2.8	1	7	-
<i>Gleditsia</i>	67	8	3	22	<i>Platytetranychus multidigituli</i>
<i>Malus</i>	50	4.3	1	9	<i>Tetranychus urticae</i> ; <i>Panonychus ulmi</i>
<i>Picea</i>	42	5	3	12	<i>Oligonychus ununguis</i>
<i>Pinus</i>	42	5.6	3	9	<i>Oligonychus ununguis</i>
<i>Platanus</i>	42	7	2	19	<i>Eotetranychus tiliarum</i>
<i>Prunus</i>	58	3.3	0.5	8	<i>Tetranychus urticae</i> ; <i>Panonychus ulmi</i>
<i>Pyrus</i>	58	6	2	9	<i>Tetranychus urticae</i> ; <i>Panonychus ulmi</i>
<i>Quercus</i>	75	9	4	16	<i>Oligonychus bicolor</i>
<i>Tilia</i>	58	5	3	7	<i>Eotetranychus tiliarum</i>
<i>Ulmus</i>	67	7	3	12	<i>Tetranychus schoenei</i>

This is a representation of the frequency at which genera appear in the top ten most planted genera for 12 eastern North American cities (Raupp et al. 2006). The average column gives the average percent of trees found in each genus relative to the total number of trees inventoried across all cities where the genus was in the top ten. *Gleditsia* is tied for the third most used genera of trees across the 12 cities. Spider mite pests associated with genera of trees comes from Jeppson et al. 1975.

CHAPTER 2. EFFECTS OF INSECTICIDES ON SECONDARY SPIDER MITE PESTS AND THEIR NATURAL ENEMIES ON HONEYLOCUST

2.1 Abstract

Spider mite outbreaks can occur in urban forests after insecticides are applied to control early season pests. We used thornless honeylocust (*Gleditsia triacanthos* var. *inermis*) and honeylocust spider mite (*Platytetranychus multidigituli*) as a model to investigate this phenomenon by applying both topical and systemic insecticides to treat early season pests of honeylocust. Impacts of these applications on populations of *P. multidigituli* and its associated predatory mites were assessed. Early season applications of bifenthrin caused outbreaks of honeylocust spider mite and drastically reduced phytoseiids but not the stigmatid mite, *Zetzellia mali*. In contrast later season applications suppressed populations of *P. multidigituli*. Pyriproxyfen, an insect growth regulator, did not affect predatory mite populations, nor did they stimulate outbreaks of spider mites. Soil applications of imidacloprid and dinotefuran were not regularly associated with spider mite outbreaks. Due to irregular irrigation and inconsistent performance against calico scale, it is difficult to determine whether the absence of outbreaks after neonicotinoid applications was due to lack of product uptake or compound activity. Historically extreme drought and high temperatures of 2012 in Indiana drastically reduced populations of phytoseiid mites but not the stigmatid mite, *Z. mali*. Overall, *Z. mali*, did not seem to impact *P. multidigituli* densities as much as the phytoseiid mites.

Densities of *Z. mali* typically followed the abundance of their spider mite prey. We conclude that outbreaks of *P. multidigituli* could be attributed to conditions that reduced the abundance of phytoseiid mites of honeylocust trees.

2.2 Introduction

Trees are an essential part of the urban landscape due to their ability to reduce energy costs, sequester atmospheric carbon dioxide, improve air quality, mitigate storm water runoff, and provide aesthetic appeal (Peper et al. 2009). Maintenance of these trees often requires pesticide use to conserve their value as they are prone to a multitude of arthropod pests. Certain urban trees can become weak or even hazardous if their health is compromised by arthropod pests. For example, emerald ash borer (*Agilus planipennis*) has killed millions of untreated trees and is likely to cost billions of dollars to manage over the next decade (Kovacs et al. 2009). Other trees like *Acer rubrum*, may require early season applications of pyrethroids to avoid damage to their tips from potato leafhoppers, *Empoasca fabae* (Prado 2013). Still, other trees attacked by sucking insects, such as soft scales, can cause significant damage when trees are coated with honeydew and sooty mold, or when they attract stinging insects. In these cases, insecticides may need to be applied at different times of year to reduce damage (Hubbard and Potter 2006).

Although many factors such as water stress (David et al. 1989) and increased nitrogen (Prado 2013) have been attributed to spider mite outbreaks, pesticides can be important triggers. Topical applications of broad spectrum insecticides directed at key pests can facilitate secondary spider mite outbreaks by drastically reducing the density of natural enemies (Penman and Chapman 1988, Gerson and Cohen 1989). More selective,

topically-applied insecticides can kill leaf arthropods without having a negative effect on spider mite natural enemies (Holt et al. 2006). However, some problems, like soft scales and leafhoppers, are proving to be more difficult to manage with these lower toxicity and narrow spectrum compounds (Hubbard and Potter, 2006; Prado 2013). For this reason, and to avoid non target effects of foliar sprays, systemic insecticides are being developed to treat these arthropods.

Uses of neonicotinoids may be problematic due to the growing body of evidence that demonstrate plants treated with neonicotinoids have larger populations of spider mites than those that are left untreated (Sclar et al., 1998; Raupp et al., 2004; Gupta and Krischik, 2007; Smith, 2010; Szczepaniec et al., 2011; Szczepaniec and Raupp, 2012). Neonicotinoids are not toxic to spider mites due to the inability of neonicotinoids to bind with spider mite acetylcholine receptors (Szczepaniec et al., 2013; Dermauw et al., 2012; Mullins, 1993). Szczepaniec et al. (2011) reported higher population levels of spider mites on elm trees, *Ulmus americana*, treated with imidacloprid citing the mechanism as stimulatory effects on reproduction and deleterious effects on insect predators. A more recent paper by Szczepaniec et al. (2013) suggests that imidacloprid alters gene expression of plant defenses freeing spider mites of bottom-up regulation which leads to larger populations.

Despite evidence that applications of pesticides can cause secondary outbreaks of spider mites, these products are still widely used to protect plant health in urban landscapes. Honeylocust (*Gleditsia triacanthos* var. *inermis*) is a common street tree with early season pests that often requires pesticide use which can result in spider mite outbreaks (Sperry et al. 2001). In the present study, we examine how the use of selected

pesticides against early season pests can contribute to outbreaks of the honeylocust spider mite (*Platytetranychus multidigituli*) in the presence and absence of a calico scale (*Eulecanium cerasorum*) infestation.

2.3 Materials and Methods

Effects of pesticides on mite populations in trees infested with calico scale. In 2011, a stand of honeylocust trees ranging in size from 8.6 to 24.6 cm DBH alongside a canal in downtown Indianapolis (39.777448 N, -86.164640 W) was designated to receive a soil or trunk-applied insecticide early in the season to target overwintering scales. City street trees ranging in size from 8.1 to 29.1 cm DBH in downtown Indianapolis (39.768263 N, -86.152452 W) received foliar applications of insecticides early in the season targeting overwintering scales. Trees at the canal site were divided into six replicate blocks. Within each block, four treatments of soil or trunk applied systemic insecticide applications (Table 2.1) were randomly assigned to each tree. Similarly, city street trees in downtown Indianapolis were divided into seven replicate blocks. The canopies of these trees were treated with six insecticides chosen based on their potential to control calico scale (Table 2.1). Trunk applications of systemic insecticides were made with a 5.68 liter hand sprayer at low pressure. Soil applications were made at 0.06 kg/m² with a 20 cm probe subsoil injector. Injections were made at four points within 0.3 m of the trunk. Imidacloprid was applied on 10 April (73 DD₁₀^{°C} Degree days base on 10 °C) and dinotefuran on 03 May (152 DD₁₀^{°C}). Foliar applications were made on 3 May, (152 DD₁₀^{°C}). Insecticides were applied directly to the tree canopy until run off using a John Bean (Philadelphia, PA) high pressure sprayer at 0.029Kg/m².

Effects of pesticide treatments on *P. multigituli* was assessed by collecting five leaves from every tree at each of four cardinal directions on 31 May, 20 June, 5 July, 19 July, 3 August, and 29 August 2011. Samples were transported to West Lafayette, IN in a cooler kept at 10-15 °C and stored in a refrigerator at 3 °C. A maximum of one week was allotted to process the samples. Ten leaves were randomly selected from the twenty leaves collected and run through a mite brushing machine (Mi Wuk Village, CA) over a Petri dish sprayed with cooking oil. All mobile stages of *P. multigituli* were counted. A LI-COR (Lincoln, NE) leaf area machine was used to measure leaf area of the ten selected leaves. *P. multigituli* counts were converted to densities of individuals per unit of leaf area.

A two year study beginning in 2012 was conducted on a cohort of trees ranging in size from 17.5 to 40.5 cm DBH in a restaurant parking lot in Fishers, IN (39.928119 N, -86.0336224 W) where trees received a foliar treatment with a John Bean (Philadelphia, PA) high pressure sprayer at 0.029kg/m² or a systemically applied insecticide after the crawler stage of scales settled on the leaves. In both years, five treatments were randomly assigned to five replicates (Table 2.1). All treatments were applied by professional applicators under our supervision on 6 June (626 DD₁₀ °C). *P. multigituli* assessments were conducted in the same way as the previous year. Predatory mite populations were analyzed by randomly pulling five leaves from the bag of 20 leaves and examining them microscopically for predatory mites. All mobile stages of *Zetzellia mali* and phytoseiid spp. were counted. These leaves were run through a LI-COR leaf surface area machine to convert predatory mite counts to individuals per unit of leaf area. Collections at this site were made on 19 June, 2 July, 16 July, 30 July, 16 August, 28 August and 17 September.

During the following year (2013), foliar applications were made earlier in the season (2 and 3 May, 143 DD₁₀ °C) by professional applicators under our supervision using a John Bean (Philadelphia, PA) high pressure sprayer at 0.029kg/m² and we applied systemic insecticides with a 20 cm probe subsoil injector at 0.06kg/m². Assessment of *P. multigituli* and predatory mites was conducted in the same manner as the previous year on 3 June, 17 June, 1 July, 17 July, 29 July, 12 August, and 26 August.

Effects of pesticides on mite populations in trees without calico scale. In 2012, studies were conducted in a stand of trees located at a nursery in Westfield, IN (40.020767 N, -86.188962 W) with trees ranging in size from 8 to 16.5 cm DBH. Treatments were assigned to trees in six replicates in a randomized complete block design. Applications of insecticides were applied on 8 June (648 DD₁₀ °C). Soil applications (Table 2.1) were made at 0.06 kg/m² with a 20 cm probe subsoil injector at four points within 0.3 m of the trunk. Due to drought conditions during the application time, trees received approximately 9.5 liters of water before and after soil injections. Foliar applications were made with a Solo 15 liter backpack sprayer until runoff. Assessment of *P. multigituli* and predatory mites was conducted in the same manner as in the Fishers, IN site on 4 June, 25 June, 9 July, 23 July, 6 August, 20 August, 4 September and 1 October.

In 2013, studies were conducted in a site in Lafayette, IN (40.415269 N, -86.829592 W) at a retailer parking lot had a stand of trees measuring from 11 cm to 27.5 cm DBH. Treatments were randomly assigned to six replicates. Soil and foliar applications were made in the same manner as the site in Westfield, IN on 7 May (132 DD₁₀ °C). A separate bifenthrin treatment was applied later on 3 July (695 DD₁₀ °C).

Assessment of *P. multidigituli* and predatory mites was conducted in the same manner as in the Fishers, IN site on 3 June, 17 June, 1 July, 17 July, 29 July, 12 August, and 26 August.

Predatory mite densities were transformed using cumulative mite days (CMD) and totals were used for analysis. The formula for CMD is as follows:

$$\text{CMD} = \sum 0.5(\text{mites/cm}^2 \text{ at obs. 1} + \text{mites/cm}^2 \text{ at obs. 2}) * \text{Days between observations}$$

Statistical analysis. Effects of treatments on *P. multidigituli* and predators were analyzed using a mixed model Analysis of Variance with a Randomized Complete Block or a Completely Randomized design as described above using PROC GLIMMIX for Generalized Linear Mixed Models (SAS® 9.3 Institute Inc., Cary, NC). LSMEANS were separated using Tukey-Kramer Test at an α -level of 0.05. Similarly, analysis of cumulative mite days for predatory mites were conducted using an Analysis of Variance and a Tukey-Kramer test with a Randomized Complete Block or a Completely Randomized design as indicated above using PROC GLIMMIX.

2.4 Results

Effects of pesticides on mite populations in trees infested with calico scale. In 2011, there was no significant effect ($F = 0.91$; $df = 3, 116$; $P = 0.4378$) of soil and trunk applied systemic insecticides on the abundance of *P. multidigituli* along the Indianapolis canal (Figure 2.1). In contrast, foliar applications of insecticides in downtown Indianapolis (Figure 2.1) significantly affected *P. multidigituli* populations ($F = 5.41$; df

= 3, 120 $P = 0.0016$). Over the course of the collection period, trees treated with bifenthrin had higher populations than the control and cyantraniliprole + surfactant based on a Tukey-Kramer test ($P < 0.05$) whereas trees treated with bifenthrin + surfactant were marginally higher than the control ($P < 0.052$). There was also a significant effect of date ($F = 6.17$; $df = 5, 120$; $P < 0.0001$) with *P. multidigituli* populations generally higher later in the season.

During 2012, insecticide applications on scale infested trees at the Fishers, IN site had no effect on *P. multidigituli* ($F = 0.98$; $df = 4, 140$; $P = 0.4185$) (Figure 2.2). Examination of individual leaves for predators revealed significant differences in the total CMD ($F = 10.84$; $df = 4, 20$; $P < 0.0001$) of *Z. mali* (Figure 2.2). Only 23 phytoseiid mites were observed on leaves during the entire 2012 season and no statistical analysis was conducted. In 2013, there was a significant effect of treatment on *P. multidigituli* ($F = 5.58$; $df = 4, 140$; $P = 0.0003$) (Figure 2.3) and a significant effect of date ($F = 6.76$; $df = 6, 140$; $P < 0.0001$). Trees treated with bifenthrin had significantly more *P. multidigituli* than the control and chlorantraniliprole ($P < 0.05$). Similarly, pyriproxyfen had significantly more *P. multidigituli* than the control and chlorantraniliprole ($P < 0.05$). Dinotefuran had significantly more *P. multidigituli* than the control ($P < 0.05$). The abundance of *Z. mali* were significantly affected by insecticide treatments ($F = 22.12$; $df = 4, 20$; $P < 0.0001$) with highest populations on trees treated with pyriproxyfen (Figure 2.3). The abundance of phytoseiid mites was not affected by insecticide treatments ($F = 0.98$; $df = 4, 20$; $P = 0.4389$).

Effects of pesticides on mite populations in trees without calico scale. During 2012, insecticides applied later in the season on trees without scales at the Westfield, IN site had significant effect on *P. multidigituli* ($F = 6.50$; $df = 5, 180$; $P < 0.0001$) (Figure 2.4). There was also significant effect of date ($F = 17.46$; $df = 25, 180$; $P < 0.0001$) as well as a correlation between treatment and date ($F = 2.48$; $df = 30, 180$; $P < 0.0001$). Trees treated with bifenthrin had significantly lower *P. multidigituli* than all other treatments ($P < 0.05$). Dinotefuran had significantly more *P. multidigituli* than the control at the peak population of the season ($P < 0.05$). CMD totals for *Z. mali* (Figure 3) were significantly different ($F = 5.92$; $df = 5, 30$; $P = 0.0006$) with pyriproxyfen having the highest totals ($P < 0.05$).

In 2013, there was no difference in treatment effects on *P. multidigituli* ($F = 1.17$; $df = 5, 240$; $P = 0.3237$) at the Lafayette, IN site (Figure 2.5). However, there was a significant difference in date ($F = 12.75$; $df = 7, 240$; $P < 0.0001$) and an interaction between date and treatment ($F = 1.56$; $df = 35, 240$; $P = 0.0282$). Populations of *Z. mali* were significantly different across treatments ($F = 28.44$; $df = 5, 30$; $P < 0.0001$) with bifenthrin having higher mite populations than all other treatments (Figure 2.5). Similarly, there was a significant interaction of treatment and phytoseiid populations ($F = 6.34$; $df = 5, 30$; $P = 0.0004$) with both bifenthrin treatments having lower CMD totals than all other treatments (Figure 2.5).

2.5 Discussion

We observed spider mite outbreaks in trees where conditions were unfavorable to phytoseiid mites. This pattern is not surprising as some phytoseiid mites have been

known to be very effective biological control agents of spider mite species (Takafuji and Chant 1976, Penman and Chapman 1988, Nyrop et al. 1998). Although multiple predators of *P. multigituli*, have been reported, phytoseiid mites seem to have the most influence on their populations. Populations of other predators found on leaves, such as *Z. mali*, are only responding to the abundance of *P. multigituli* and have little capacity to reduce spider mite density by themselves. This is consistent with Prado (2013) which observed phytoseiid mites to have greater impacts on maple spider mite densities than *Z. mali*. Other mechanisms contributing to the reduction of phytoseiid mites in our study were lethal exposure to insecticides, excessive honeydew, and hot, dry weather.

Trees without calico scales that were treated with bifenthrin early or late in the season during 2013 had significantly higher populations of *P. multigituli* and lower numbers of phytoseiid mites compared to the control. This suggests that applications of bifenthrin have a negative effect on phytoseiid mites and alleviate top down pressures on *P. multigituli*. Other studies have observed a similar phenomenon where pyrethroids caused spider mite outbreaks (Penman and Chapman 1988, Gerson and Cohen 1989). In 2011, trees treated early in the season with bifenthrin resulted in outbreaks of *P. multigituli* (Figure 2.1). Although predatory mite populations were not measured in 2011, the pattern is consistent with 2013.

In 2012, Indiana experienced record high temperatures and drought conditions throughout much of the growing season (National Climatic Data Center). Although some studies have indicated that drought stress could directly enhance spider mite population growth, negative effects of the drought on the phytoseiids have also been shown to contribute to mite outbreaks (English-Loeb 1990). Not surprisingly, phytoseiid mites

were very scarce during this season as they tend to favor cooler, wetter climates (Holtzer et al. 1988, Broufas and Koveos 2001, Stavrinides et al. 2010). Consequently, we experienced very large outbreaks of *P. multidigituli* among all treatment groups, except for trees treated with bifenthrin. Since we applied insecticides later in the 2012 season compared to other years, we suspect bifenthrin's miticidal activity suppressed *P. multidigituli* populations for most of the collection period (Figure 2.4). *Z. mali* were abundant throughout this season which supports the hypothesis that these stigmatid mites have much less impact on *P. multidigituli* than phytoseiids.

We observed higher populations of *P. multidigituli* on trees treated with dinotefuran and imidacloprid during late season collections in 2013 at the Lafayette, IN site. Interestingly, phytoseiid mite populations were not different from the control in either neonicotinoid treatment (dinotefuran and imidacloprid). This would support recent work by Szczepaniec et al. (2013) who suggest that applications of neonicotinoids are relatively non-toxic to phytoseiids and that they cause outbreaks by improving the quality of plants for spider mites by interfering with their capacity to produce plant defenses.

Some insecticides did not increase the abundance of spider mites. In the absence of calico scales, trees treated with pyriproxyfen did not have higher numbers of *P. multidigituli* or phytoseiid mites when compared to the controls. When bifenthrin was applied to target spider mites later in the season, phytoseiids and *P. multidigituli* were lower than the controls. This suggests that both pesticides are capable of being used on trees without causing outbreaks of spider mites. Pyriproxyfen's selectivity does not disrupt the mite community whereas bifenthrin's miticidal activity killed both mite species.

At the Fishers, IN site in 2012 and 2013, the heavy infestation of calico scales negatively affected populations of mite species. Low numbers of phytoseiid mites were observed across all treatments (Figure 2.3) when compared with the much higher numbers observed at the scale-free site in Lafayette, IN (Figure 2.2). The presence of a thick, sugary film on leaves is a significant difference between the two sites, but other site differences may play a role in the variation of mite densities.

Interestingly, in the presence of scales, trees treated with pyriproxyfen had higher numbers of *P. multidigituli* when compared to the controls. However, it seems likely that this increase was due to the reduction of honeydew present on leaves as pyriproxyfen is an effective insecticide against calico scale (Quesada 2013). This opened available habitat for mite species on treated plants which may have contributed to the larger populations of *P. multidigituli* compared to the control. Similarly, trees treated with bifenthrin had the same pattern as pyriproxyfen treated trees, but with an even greater increase than the control. Populations of both predatory mites were lower or the same as the control in trees treated with bifenthrin. In contrast, predatory mites were either higher or the same in trees treated with pyriproxyfen (Figure 2.3) which supports the previous conclusion that pyriproxyfen does less to disrupt the mite community than bifenthrin.

Surprisingly, no outbreaks were observed on any of the three neonicotinoid treatments made in 2011 when rainfall was not scarce during application time. This is contradictory to recent studies that have correlated spider mite outbreaks to application of neonicotinoids (Sclar et al. 1998, Sczcepaniec et al. 2011). Since we did not measure predator abundance in this year, it is difficult to postulate a mechanism for the low abundance of *P. multidigituli* in Indianapolis, IN.

Trees treated with chlorantraniliprole, an anthranilic diamide, never resulted in outbreaks of *P. multigituli* nor did applications affect phytoseiid mites. However, trees treated with another anthranilic diamide, cyantraniliprole, in 2011 experienced high densities of *P. multigituli* late in the season, but there was no overall significant treatment effect between cyantraniliprole and the control.

In conclusion, strategies for managing early season pests of honeylocust should consider potential effects of pesticides on predators of spider mites. Problems with their use can be reduced by avoiding applications through use of threshold densities to determine whether applications are needed against early season pests (Prado 2013) or by using more selective pesticides. If an early season treatment with bifenthrin is necessary to control a pest, a follow-up application of bifenthrin may be needed later in the season to avoid outbreaks of spider mites.

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Table 2.1 Formulations, rates, field sites, application dates and methods used to apply insecticides on honeylocust trees targeting early and later season pests. Trees in Indianapolis and Fishers, IN had infestations of *Eulecanium cerasorum*.

Active Ingredient (and trade name)	Rate (A.L.)	Growing DD _{10c} Target	2011		2012		2013	
			Mid-Spring	Late Spring	Mid-Spring	Summer		
			Site location	Indianapolis, IN	Westfield, IN	Fishers, IN	Fishers, IN	Lafayette, IN
		Application Method						
Bifenthrin (Talstar S)	0.118 g/L	Foliar	3-May	8-Jun	6-Jun	3-May	7-May	3-Jul
Bifenthrin + S ^a	0.118 g/L	Foliar	3-May	-	-	-	-	-
Cyantraniliprole (HWG-355) + S	0.058 g/L	Foliar	3-May	-	-	-	-	-
Chlorantraniliprole (Acelepryn)	0.058 g/L	Foliar	-	8-Jun	6-Jun	2-May	-	-
Pyriproxyfen (Distance)	0.099 g/L	Foliar	-	8-Jun	6-Jun	2-May	7-May	-
Dinotefuran (Transtect 70WSP)	0.567 g/cm ^b	Soil	3-May	-	6-Jun	3-May	7-May	-
Imidacloprid (Xytect 75WSP)	0.580 g/cm	Soil	10-Apr	8-Jun	-	-	7-May	-
Dinotefuran (Safari 2G)	0.470 g/cm	Soil	-	8-Jun	-	-	-	-
Dinotefuran (Transtect 70WSP) + S	0.470 g/cm	Trunk	3-May	-	-	-	-	-
Water			3-May	8-Jun	6-Jun	2-May	7-May	-

^a S refers to surfactant applied at 0.599g/L.

^b cm of DBH (diameter at breast height)

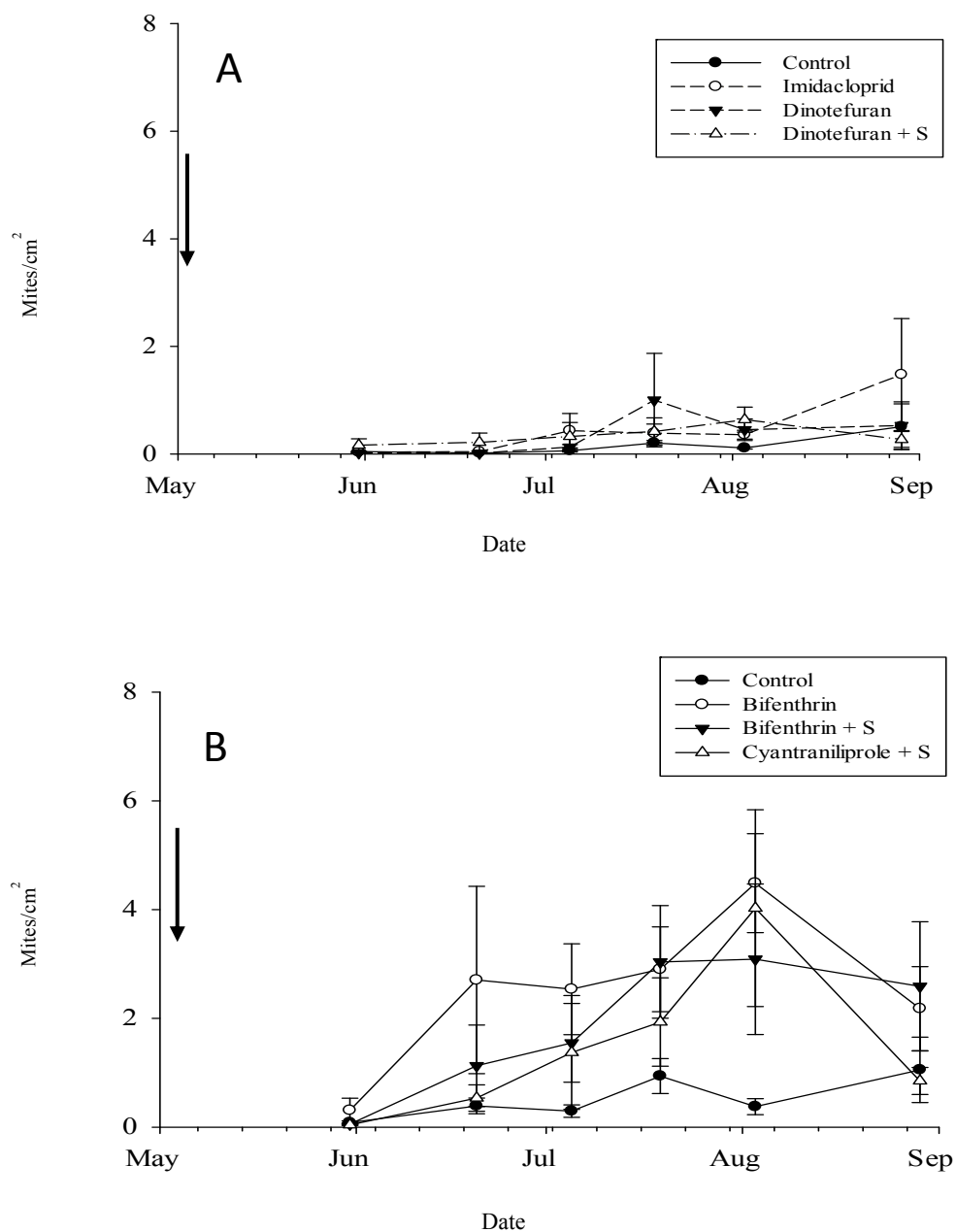


Figure 2.1 *P. multidigituli* densities on honeylocust trees infested with *Eulecanium cerasorum* in Indianapolis, IN along the canal (A) and on streets downtown (B) over the course of the 2011 season. Arrows indicate when insecticides were applied except for imidacloprid for reasons described in the text.

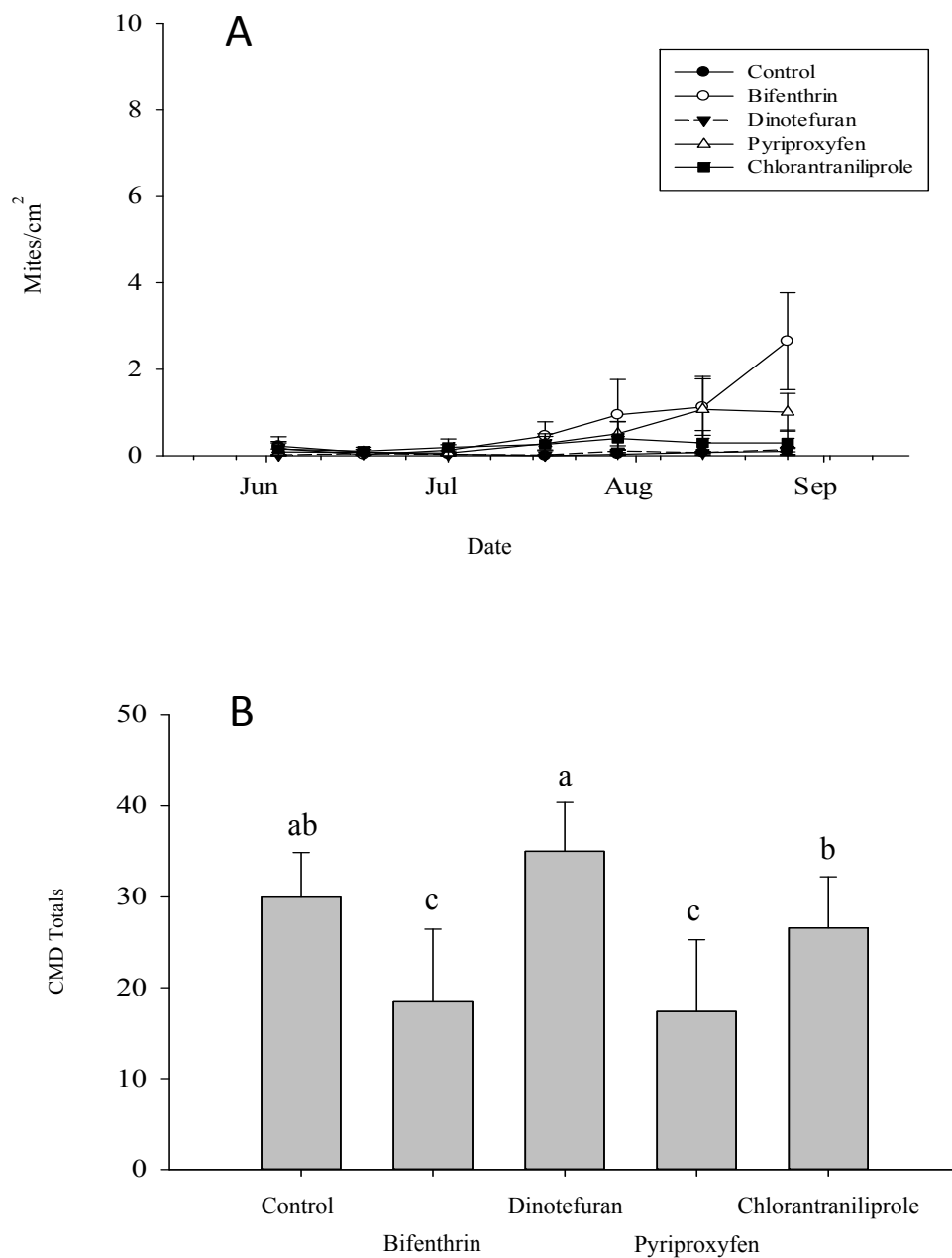


Figure 2.2 (A) *P. multidigituli* densities on honeylocust trees infested with *Eulecanium cerasorum* in Fishers, IN over the course of the 2012 season after applications were made in on 6 June. (B) Total cumulative densities of *Z. mali* on these trees over the course of the season.

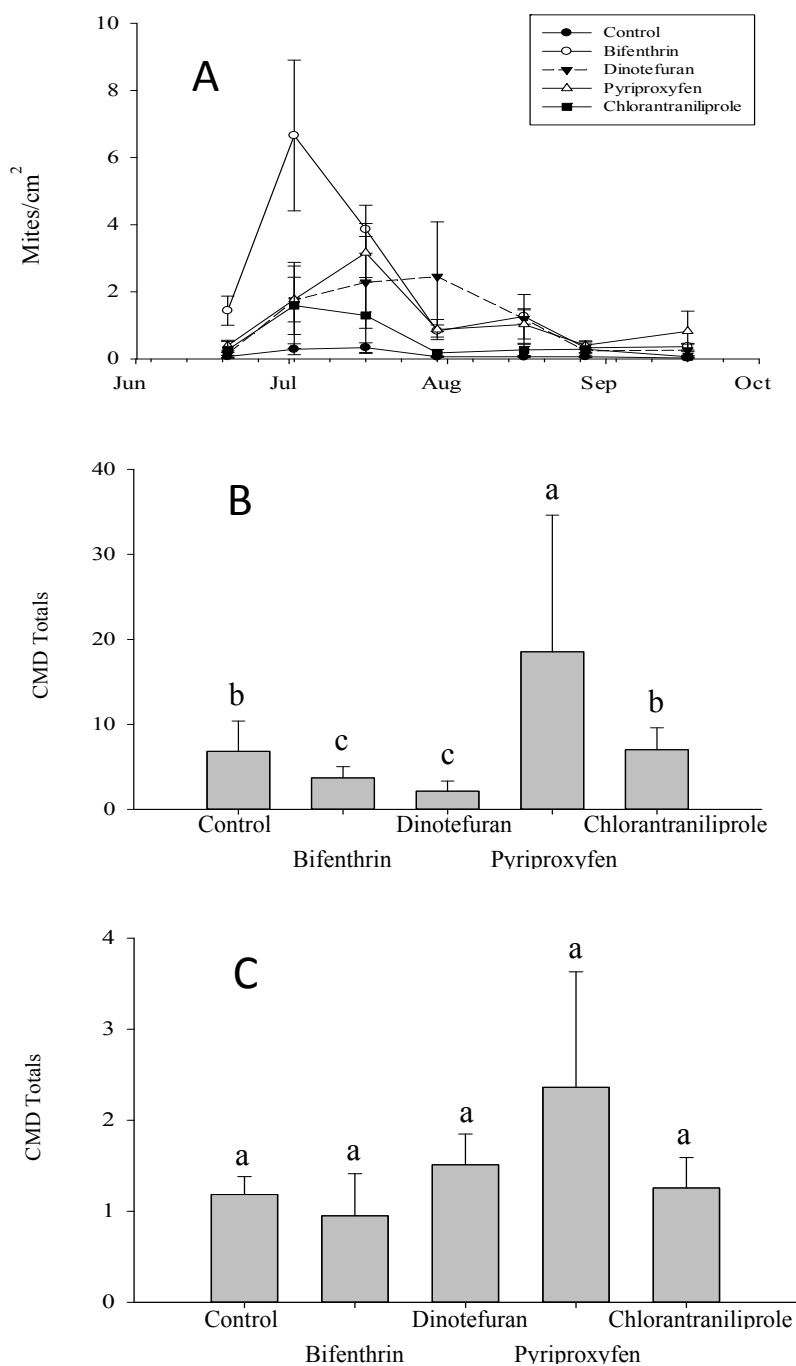


Figure 2.3 (A) *P. multidigituli* densities in on honeylocust trees infested with *Eulecanium cerasorum* in Fishers, IN over the course of the 2013 season. Total cumulative densities of *Z. mali* (B) and phytoseiids (C) on these trees over the course of the season. All applications were made on 2 or 3 May.

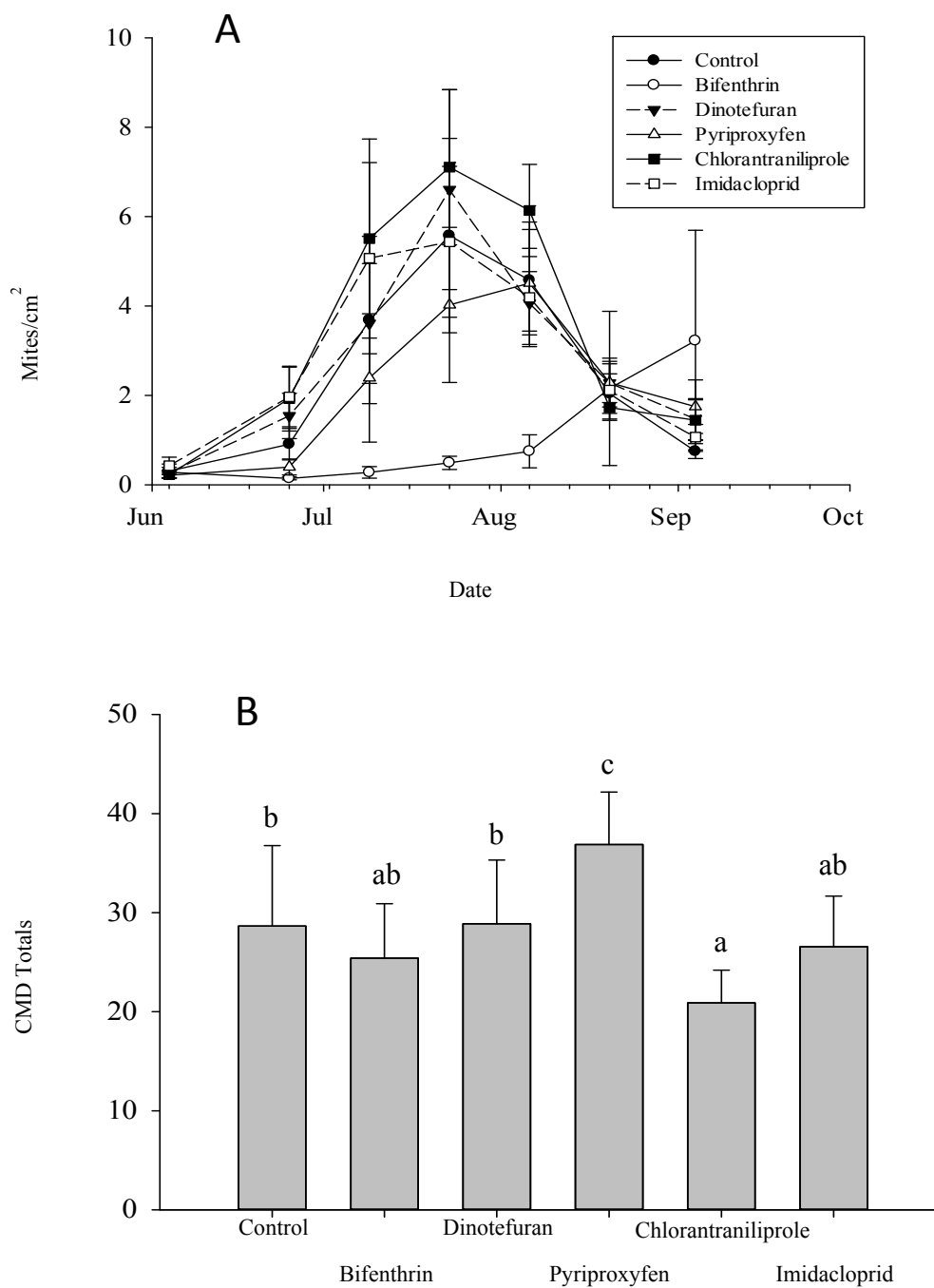


Figure 2.4 (A) *P. multidigituli* densities on honeylocust trees at a nursery in Westfield, IN over the course of the 2012 season after applications were made in on 8 June. (B) Total cumulative densities of *Z. mali* on these trees over the course of the season.

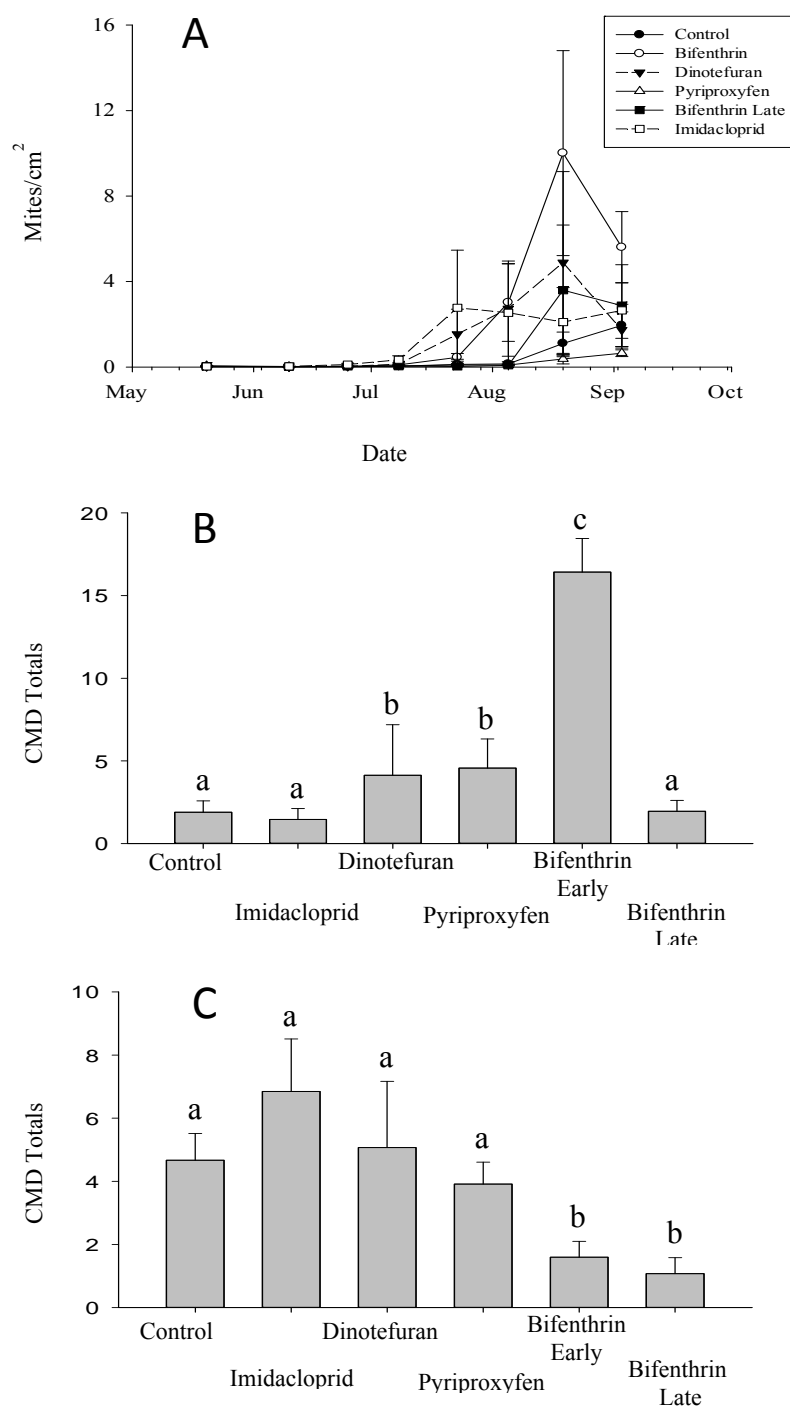


Figure 2.5 (A) *P. multidigituli* densities on honeylocust trees in Lafayette, IN over the course of the 2013 season. Total cumulative densities of *Z. mali* (B) and phytoseiids (C) on these trees over the course of the season. All but the late bifenthrin applications were made on 7 May. Late bifenthrin applications were made on 3 July.

CHAPTER 3. SUMMARY

Insecticides are sometimes necessary to treat pests in urban forests. However, their effects on spider mites and natural enemies of spider mites vary between classes of insecticides and timing of application. Although others have reported spider mite outbreaks to occur when neonicotinoids are applied to trees, the frequency and extent of this phenomenon varies (Szczepaniec personal communication). We also observed some insecticides (i.e. bifenthrin) to be very effective at killing all mite fauna in honeylocust trees. Yet, when bifenthrin is applied early we notice outbreaks of *P. multidigituli* because their populations recover more quickly than their natural enemy counterparts. In contrast, bifenthrin treatments applied later in the year do not cause outbreaks because *P. multidigituli* populations do not rebound to damaging levels before fall leaf drop. Urban tree caretakers should be cautious about applying broad spectrum insecticides early in the season to control pests of honeylocust.

Some of the commonly occurring pests of honeylocust trees that are treated for with insecticides in Indiana are honeylocust plant bug (*Diaphnocoris chlorionis*), mimosa webworm (*Homaduala anisocentra*), and calico scale (*Eulecanium cerasorum*). Using more selective pesticides when possible to control honeylocust pests may reduce negative effects on spider mite natural enemies such as *Z. mali* and phytoseiid mites. Limited research is available on the effectiveness of the insecticides used in this study against the

previously mentioned pests. However, *D. chlorionis* causes the most damage in the nymphal stage. Therefore, pyriproxyfen (an insect growth regulator) could be effective at killing this pest because IGR's inhibit proper adult formation by disrupting the endocrine system of insects. Also, pyriproxyfen has been shown to effectively kill other Hemipteran pests such as whiteflies (Ishaaya and Horowitz 1995) and calico scale (Quesada 2013). Chlorantraniliprole is labeled for treatment of multiple Lepidopteran pests including *H. anisocentra*. Its translaminar properties could make it effective at reaching the larvae, but this would need to be researched further.

Imidacloprid is recommended to treat for *D. chlorionis* (Krischik and Davidson 2004), but has been known to cause outbreaks of *P. multigituli* on honeylocust (Sclar et al. 1998). We also observed this phenomenon once over our three year study, but population sizes were much less than other treatments like bifenthrin. Imidacloprid is also ineffective at killing Lepidopteran pests. Similarly, dinotefuran could be used to treat for the two Hemipteran pests mentioned earlier, but potential for *P. multigituli* outbreaks is possible. Dinotefuran is also effective at killing *H. anisocentra* when applied a couple weeks prior to egg hatch (Sadof personal communication). Bifenthrin (a broad spectrum pyrethroid) is labeled for all of these honeylocust pests. However, this insecticide too has the ability to cause spider mite outbreaks.

The next step of this research should focus on identifying a density threshold for the common honeylocust pests discussed and determining when insecticide intervention could be warranted. Thresholds should be based on aesthetic damage, or loss of roughly 5-10% of the canopy to a pest (Sadof and Raupp 1997). Infestations of these pests are mostly aesthetic and do not typically cause death to hosts. By using density thresholds,

tree caretakers would have a monitoring tool that allows them to determine when insecticide intervention is necessary. Outbreaks of *P. multidigituli* later in the season could then be avoided by conserving natural enemies when the threshold is not reached, or by using only selective pesticides.

Perhaps assessing the LD50 for the pesticides used in this study on *P. multidigituli* and predatory mites could provide further insight to this system as we saw variability in density responses of mite species to insecticides. These inconsistencies may be better answered if a closer look is taken at direct exposure of mite species to the insecticides used in this experiment. This type of experiment proved difficult as mites are difficult to assess in a controlled arena due to their mobility and small size. Other studies assessing these effects have been conducted by placing mites on tape and then treating them to determine if they are sensitive to the compound. However, this method addresses the question in an extremely modified system.

Lastly, conducting predatory mite feeding assays on *P. multidigituli* would be interesting to see if similar interactions occur on domatia-covered honeylocust leaves as they do on *Acer rubrum* 'Red Sunset.' In Red Sunset maple trees, domatia reduces the interaction of predatory mite species leading to an additive effect on spider mite prey reduction (Prado 2013). This interaction on honeylocust would be helpful to determine whether it is necessary if both predators should be preserved in order to maintain stability or if only the presence of one predatory mite (i.e. phytoseiid mites) is necessary to keep spider mite populations from rapidly increasing.

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