ABSTRACT

Title of Dissertation: MECHANISMS UNDERLYING OUTBREAKS OF SPIDER MITES FOLLOWING APPLICATIONS OF IMIDACLOPRID.

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Imidacloprid is a widely used neonicotinoid insecticide with high efficacy and long residual activity, and it is frequently applied to manage insect pests in urban landscapes. Recent reports of secondary outbreaks of spider mites after imidacloprid applications have prompted research endeavors to explain the driving force of the abrupt increases in abundance of mites. In this research, I documented outbreaks of spider mites in field and greenhouse experiments, and explored the three main mechanisms that have been proposed to explain the outbreaks: elimination of natural enemies, direct stimulation of spider mite fecundity and changes in plant quality, specifically, changes in defense pathways. To this end, I examined if the outbreaks occur in field and greenhouse experiments, and tested if imidacloprid applications disrupted communities of beneficial insects and caused increased reproductive performance of spider mites in two woody ornamental systems, elm trees and boxwood shrubs. Additionally, I used a model

organism, tomato plants, to address the hypothesis of altered plant defenses in plants treated with imidacloprid.

I found overwhelming evidence that outbreaks of spider mites occur consistently following applications of imidacloprid in landscape and greenhouse experiments. Moreover, surveys of arthropods on elms and boxwoods showed no evidence of disruption of a key predator of spider mites that could explain the outbreaks. Importantly, I found a plant-mediated effect of imidacloprid on fecundity of spider mites, while there was no evidence that the insecticide applied directly to the mites exerted the same effect on their reproductive performance. Lastly, two genes involved in jasmonic and salicylic acid showed a differential expression in tomatoes treated with imidacloprid, indicating that it affected plants' defense pathways in ways that could render plants more suitable for spider mites. This research demonstrated that changes in quality of plants brought about by imidacloprid seem to be the driving mechanism of secondary outbreaks of spider mites.

MECHANISMS UNDERLYING OUTBREAKS OF SPIDER MITES FOLLOWING APPLICATIONS OF IMIDACLOPRID.

By

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Chapter 1: Abundance of a non-target pest, spider mites (Acari: Tetranychidae), increases abruptly following applications of imidacloprid to woody ornamental plants.

Abstract

Imidacloprid, a neonicotinoid insecticide, has been used worldwide since its development in the early 1990's. It is a highly efficacious insecticide with long residual activity against a wide range of target pests. Recently, economic and environmental benefits of imidacloprid have been overshadowed by reports of secondary outbreaks of spider mites (Acari: Tetranychidae) on plants treated with imidacloprid. The objectives of this study were to quantify differences in abundance of teranychids on commonly grown woody ornamental plants. Field and greenhouse experiments were conducted using elm trees and boxwood plants in a managed urban landscape and potted boxwoods and cotoneasters in a greenhouse. Abundance of *Tetranychus schoenei* McGregor on elm, *Eurytetranychus buxi* Garman on boxwood, and *Oligonychus ilicis* McGregor on cotoneaster was compared between treatments. Spider mites in the field and greenhouse experiments were more numerous on plants that received imidacloprid. Additionally, there is some evidence of increase in numbers of eriophyid mites (*Peralox insolita* Keifer, Acari: Diptilomiopidae) and tydeid mites (*Homeopronematus anconai* Baker, and *Lorryia spp* Oudemans, Acari: Tydeidae) on elm trees treated with imidacloprid. Abundance of a key predator of *T. schoenei*, *G. herbertae* (Acari: Phytoseiidae) was reduced on elms treated with imidacloprid. Implications of these findings to possible mechanisms of secondary outbreaks of spider mites are discussed.

Introduction

Outbreaks can be defined as dramatic increases in the abundance of arthropod pests that occur in relatively short periods of time that negatively affects some aspect of human well-being (Berryman 1987, Barbosa and Schultz 1987). Outbreaks of herbivorous arthropods that successfully compete with humans for valued resources have attracted considerable attention from scientists (Logan et al. 2003). Sudden and unpredictable spikes in abundance of pests are thought to arise from such factors as changes in physical environment, abundance and quality of host plants, inherent genetic propensity of the organisms, and disruptions of natural enemies that allow pests to escape regulation by predators and parasites (Berryman 1982, Barbosa and Schultz 1987, Wallner 1987, Logan et al. 2003, Raupp et al. 2009).

Climate is one of the most powerful factors in shaping geographical distribution of arthropods and alters the frequency of outbreaks (Logan et al. 2003). On a global scale, weather and rainfall are the most important predictors of insect distribution (Wallner 1987). Temperature fluctuations and drought have been recorded to precede insect outbreaks. For example, Powers et al. (1999) found that in addition to topography and vegetation type, weather phenomena played a role in outbreaks of bark beetles on Douglas fir in Oregon forest. Increased abundance of giant phasmids and chrysomelid beetles on eucalyptus was linked to temperature and rainfall in Australia (Nylin 2002). Temperature and patterns of rainfall are linked to outbreaks of herbivores driven by qualitative and quantitative changes in their host plants and disruption of predator-prey interactions that normally suppress pest populations (Berryman 1982, Barbosa and Schultz 1987, Wallner 1987, Logan 2003, Raupp et al. in press).

While natural events can dramatically destabilize trophic interactions, anthropogenic practices can have impacts equally damaging in consequences. Widespread use of pesticides to control herbivorous arthropods in urban landscape has been implicated in resurgence of primary pests and outbreaks of secondary herbivores (Ripper 1956, Roberts et al. 1973, McClure 1977, Dreistadt and Dahlsten 1986, Godfray and Chan 1990, Raupp et al. 1992, Raupp et al. 2001, Raupp et al. in press, Amalin et al. 2001, Marquini et al. 2002, Devotto et al. 2006, Frampton et al. 2007, Liang et al. 2007).

Documented cases of outbreaks of secondary pests are of particular relevance to my research. In 1975 Luck and Dahlsten reported that mosquito-fogging programs resulted in increased abundance of pine needle scale, *Chionaspis pinifoliae.* A secondary outbreak of another scale insect, European fruit lecanium, followed application of an insecticide aimed to control filth-flies (Merritt et al. 1983). Similar cases of increased abundance following insecticide applications were reported for citrus red mite, honeylocust spider mite, woolly whitefly, purple scale, citrus mealybug (Debach and Rose 1977, Sclar et al. 1998, Raupp et al. 2004, Raupp et al. 2008). It was the onset of widespread use of pesticides after World War II that contributed to the rise of spider mites (Acari: Tetranychidae) to a status of a worldwide pest (Kropczynska-Linkiewicz 1984). Population levels of tetranychids usually remain low until pesticides are applied (Prischmann 2005).

In general, outbreaks of pests following applications of insecticide are generally thought to arise due to a few mechanisms. These include insecticide-induced elimination of natural enemies or decrease of their foraging abilities, increased herbivore fecundity either by hormoligosis, the direct, sublethal effect of stress agent on reproductive ability

or trophobiosis, insecticide-driven changes in plant physiology that increase plant's nutritional value, elimination of competition and lastly, shift toward female-biased sex ratio that results in greater number of eggs are thought to cause the outbreaks of pests (Jones and Parella 1984, Trichilo and Wilson 1993, Hardin et al. 1995).

Elimination or decrease in foraging ability of natural enemies has been extensively studied with respect to pest outbreaks following application of insecticides (Roberts et al. 1973, Luck and Dahlsten 1975, DeBach and Rose 1977, Merritt et al. 1983, Dreistadt and Dahlsten 1986). Outbreaks of tetranychids often follow when pesticides remove predators and release mites from their regulating pressure (Ripper 1956, Croft and Brown 1975, Pimentel and Edwards 1982). Following the application of pyrethroid insecticides, Trichilo and Wilson (1993) noted a 12-fold increase in abundance of mites on treated plants. Natural enemy release and an increase in fecundity of mites where mechanisms underlying these dramatic increases. Applications of an organophosphate insecticide to wine grapes lead to increased abundance of spider mites that was linked to lower numbers of predatory phytoseiid mites (Acari: Phytoseiidae) (Prischmann et al. 2005). Phytoseiids are key predators of tetranychids (McMurtry et al. 1970, Helle and Sabelis 1985). Stavrinides and Mills (2009) observed higher levels of spider mites and lower numbers of predatory mites on grapes that were treated with imidacloprid, a neonicotinoid insecticide. However, many studies illustrated only the post-treatment effect of pesticides on predators, and failed to demonstrate that the specific beneficial arthropod was vital to regulating spider mite populations in a density-dependent relationship in the studied system (Ripper 1956, Hardin et al. 1995).

In addition to examples of disruption of natural enemies of spider mites, there are numerous reports of insecticides that directly affect mites. Saini and Cutkomp (1966) and Dittrich et al. (1974) reported that DDT increased oviposition and resulted in female-biased ratio in spider mites. Methyl carbamate applications had a stimulatory effect on spider mites as well (Dittrich et al. 1974, Boykin and Campbell 1982, Costa et al. 1988, Calabrese 1999). Another insecticide class, pyrethroids, affected tetranychids in a similar way. Application of synthetic pyrethroids resulted in higher fecundity, female-biased sex ratio, decreased generation time and delayed diapause (Iftner and Hall, 1984, Jones and Parella 1984, Costa et al. 1988, Gerson and Cohen 1989, Ayyappath 1997).

Moreover, in addition to effects of insecticides on natural enemies and reproductive stimulation of tetranychid mites, there is some evidence that insecticidal chemicals promote changes in plant quality that may lead to outbreaks of mites. Insecticides are known to have positive effects on plant growth (Pless et al. 1971, Wheeler and Bass 1971, Chelliah and Heinsrich 1980, Oosterhuis and Brown 2003, Gonias et al. 2006, Tenczar and Krischik 2006, Gonias et al. 2008), and there are several studies suggesting that changes in plant physiology have positives effect on abundance of spider mites. Boykin and Campbell (1982) found changes in physiology of peanut plants after carbaryl applications resulted in elevated populations of spider mites. This effect was also observed on soybean plants treated with carbofuran (Mellors et al. 1984). More recently, Gupta and Krischik (2007) reported that rose plants treated with imidacloprid had elevated indices of chlorophyll and leaf area and housed higher numbers of spider mites than untreated plants.

Elevated numbers of tetranychid mites after applications of imidacloprid have been observed in the past. Sclar et al. (1998) first described the phenomenon of increased populations of spider mites following the application of imidacloprid on honeylocust. Elevated populations of spider mites were later reported on hops and hemlocks (James et al. 2001, Raupp et al. 2004). Because of imidacloprid's widespread use in management of herbivorous insects (Li et al. 2001, James and Price 2002, Rogers et al. 2007), it is crucial to document the secondary outbreaks of mites and understand their underlying mechanisms.

Imidacloprid, [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine], was the first neonicotinoid that came into widespread use (Mullins 1993). It is similar in structure to nicotine, and acts as an agonist at the nicotinic acetylcholine receptor (nAChR) distributed throughout the nervous system of insects (Tomizawa and Casida 2003). Insect nAChRs are involved in rapid neurotransmission. Binding of the primary ligand, acetylcholine, to the extracellular domain of the receptor in the postsynaptic region results in conformational change of the receptor and subsequently an action potential is generated by influx of Na^+ ions and efflux of K^+ ions (Tomizawa and Casida 2003). After the acetyl group of the ligand is cleaved by acetylcholinesterase, choline leaves the receptor and membrane repolarizes (Tomizawa and Casida 2003). Imidacloprid has been found to depolarize and block transmission between synapses of the receptor at the postsynaptic membrane. It binds to the receptor causing an action potential, but then is not recognized by acetylcholinesterase and remains bound to the receptor (Matsuda et al. 2001). Neonicotinoids do not ionize at physiological pH, and are thus more hydrophobic and better at penetrating membranes (Tomizawa and Casida

2003). Moreover, the insecticide exhibits target site specificity for insect nAChR. This is attributed to the fact that imidacloprid's structure enhances its reactivity with insect nAChR (Tomizawa and Casida 2003, Matsuda et al. 2001). Imidacloprid contains bridgehead nitrogen and a strong electron withdrawing nitro group that are thought to strengthen interactions with particular amino acids of insect nAChR (Matsuda et al. 2001). This was confirmed by Matsuda et al. (2001) in an experiment that involved synthesis of a chemical that differed from imidacloprid only by absence of the bridgehead nitrogen and the nitro group. This molecule did not show high affinity for insect nAChR. Moreover, Zhang et al. (2000) found that the specificity of imidacloprid for the binding site on acetylcholine receptor was conserved between two different aphid species, a housefly and a fruit fly. This indicates that the high specificity of imidacloprid is conserved among insect species and families.

Imidacloprid’s chemical characteristics translate to very important practical benefits such as reduced environmental impact, high efficacy and long residual activity. Imidacloprid's high affinity for insect nAChRs increases its safety to humans and other mammals. The Environmental Protection Agency has found the insecticide to have no acute, reproductive, or carcinogenic toxicity (EPA 2000). A great advantage of the pesticide is that it can be applied as a soil drench or soil injection, which minimizes exposure of non-target arthropods to the chemical. Imidacloprid is a systemic insecticide, and it is absorbed through the roots into the vascular system, and distributed to plant tissues (Mullins 1993, Gill et al. 1999). It has been reported to control sucking insect pests such as aphids, whiteflies, lace bugs, adelgids as well as several species of Coleoptera and Diptera (James 1997, Gill et al. 1999, d'Eustachio and Raupp 2001, James

and Vogele 2001, Webb et al. 2003, Raupp et al. 2004, Szczepaniec and Raupp 2007). High effectiveness and low mammalian toxicity of imidacloprid stimulated research on other neonicotinoids: thiamethoxam, nitenpyram, acetamiprid, dinotefuran, clothianidin, all of which are currently available on the market. Neonicotinoid seed treatments have been shown to provide an effective control of potato leafhopper (Nault et al. 2004). In addition to providing excellent short-term control of insect pests, imidacloprid has shown exceptionally long activity: absence of pests and toxicity of foliage was observed up to three years following application in potted cotoneaster plants (Szczepaniec and Raupp 2007) and up to almost three years in established hemlock trees (Raupp et al. 2004).

The objective of this study was to document empirically the occurrence of spider mite outbreaks following applications of imidacloprid to plants growing in urban landscapes. To this end, the abundance of tetranychid mites was observed on elm trees and boxwood shrubs in managed landscapes and common gardens, and on boxwoods and cotoneasters in greenhouse studies. Spider mites are parenchyma-sucking arthropods that feed on cell contents of their host plants by piercing the cell walls with long stylets (Helle and Sabelis 1985). Another cell-contents feeders, lace bugs (Hemiptera: Tingidae) are known to be susceptible to imidacloprid (Gill et al. 1999, d'Eustachio and Raupp 2001), which implies that arthropods can come in contact with imidacloprid by sucking out the contents of plant cells. This allowed me to assume that spider mites were exposed to imidacloprid by consuming plants treated with the neonicotinoid. Species of mites used included *Tetranychus schoenei* (McGregor) on elm, *Eurytetranychus buxi* (Garman) on boxwoods and *Oligonychus ilicis* (McGregor) on cotoneasters. With the exception of a study of boxwoods conducted in a greenhouse, no mites other than naturally occurring

populations were introduced onto the experimental units. In addition to tetranychid mites, abundances of other mites were recorded in experiments conducted with elms in the field.

Methods

Study system: *Tetranychus schoenei* McGregor (Acari: Tetranychidae) and *Ulmus americana* Linn. (Urticales: Ulmaceae)

Herbivore and its natural enemies. The *T. schoenei*, (*Tetranychus schoenei* McGregor) is a polyphagous tetranychid distributed over the eastern and southeastern US (Reeves 1963). It shares many traits of its natural history with a close relative, the twospotted spider mite, *Tetranychus urticae* (described in Chapter 4). Similar in this respect to the twospotted mite, the *T. schoenei* has four developmental stages, larva, protonymph, deutonymph and adult. (Jeppson et al. 1975). There are morphological differences between different developmental forms of this mite. While deutonymphs and adult forms of *T. schoenei* have four dark spots, two located on each side of their bodies, larvae and protonymphs have only two spots, one on each side. The complete life cycle can take place in seven days at optimum temperature (25-28 °C), and there is an average of nine generations per year. At their maximum reported longevity, 36 days, *T. schoenei* females may lay over 100 eggs (Jeppson et al. 1975). *T. schoenei* feeds mainly on the underside of leaves and produces variable webbing. Heavy infestations of the pest cause yellowing of the leaves and leaf drop. This mite overwinters as mated females, and diapausing individuals can be distinguished by their bright orange coloration (Jeppson et al. 1975).

The natural enemies of this mite are shared by most tetranychids, and include phytoseiid mites (Acarina: Phytoseiidae) (Helle and Sabelis 1985, Dicke et al. 1999, Roda et al. 2000), minute spider mite destroyer, *Stethorus punctum* (Coleoptera: Coccinellidae) (Helle and Sabelis 1985, Roda et al. 2000, Rott and Ponsonby 2000, Roy et al. 2002, Roy et al. 2003), lacewing larvae (Neuroptera: Chrysopidae) (Reddy 2001, Rosenheim et al. 2004), dusty wings (Neuroptera: Coniopterygidae) and ceccidomyid larvae (Diptera: Ceccidomyiidae) (Huffaker and Messenger 1976). There are no reports of a specialist natural enemy feeding exclusively on the *T. schoenei.*

Host plant. The American elm (*Ulmus americana),* also known as water elm and white elm, is a deciduous tree native to North America (United Stated Department of Agriculture 2009a). It is distributed from Nova Scotia to Florida, and occurs as far west as Manitoba and down to central Texas (United Stated Department of Agriculture 2009a). Elms are fast-growing trees that are adapted to various types of soil and have medium drought tolerance (United Stated Department of Agriculture 2009b).

Elms' low - maintenance and highly aesthetic appearance made it one of the most popular landscape trees until the onset of Dutch elm disease (DED) in the 1930's (McLeod et al. 2005, Newhouse et al. 2007). Among other pests of American elms are leafhoppers, aphids, elm lace bugs, leaf miners, fall webworm, elm leaf beetle, eriophyid mites and spider mites (Johnson and Lyon 1991). Recently, an introduced species of a boring beetles, Asian longhorned beetle (ALB), *Anoplophora glabripennis,* has killed many elm trees and forced the removal of thousands of others in Illinois, New Jersey, New York and Massachusetts (United Stated Department on Agriculture 1996, 1998,

2003, 2007, 2008). ALB eradication efforts include prophylactic applications of imidacloprid in quarantine zones, which resulted in unusually high abundance of spider mites on American elms in Central Park, New York, NY (Raupp et al. 2008).

Study system: *Eurytetranychus buxi* Garman (Acari: Tetranychidae) and *Buxus sempervirens* Linn. (Euphorbiales: Buxaceae)

Herbivore and its natural enemies. *E. buxi* is a specialist and feeds only on boxwoods (Jeppson et al. 1975). Its developmental stages do not differ from other tetranychids. However, boxwood mites tend to have a longer life cycle. It takes about 20 days from the time an egg is laid for a mite to mature. Fecundity of *E. buxi* is also significantly lower than *T. schoenei.* Boxwood mites lay an average of 30 eggs in the span of their lifetime, which varies from two to five weeks (Jeppson et al. 1975). *E. buxi* prefers to feed on the upper side of young boxwood leaves, and in heavy infestations, the leaves may appear yellow from coalesced stippling injury. Boxwood spider mite does not produce webbing, and prefers high temperatures with low humidity (Jeppson et al. 1975). After approximately 8 generations a year, boxwood spider mites overwinter as eggs, and hatch in early spring (Jeppson et al. 1975).

As with other tetranychids, the key predator of this mite are phytoseiids (Acarina: Phytoseiidae) (Helle and Sabelis 1985, Dicke et al. 1999, Roda et al. 2000). Other important natural enemies of boxwood mites are minute spider mite destroyer, *Stethorus punctum* (Coleoptera: Coccinellidae) (Helle and Sabelis 1985, Roda et al. 2000, Rott and Ponsonby 2000, Roy et al. 2002, Roy et al. 2003), lacewing larvae (Neuroptera: Chrysopidae) (Reddy 2001, Rosenheim et al. 2004), dusty wings (Neuroptera:

Coniopterygidae) and ceccidomyid larvae (Diptera: Ceccidomyiidae) (Huffaker and Messenger 1976).

Host plant. Boxwoods (*Buxus sempervirens)* are one of the most popular woody ornamental shrubs grown in urban landscapes (Jagdale et al. 2002, Raupp et al. 1985). This introduced evergreen is distributed throughout the Continental US (ITIS 2009), but it originated in the Mediterranean region of Eurasia (Roberts and Wink 1998). Boxwood leaves are opposite, shiny and dark green, and contain toxic alkaloids such as cyclobuxine and buxanine (Roberts and Wink 1998). These compounds are derived from cholesterol skeletons and had medicinal use in ancient and medieval times (Roberts and Wink 1998). This slow-growing plant can reach up to ~15 feet in height, and clay and or loamy soils are the most suitable for growth (Gilman 1999).

A few key pests attack boxwoods. Leafminer *Monarthopalpus flavus* (Diptera: Cecidomyiidae) infestations are particularly troublesome because they cause yellowing and blistering of the leaves, which diminishes the aesthetic value of boxwoods (d'Eustachio and Raupp 2001a). Distortion of leaves is caused by a psyllid, *Psylla buxi*, (Hemiptera: Psyllidae) which is another troublesome boxwood pest. Psyllid feeding causes significant, visible damage by cupping of the leaves in which immatures of the pest develop. A few other pests such as various scale insects and a specialized tetranychid, boxwood spider mite, may inflict significant damage (Johnson and Lyon 1991). Imidacloprid applications are commonly administered to control boxwood leafminer and psyllid infestations (d'Eustachio and Raupp 2001b).

Study system: *Oligonychus ilicis* McGregor (Acari: Tetranychidae) and *Cotoneaster salicifolius* Franch (Rosales: Rosaceae)

Herbivore. Southern red mites, *O. ilicis,* are oligophagous pests with a worldwide range (Jeppson et al. 1975). It is the most destructive and widespread tetranychid pest of broad-leaved evergreens, and it attacks a variety of economically important plants (Jeppson et al. 1975, Johnson and Lyon 1995). As their common name suggests, southern red mites are red to purple in color, and lay eggs of a similar coloration (Jeppson et al. 1975). They can complete their development in two weeks at 22-24° C and have multiple, overlapping generation during periods of their activity (Jeppson et al. 1975). Feeding by these mites results in bronzing of the foliage, especially along the mid-rib (Jeppson et al. 1975). Southern red mite prefers cooler season and aestivates as eggs during the summer months (Jeppson et al. 1975, Johnson and Lyon 1995). *O. ilicis* was found to be most abundant in late spring and early fall in Massachusetts and Kentucky (Jeppson et al. 1975, Potter and Kimmerer 1989). Phytoseiids (Acarina: Phytoseiidae) are a key natural enemy of this spider mite (Helle and Sabelis 1985, Dicke et al. 1999, Roda et al. 2000)

Host plant. Cotoneaster (*Cotoneaster salicifolius)* is a shrub commonly grown in urban landscape. It reaches a maximum height of 0.3 m, has attractive dark green leaves and produces abundant red berries in the winter months (USDA 2009b). It is native to central Asia, and has escaped cultivation and become an invasive species in parts of California and Hawaii (Starr et al. 2003). Cotoneasters are attacked by hawthorn lace bugs, *Corythuca cydoniae* Fitch (Hemiptera: Tingidae), whose feeding may lead to

severe discoloration of leaves and eventual leaf drop in high infestations (Schultz 1983). This serious key pest is successfully controlled with imidacloprid (Gill et al. 1999, Szczepaniec and Raupp 2007).

Effect of imidacloprid applications on abundance of spider mites on elm trees and boxwood shrubs in managed landscapes

Field experiment: Elm. To investigate how applications of imidacloprid applied to the soil affected spider mite populations on elms, 18 elm trees were planted in a common garden at the University of Maryland Turf Research Farm in College Park, Maryland in May 2005. The trees were purchased from a nursery, and had a trunk diameter at breast-height (DBH) of approximately 2.5 cm at the time of planting. They were hand-watered as needed throughout each season, and received applications of 15 g of a slow-release fertilizer Osmocote® (N:P:K of 17:7:12) once a year. In a completely randomized block design, nine elms were treated with imidacloprid (Merit® soluble powder formulation, 750g of imidacloprid/kg, Bayer, Kansas City, MO) at the label rate of 1.4 tsp (~2 g) per 2.5 cm DBH dissolved in 1L of water. Nine other elms were designated as untreated controls. I applied imidacloprid on 06/05/2006, and repeated the applications on 05/11/2007 and 05/19/2008.

Numbers active stages and eggs of the spider mite, *T. schoenei*, and other mites were counted from June to September in 2007 and 2008. Four terminal leaves were removed from two branches on each tree using hand and pole pruners. Foliage was brought back to the laboratory in a cooler filled with ice where spider mites on both sides of the leaves were counted using a dissecting microscope. To compute density of mites, leaf area was measured using LI-31100C area meter (Li-Cor® Biosciences USA) and

arthropod abundance was expressed as the number per cm^2 of leaf area. Spider mites feed by sucking out contents of plant cells, and their feeding has little effect on biomass of the hosts unless populations reach extremely high levels. Thus, density of the mites was the most suitable parameter to detect fluctuations in mite abundance.

Field experiment: Boxwood. Responses of *E. buxi* to applications of imidacloprid were evaluated on boxwoods, *B. sempervirens,* in a managed landscape on campus of the University of Maryland, College Park, MD, U.S.A. Boxwoods in this garden were not trimmed during the study and they received no supplemental water other than rain. I used 20 boxwoods that were approximately 0.6 m tall, and grew in rows separated by about 0.3 m between individual plants. The experiment was a completely randomized design with two boxwoods between shrubs assigned to treatments serving as buffers. Ten plants received imidacloprid and ten boxwoods were designated as untreated controls. Imidacloprid was administered to the plants on 05/17/2005. Imidacloprid was applied as a soil drench formulation (Merit® water soluble powder, 750 g of imidacloprid/kg, Bayer, Kansas City, MO) at the label rate of 1.4 tsp (~ 2 g) per 0.3 m of height dissolved in 1L of water. Spider mite abundance was evaluated throughout the growing season in 2005 as described above, with the exception that mites on five leaves rather than four were recorded. Leaf area was measured using Li-Cor® LI-31100C area meter and abundance of mites was expressed as the number per cm^2 of leaf area.

Effect of imidacloprid applications on abundance of spider mites on boxwoods and cotoneasters in a greenhouse

Greenhouse study: Boxwood. This experiment was conducted at the Research Greenhouse Complex at the University of Maryland, College Park, MD, U.S.A. Containerized boxwoods, *B. sempervirens,* measuring 0.3 – 0.5 m in height, were purchased from a commercial supplier. Shrubs were potted in 3.7 L containers, they were maintained at 18 – 22° C, and received approximately 0.2 L of water every day delivered by drip irrigation. To evaluate how soil drench applications of imidacloprid affected abundance of spider mites, 10 boxwood plants free of spider mites received imidacloprid (Marathon® soluble powder formulation, 600 g of imidacloprid/kg, Bayer, Kansas City, MO) at the high label rate of 0.33 g per pot dissolved in 0.1 L of water. Ten other boxwoods were designated as untreated controls. Boxwoods in both treatments were then interspersed with shrubs heavily infested with boxwood mite to create infestations on experimental units. Imidacloprid was administered in February 2008, and numbers of boxwood mites were recorded four months later. Branch samples and mite counts were obtained in the same manner described above for the field experiment.

Greenhouse study: Cotoneaster. This experiment was conducted at the Research Greenhouse Complex at the University of Maryland, College Park, MD, U.S.A. Plant material for this study was purchased at a retail nursery. Cotoneasters, *C. salicifolius,* were in 3.7 L pots, and measured approximately 0.5 m in height. Plants were maintained in the conditions described previously for boxwoods, and were watered every other day with a hand-held hose until leaching was observed. Ten cotoneasters were randomly

assigned to receive imidacloprid and 10 were designated as untreated controls. The insecticide was administered on 05/17/2005 as a soil drench formulation at the high dose described on the label as described previously. Southern red mites, *O. ilicis*, were recorded on treated and untreated cotoneasters in August by sampling five terminal leaves from four branches chosen at random from each experimental unit. Branches were not excised from cotoneasters, and a hand lens (10X, Hasting Triplet, Bausch & Lomb®) was used to count spider mites and their eggs on both sides of each leaf. Spider mite density was expressed as number of mites per terminal.

Statistical analyses

Results of the field experiments that spanned entire growing seasons were analyzed using repeated measures model of analysis of variance (SAS 2008). If time by treatments interactions were statistically significant, then one-way ANOVA tests were performed at each date of sampling, (Statistix 2005). For each of the greenhouse experiments, normality of distribution was tested using a Shipiro-Wilk test, and homogeneity of variance was evaluated according to Levene's test (SAS 2008). One-way analysis of variance was performed on data that were normally distributed and homoschedastic. Abundance was compared using a nonparametric Kruskal – Wallace test if assumptions of ANOVA could not be met by raw or transformed data (Ott and Longnecker 2001).

Results

Effect of imidacloprid applications on abundance of spider mites on elm trees and boxwood shrubs in managed landscapes

Field experiment: Elm. Abundance of spider mites on elms differed between treatments in both sampling years (Figure 1.1). In 2007, there was a significant interactive effect of treatment and time ($F_{2,52} = 9.17$, $P = 0.0012$). Treated elm trees housed more mites than untreated trees on two out of the three sampling dates in 2007 (Figure 1.1, Table 1.1). Similarly, in 2008 there was a significant treatment by time interaction on tetranychid abundance ($F_{3,71} = 5.10$, $P = 0.0053$) (Figure 1.1). With the exception of the first sampling in June, *T. schoenei* were more numerous on elms treated with imidacloprid (Table 1.1).

In addition to tetranychid mites, elms housed three other mites that were relatively common. Phytophagous mites from the super family Eriophyoidea responded positively to imidacloprid applications. *Peralox insolita* Keifer (Acari: Diptilomiopidae) was significantly more abundant on imidacloprid treated elms in 2007 ($F_{1,24} = 11.57$, $P = 0.0023$) and there was no significant interactive effect of time and treatment on abundance of these mites ($F_{2,52} = 2.64$, $P = 0.0922$) (Figure 1.2). *P. insolita* were significantly more numerous on treated elms only on the first sampling date (Table 1.1). However, a trend for greater abundance on elms that received imidacloprid application continued throughout the season. In contrast, imidacloprid had no effect on eriophyids during the following year ($F_{1,32} = 0.01$, $P = 0.9232$) (Figure 1.2). In 2008, eriophyid mites were more abundant on control plants on the first sampling date (Table 1.1), while the trend reversed one month later. In both years, eriophyid mites populated trees in

greater numbers during June and July and decreased in the month of August and early September.

Abundance patterns of another acarian, tydeid mites *Homeopronematus anconai* Baker and *Lorryia sp.* Oudemans (Acari: Tydeidae), was somewhat similar to *P. insolita*. Majority of tydeids collected on the elms were in the species *H. anconai*. In 2007, tydeid mites were significantly more abundant on elms that received imidacloprid ($F_{1,24} = 4.79$, $P = 0.0387$), and there was no interactive effect of time and treatment on tydeid numbers ($F_{2,52} = 0.88$, $P = 0.4260$) (Figure 1.3). Treated elms had consistently greater abundance of tydeids in 2007 (Table 1.1). However, the effect of imidacloprid on these mites was not repeated in 2008. Tydeids tended to be greater on treated trees on most dates, but the difference between treatments was not significant ($F_{1,32} = 0.01$, $P = 0.9363$) (Figure 1.3). In fact, mites were more numerous on untreated elms on the last sampling date.

Importantly, the opposite trend was observed for the effect of imidacloprid on abundance of predatory mites, *Galendromus herbertae* Chant and *Galendromus halvelous* Chant (Acari: Phytoseiidae), which are key natural enemies of spider mites. *G. herbertae* was more common of the two species collected. These species could not be readily separated visually during the processing of samples, but the ratio of *G. herbertae* to *G. halvelous* was approximately 8:1. In both sampling years, phytoseiid numbers were significantly different between treatments ($F_{1,24} = 5.06$, $P = 0.0340$, $F_{1,32} = 20.34$, $P = 0.0001$ in 2007 and 2008, respectively) (Figure 1.4). In addition, interaction between time and treatment was not significant in either year ($F_{2,52} = 2.03$, $P = 0.1529$ and $F_{3,71} = 3.70$, $P = 0.217$ in 2007 and 2008, respectively). In both years, the trend for higher abundance of phytoseiids on untreated elms persisted throughout the season, and

Galendromus sp. were significantly more abundant on two of the sampling dates in each year (Table 1.1).

Field experiment: Boxwood. Boxwood spider mites responded in a pattern similar to *T. schoenei*. Imidacloprid had a significant effect on abundance of *E. buxi* ($F_{1,72}$ = 11.03, P = 0.0013) (Figure 1.5). However, in case of the boxwood mite, time and treatment did not have an interactive effect on the spider mites ($F_{7,159}$ = 0.63, P = 0.7310). Mean numbers of the mite were higher on imidacloprid-treated shrubs on most sampling dates. Mite abundance declined sharply in both treatments in mid-July.

Effect of imidacloprid applications on abundance of spider mites on boxwoods and cotoneasters in a greenhouse.

Greenhouse studies: Boxwood and Cotoneaster. Abundance of boxwood spider mites in the greenhouse differed significantly between treatments ($F_{1,18}$ = 12.56, P = 0.0023) (Figure 1.6). Treated boxwoods housed a greater number of mites 4 mo after imidacloprid was applied to the shrubs. Similarly, southern red spider mites were more numerous on cotoneasters treated with imidacloprid ($F_{1,18}$ = 6.53, P = 0.0199) (Figure 1.7). *O. ilicis* were nearly 20 times more abundant on cotoneasters treated with imidacloprid.

Discussion

Applications of imidacloprid to elms and boxwoods resulted in outbreaks of tetranychid mites on plants treated in the field. Abundance of mites on imidacloprid-treated elms was ten-fold greater than on untreated plants on three sampling dates over

the course of the experiment. By mid-summer in both sampling years, densities of *T. schoenei* on trees treated with imidacloprid surpassed those of untreated elms, and while date of sampling had a significant effect on abundance of mites, it does not explain elevated numbers of mites on treated plants.

While the magnitude of difference in abundance was not as pronounced as on elms, spider mites on boxwoods also responded differentially to imidacloprid applications. Additionally, numbers of mites on imidacloprid-treated boxwoods were not dependent on date of sampling. *E. buxi* were significantly more abundant on treated shrubs on all sampling dates past mid-July. Relative to the earlier sampling dates, mites on untreated shrubs declined, while mean number of tetranychids on treated boxwoods remained unchanged. Patterns of abundance of this mite suggests it is a cool-season mite, and imidacloprid-containing plants seem to provide better conditions for this arachnid in the hotter, more humid environment of Maryland summers.

Perhaps the most important finding that helps untangle mechanisms leading to outbreaks of spider mites after imidacloprid treatments are the results of greenhouse experiments. On boxwoods and cotoneasters grown in the greenhouse, abundances of *E. buxi* and *O. ilicis* were significantly greater when plants received applications of imidacloprid. These plants housed elevated populations of mites from two genera in the relative absence of natural enemies. This provides strong evidence that while natural enemies may play a role in outbreaks; there is a strong bottom-up force that drives a positive response of mites to plants treated with the imidacloprid. Either imidacloprid in plant tissues affects mites directly, or it promotes changes in the quality of plants that render it nutritiously more suitable or less defended.

While there is only one study that found empirical evidence that imidacloprid enhances spider mite fecundity directly (James and Price 2002), the literature offers compelling support for the plant-mediated mechanisms of the outbreaks. Most recently, Gupta and Krischik (2007) described rose plants that received three times the label dose of imidacloprid with a greater total chlorophyll index, leaf nitrogen content, and leaf area than the untreated plants. While greater chlorophyll index translates into increased photosynthetic capacity of plants (Campbell and Reece 2002) nitrogen content has been positively correlated with increased spider mite fecundity and shorter developmental time (Kropczynska-Linkiewicz 1984, Helle and Sabelis 1985, Wilson et al. 1988). In addition to these studies, Tenczar and Krischik (2006) found poplar trees to have an increased rate of growth between one and four years after applications of imidacloprid. Moreover, imidacloprid has been reported to have a positive effect on yield and growth rates of cotton. Gonias et al. (2006) found that cotton treated with imidacloprid had increased yield of 7% and elevated dry weight of 16%. In addition, it was later demonstrated that imidacloprid-treated cotton had greater photosynthetic rates and chlorophyll index than untreated plants (Gonias et al. 2008). This response was amplified when plants experienced temperature and water stress, suggesting that imidacloprid enhanced tolerance of cotton to stress, a possibility alluded to by Thielert (2006). It is important to note that the body of research cited here illustrates that applications of imidacloprid may promote changes in plant physiology that could be involved with outbreaks of spider mites.

Imidacloprid also had an effect on populations of eriophyid mites, *P. insolita.* Eriophyid mites are small (0.1-0.3 mm), spindle-shaped mites with two pairs of legs

(Jeppson et al. 1975). They are phytophagous, and feed primarily on succulent plant tissue causing fine stippling that coalesces into larger brown spots when populations reach high numbers (Jeppson et al. 1975). Some species secrete chemicals that change plants' growth patterns resulting in galls and curling of leaves inside of which the mites feed and reproduce (Jeppson et al. 1975). There are three families in the superfamily Eriophoidea: Phytoptidae, Eriophyidae and Diptilomiopidae, and they are distributed worldwide (Jeppson et al. 1975; Linquist et al. 1996; Childers and Achor 1999). *P. insolita* (Diptilomiopidae) is a common vagrant on elm leaves and its feeding can result in fine stippling, while another eriophyid mite attacking elms, *Eriophyes ulmi* Gar (Acari: Eriophyidae) causes formation of small, thin galls on the upper side of leaves (Johnson and Lyon 1991).

In this experiment, in addition to tetranychid outbreaks, an interesting trend for increased numbers of eriophyid mites on treated elm trees emerged. Previously, the positive effect of imidacloprid applications on colonization of eriophyid mite, *Aceria tosichella* Keifer, was reported on wheat (Harvey et al. 1998). Moreover, Raupp et al. (2004), illustrated increased rust mite (Acari: Eriophyidae) damage and one case of increased abundance of hemlock rust mites, *Nalepella tsugifolia* Keifer on imidacloprid-treated trees. However, Raupp et al. (2004) also found no increase in rust mite abundance in a second study. Similarly, a trend for greater abundance of eriophyids was observed in a survey of arthropod fauna on imidacloprid-treated elms in a managed landscape in Maryland (Chapter 2). In this study, however, eriophyids were more abundant on imidacloprid-containing trees. Notably, in this as well as other studies, eriophyids do not always respond to imidacloprid exposure consistently.

Tydeid mites on elms follow abundance patterns similar to those of the eriophyid mite. Tydeid biology is not fully known, even though they are one of the most commonly encountered family of mites on leaves (Walter and Behan-Pelletier 1999). However, *H. anconai,* which was the most abundant tydeid collected, is known to feed on eriophyid mites (Hessein and Perring 1986, Hessein and Perring 1988, Aguilar et al. 2001, Kawai 2002, Mainul and Kawai 2003), while *Lorryia sp.* are plant and fungi feeders (Jeppson et al. 1975, Mendel and Gerson 1982, Badii et al. 2001). On nearly all of the sampling dates, imidacloprid-treated elms housed more tydeids than untreated trees. Increased incidence of these mites could be caused by greater availability of eriophyid mites in case of *H. anconai,* or changes in quality of plants or fungal resources in case of tydeids in *Lorryia sp*. Effects of imidacloprid on interactions between tydeid mites and their prey deserve a closer look in future research.

The results of my study indicated that applications of imidacloprid to elms might harm phytoseiid mites. Most phytoseiids collected on the elms belonged to the species *G. herbertae* , which is a selective predator of Tetranychidae (McMurtry and Croft 1997). Phytoseiids responded fairly consistently in both sampling years. These results suggest that either consuming prey that are toxic or exposure to foliage of treated elms is detrimental to this mite. Imidacloprid has been shown previously to have negative impacts on abundance of phytoseiid mites (James and Coyle 2001, James 2003, Kimm et al. 2005, Stavrinides and Mills 2009). Because phytoseiid mites are key predators of spider mites (Helle and Sabelis 1985, Dicke et al. 1999, Roda et al. 2000), it is conceivable that the release of spider mites from phytoseiid regulation contributes to mite outbreaks.

It is noteworthy, however, that Phytoseiidae respond ambiguously to exposure to imidacloprid. There is one report of stimulated reproduction of a phytoseiid mite exposed to sprays of imidacloprid. James (1997) reported that *Amblyseius victoriensis* (Acari: Phytoseiidae) laid more eggs in laboratory experiments when directly exposed to imidacloprid sprays than its untreated counterparts. This experiment was not replicated by other researchers, however, and remains the singular case of a positive effect of imidacloprid on a phytoseiid mite.

Additionally, phytoseiids do not always suppress populations of spider mites in urban settings. Ehler and Frankie (1979) found well developed communities of predatory mites on oaks that were not able to prevent spider mite outbreaks in an urban landscape. The authors suggested that mite outbreaks on these trees were related to plant stress rather than release from natural enemies. Kropczynska et al. (1986) reported a similar finding for populations of spider mites outbreaking despite an assemblage of phytoseiid predators on lindens in Warsaw, Poland. Enhanced nutritional quality of lindens along streets elevated fecundity of *Eotetranychus tiliarum* (Acari: Tetranychidae) and resulted in subsequent outbreaks of mites. Additionally, a few studies report lack of numerical response of phytoseiids to increasing prey availability. Such examples include interactions between *Amblyseius potentillae* (Garman) (Acari: Phytoseiidae) and *T. urticae* on rose plants, and *Amblyseius ovalis* (Evans) (Acari: Phytoseiidae) and *Tetranychus kanzawai* (Kishida) (Acari: Tetranychidae) on maize (Halle and Sabelis 1985).

Tables

Table 1.1. Results of analyses of variance (F_{df}) and non-parametric Kruskal-Wallis test (X^2 (df)) comparing abundance of arthropods within each sampling date on imidacloprid-treated elms and untreated elms.

Date	**6/13/07**		**8/01/07**		**9/01/07**	
Taxa	Test	*P*	Test	*P*	Test	*P*
T. schoenei	X^2= 4.86 (1)	0.028	X^2= 9.85 (1)	0.002	X^2= 8.24 (1)	0.004
P. insolita	$F_{1,18}$= 18.52	0.001	$F_{1,18}$= 2.86	0.120	$F_{1,18}$= 0	0.952
Tydeidae	$F_{1,18}$= 3.34	0.086	$F_{1,18}$= 4.64	0.042	X^2= 0.33 (1)	0.564
Galendromus sp	X^2=0.01 (1)	0.963	$F_{1,18}$= 5.85	0.028	$F_{1,18}$= 3.39	0.084

Date	**6/24/08**		**7/21/08**		**8/11/08**		**9/11/08**	
Taxa	Test	*P*	Test	*P*	Test	*P*	Test	*P*
T. schoenei	X^2= 1.84 (1)	0.175	X^2= 12.29 (1)	0.001	$F_{1,18}$= 15.43	0.001	X^2= 11.68 (1)	0.001
P. insolita	$F_{1,18}$= 12.71	0.003	X^2= 1.031 (1)	0.310	X^2= 0.049 (1)	0.825	$F_{1,18}$= 0.48	0.498
Tydeidae	X^2= 0.03 (1)	0.859	$F_{1,18}$= 0.4	0.537	X^2= 0.2 (1)	0.659	$F_{1,18}$= 16.3	0.001
Galendromus sp	X^2= 0.66 (1)	0.418	X^2= 1.87 (1)	0.171	$F_{1,18}$= 10.13	0.006	X^2= 12.80 (1)	0.001

Figures

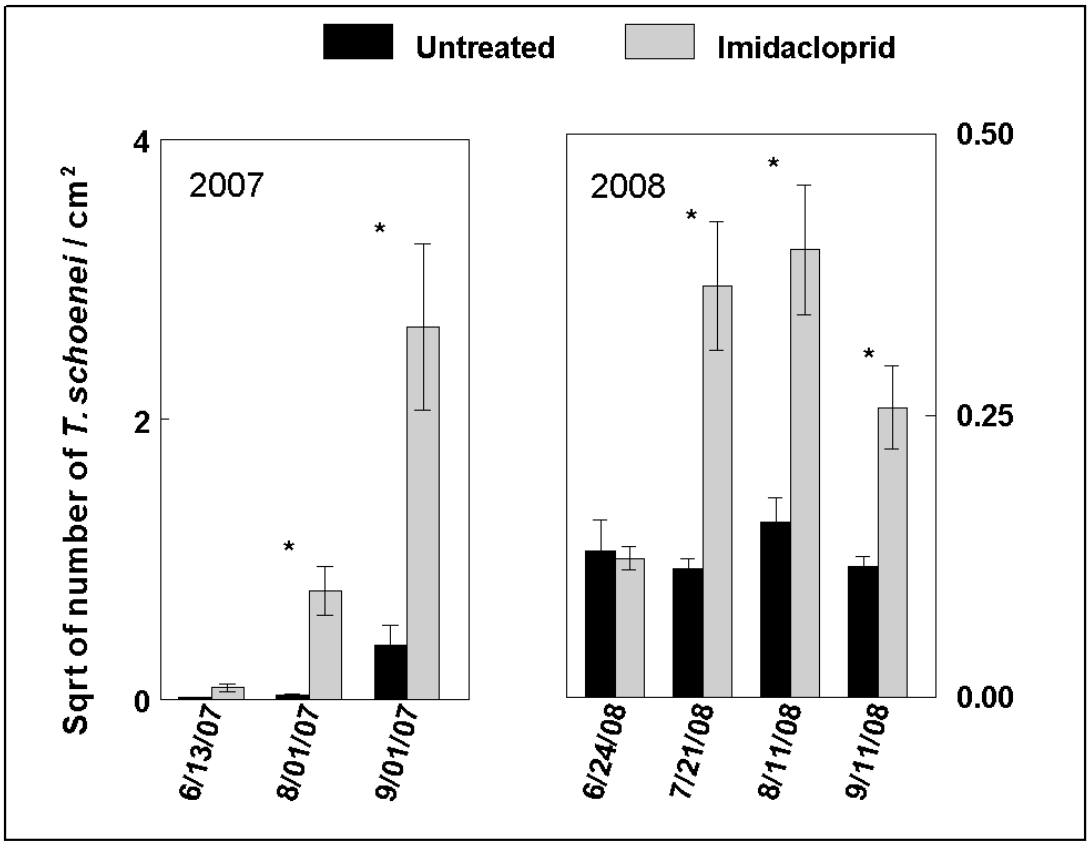

Figure 1.1. Abundance of *T. schoenei* imidacloprid treated and untreated elms in College Park, MD in 2007 and 2008. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

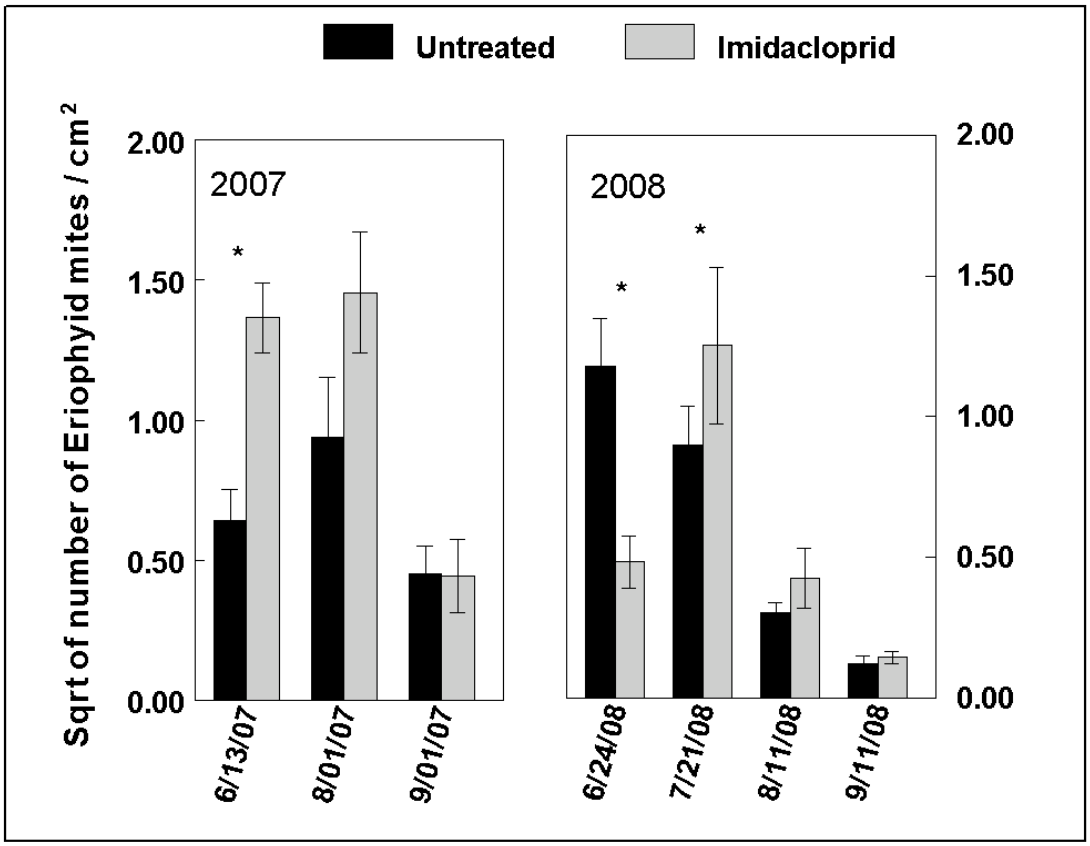

Figure 1.2. Abundance of an eriophyid mites on imidacloprid treated and untreated elms in College Park, MD in 2007 and 2008. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

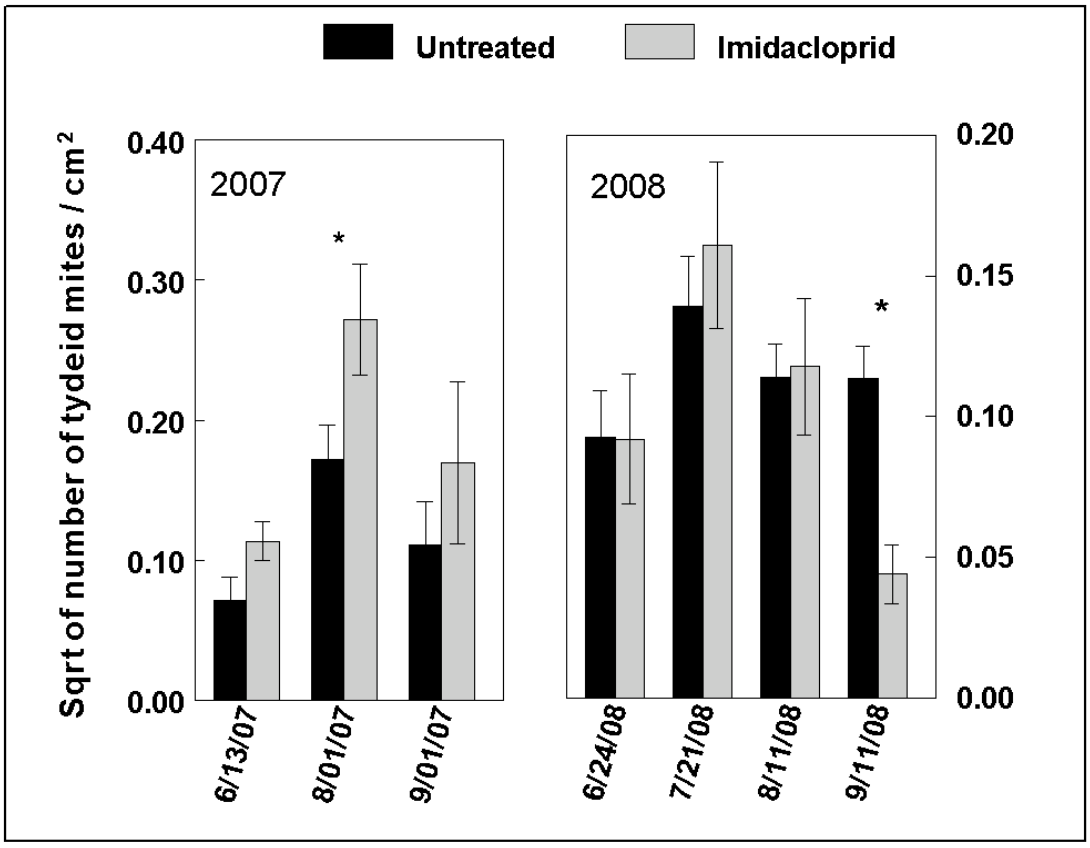

Figure 1.3. Abundance of tydeid mites on imidacloprid treated and untreated elms in College Park, MD in 2007 and 2008. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

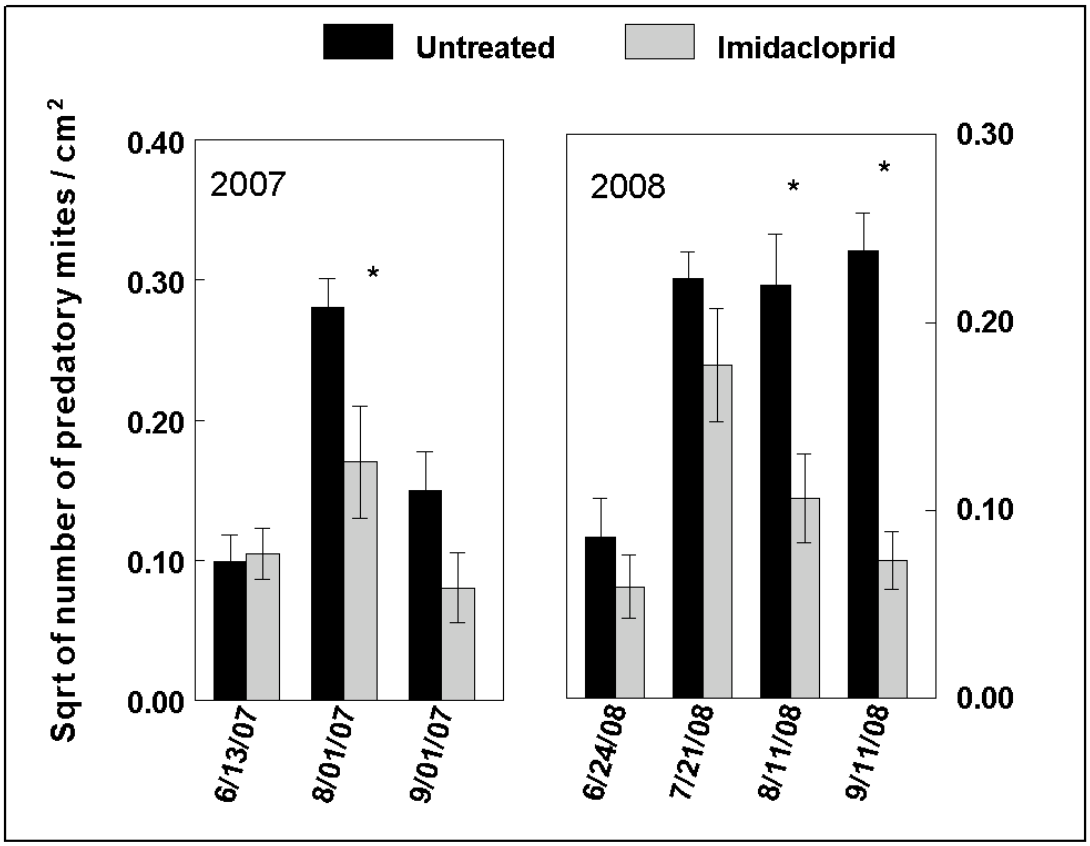

Figure 1.4. Abundance of predatory mites on imidacloprid treated and untreated elms in College Park, MD in 2007 and 2008. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

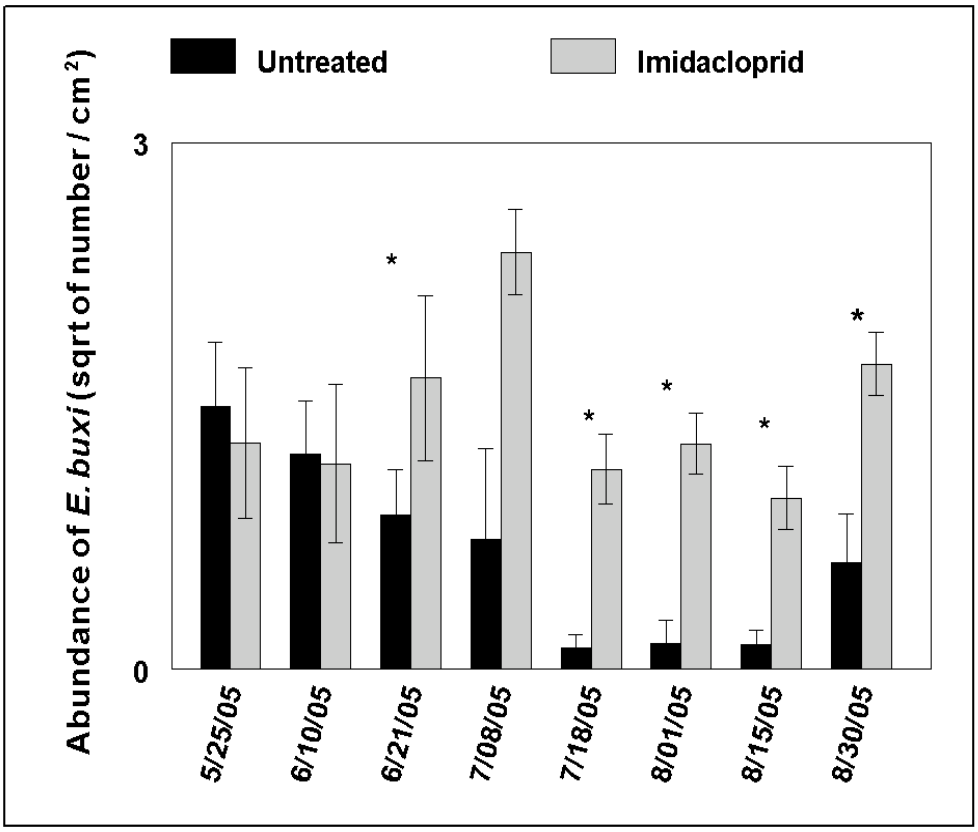

Figure 1.5. Abundance of boxwood spider mite imidacloprid treated and untreated boxwoods in College Park, MD in 2005. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

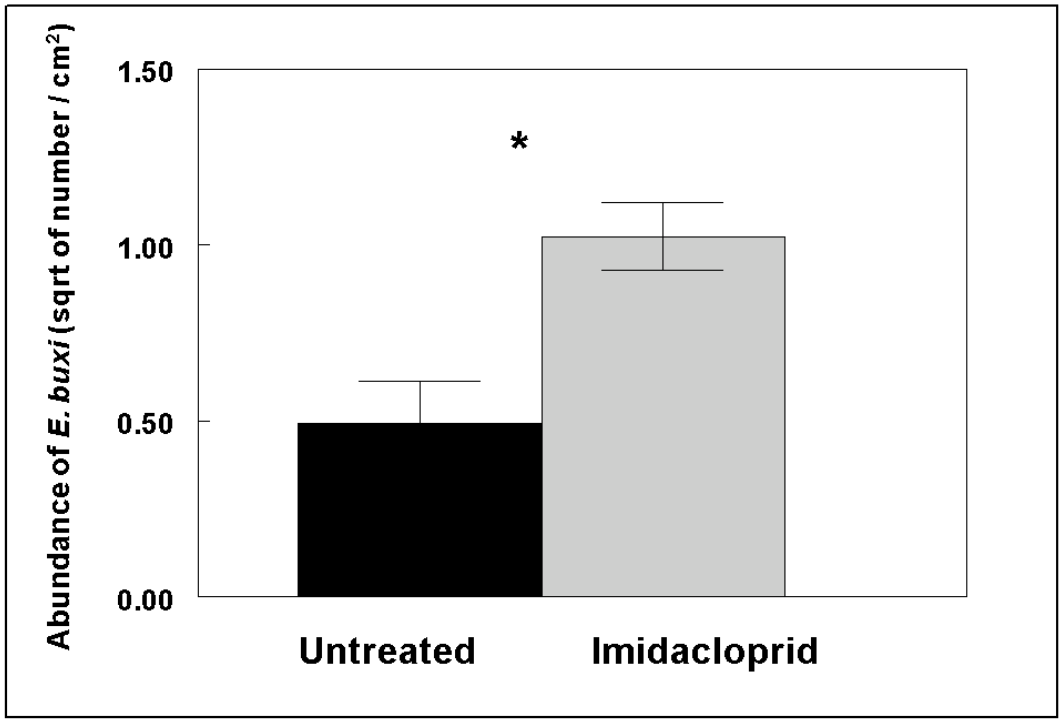

Figure 1.6. Abundance of *E. buxi* on imidacloprid treated and untreated boxwoods in a greenhouse. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

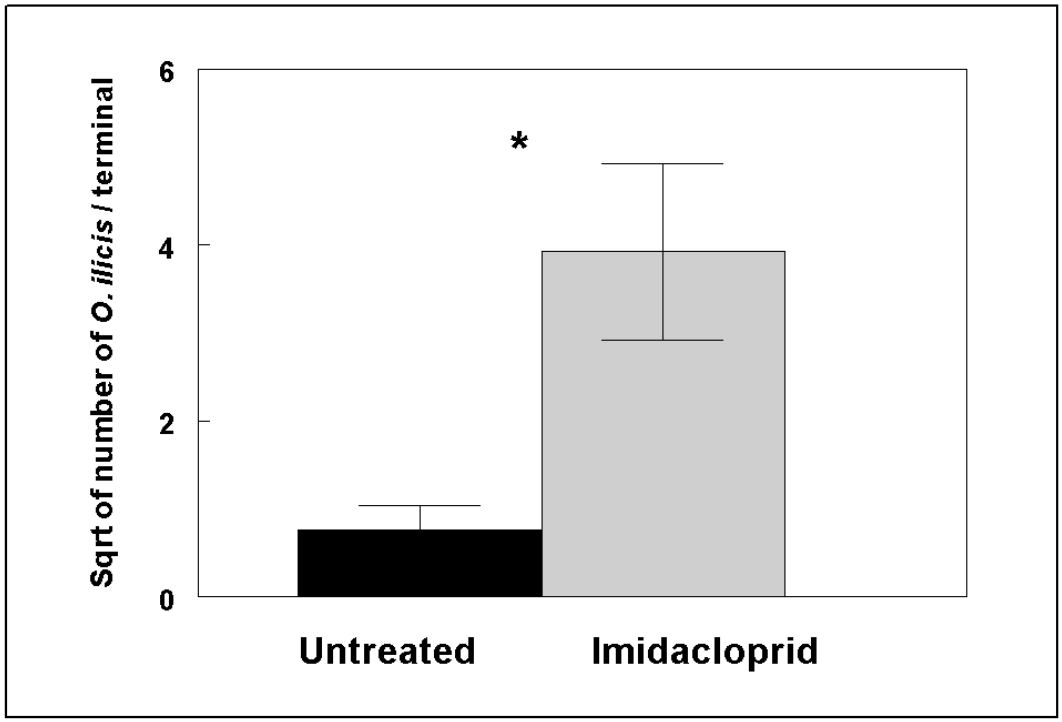

Figure 1.7. Abundance of southern red mite (*O. ilicis)* on imidacloprid treated and untreated cotoneasters in a greenhouse. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent standard errors of the mean. Asterisks mark means that were significantly different within each date at P = 0.05.

Chapter 2: Effects of imidacloprid on the community of arthropods associated with elm trees and boxwood shrubs in the urban forest.

Abstract

Reports of secondary outbreaks of spider mites after applications of imidacloprid to control an invasive pest, Asian longhorned beetle (ALB), prompted interest in the effects of the insecticide on communities of beneficial arthropods. Elimination of key predators of spider mites was suggested as the mechanism underlying the outbreaks. To evaluate the impact of imidacloprid on the assemblage of arthropods in general and natural enemies in particular, the arthropod community on elm trees and boxwood shrubs was compared between treated and untreated plants. In both study systems and across locations, the arthropod community responded positively to imidacloprid applications. However, high numbers of spider mites drove the response curves. Spider mites on elm trees and boxwood shrubs, *T. schoenei* and *E. buxi* were significantly more abundant on plants that received imidacloprid applications. Additionally, another phytophagous mite, *P. insolita* (Acari: Eriophyoidea: Diptilomiopidae) tended to be more numerous on imidacloprid-treated elms at one of the locations, but its response was not consistent across the sampling years. In general, treated elms housed lower numbers of an omnivorous tydeid mite (Acari: Tydeidae), while arthropods on boxwood responded variably. Neither of the plant systems exhibited any evidence of detrimental effects of imidacloprid on the community of beneficial arthropods. Abundance of a predator of spider mites, *Gelendromus* sp. (Acari: Phytoseiidae) did not differ significantly between treated and untreated elm trees and varied between the two study sites. Elimination of

key predators of spider mites does not appear to be the sole mechanism underlying secondary outbreaks of spider mites following imidacloprid applications.

Introduction

Attacks of invasive species of insects have always prompted serious measures on the part of government and local authorities to halt the spread of the pest (Invasive Species Act 1999). A recent invasion of trees in several states in the United States by an exotic cerambicid borer, the Asian long-horned beetle (ALB) (*Anoplophora glabripennis*, Coleoptera: Cerambycidae) resulted in quarantines, removal of infested trees and preventive insecticide treatments in Illinois, New Jersey, New York and Massachusetts (USDA 1998, 2003, 2007, 2008). One of the most significant urban parks in the US, New York City's Central Park found itself at the heart of the battle against this invasive cerambycid. Between 2002 and 2007, thousands of elms were treated with a systemic neonicotinoid insecticide, imidacloprid, as part of the Asian long-horned beetle eradication effort (USDA 2007). While these preventative treatments may have slowed down the invasive pest's progress, they had a surprising effect on another arthropod. Abundance of a spider mite, *T. schoenei*, erupted on trees that received treatments of the systemic insecticide, imidacloprid. Unusually high densities of this mite resulted in yellowing of the foliage and premature leaf drop.

Sudden outbreaks of pests following insecticide applications have been documented in urban settings previously (Raupp et al. 1992, 2009). In 1975 Luck and Dahlsten reported that mosquito-fogging programs resulted in increased abundance of pine needle scale, *Chionaspis pinifoliae* through elimination of parasitoids that otherwise successfully control this pest. A secondary outbreak of another scale insect, European

fruit leucanium, followed application of an insecticide aimed to control filth-flies (Merritt et al. 1983). Similar cases of increased abundance following insecticide applications were reported for citrus red mite, honeylocust spider mite, woolly whitefly, purple scale, citrus mealybug (Debach and Rose 1977, Sclar et al. 1998).

Despite imidacloprid's selectivity and systemic mode of action, it has been proposed that detrimental effects of imidacloprid on the community of natural enemies may cause the outbreaks of spider mites (Sclar et al. 1998). Investigations of imidacloprid and natural enemies found some evidence supporting the contention that imidacloprid inflicts mortality and impairs foraging activity of key beneficial insects. Imidacloprid has been found to be harmful to certain species of anthocorids, coccinellids, lacewings, seed bugs, some parasitic wasps, such as Braconidae and Aphelinidae, and pentatomids (Mizell and Sconyers 1992, Stark et al. 1995, Sclar et al. 1998, Stapel et al. 1999, Elzen 2001, James and Vogele 2001, Lucas et al. 2003, Rebek and Sadof 2003). James and Vogele (2001) also observed a reduction in the abundance of coccinellid and neuropteran larvae four to nine weeks after imidacloprid was applied. Early studies focused on foliar applications of imidacloprid, but there are now reports of experiments that examined the effects of imidacloprid when applied through the soil. Smith and Krischik (1999) found *Coleomegilla maculata* (Coleoptera: Coccinellidae) that consumed pollen of plants treated with soil application of imidacloprid experienced reduced longevity, reduced general mobility, and increased time to first oviposition. Decreased longevity and survival has also been shown in minute pirate bug (*Orius insidiosus,* Hemiptera: Anthocoridae) feeding on plants that received soil drench applications of imidacloprid (Sclar et al. 1998). The minute pirate bug is an omnivore, and it has been

observed to eat and develop on plant tissue when prey are not available (Coll 1996, Armer et al. 1998, Ferkovich and Shapiro 2004).

Notably, there is some evidence that imidacloprid may harm a key predator of tetranychids, predatory mites in the family Phytoseiidae. Mortality of *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) increased after application of imidacloprid in a laboratory study (Kimm et al. 2005). Additionally, James (1997) demonstrated a decrease in number of a predatory mite from the same genus following foliar application of imidacloprid in a field study conducted in an orchard. Notably, James (1997) also found that in laboratory bioassays females of the predatory mite sprayed with imidacloprid laid more eggs than unsprayed mites. When the predator was exposed to ten-fold increase in imidacloprid, however, 34% mortality was observed. This study was replicated in 2001, using two species of phytoseiid mite from a different genus, *Typhlodromus dossei* and *T. doreenae* and included both laboratory bioassays and field experiments using foliar applications of imidacloprid (James and Vogele 2001). In this experiment, fecundity of the predators was not monitored, and the authors reported no effect of the insecticide on either predator species when imidacloprid was applied at the label rate. At ten times the label rate, 19% mortality of *T. doreenae* was observed, but *T. dossei* was not affected. Inconsistent reports on imidacloprid's toxicity to predatory mites from different genera imply that the phytoseiid mites do not respond to imidacloprid as uniformly as spider mites, which have been found to increase in numbers across different genera within family Tetranychidea (Sclar et al. 1998, Gupta and Krischik 2007, Raupp et al. 2004, 2008).

Here, I investigate how imidacloprid applications alter the community of arthropods in general. In particular, I was interested in determining how key predators of spider mites responded to introduction of imidacloprid to plants they occupied. The experiments were conducted using two plant systems common in urban landscape, elm trees, *U. americana,* and boxwood shrubs, *B. sempervirens*. Experiments involving elms spanned three years at two urban locations, in Central Park, New York, NY, and on campus of the University of Maryland, College Park, MD. The boxwood study was carried out in College Park, MD, and lasted one growing season.

Methods

Structure of arthropod assemblage on elm trees treated with imidacloprid.

Central Park, New York, NY. A preliminary sample of elm trees in New York City's Central Park was conducted in 2004. Comprehensive studies of the community structure in elm canopies and abundance of *T. schoenei* on elms in Central Park were conducted in 2005, 2006, and 2007. The experimental design in Central Park was inherently challenging, because treatments were not randomly assigned to experimental subjects. Since 2001, USDA-APHIS, US-Forest Service, State and City cooperators in New York designate elm trees for treatment with imidacloprid in Central Park based on their proximity to known infestations of ALB in Manhattan (USDA 2005). Three insecticides with imidacloprid as the active ingredient were applied from 2004 to 2006. In 2004 the elms were treated with Imicide HP® (J.J. Mauget Co, Arcadia, CA, 10% imidacloprid) delivered by trunk injections (4 ml per 2.5 cm DBH), while in 2005 and 2006 trunk injections of Imicide HP®, soil injections of Merit® 75 WSP (Bayer, Kansas City, MO, water soluble powder, 75% imidacloprid, 2 g per 2.5 cm DBH) and Bandit® 75

WSP (Bayer, Montvale, NJ, 75% imidacloprid, 2 g per 2.5 cm DBH), and soil drenches of Bandit® 75 WSP (75% imidacloprid, 2 g per 2.5 cm DBH) were administered to elms in quarantine area. Bandit® 75 WSP and Imicide® were applied to all treated elms in 2007. In 2004, 924 elm trees south of 65th Street were treated with imidacloprid and trees north of 65th Street were untreated. In 2005, 2006, and 2007, the treatment zone shifted north to 86th Street. The number of trees that received imidacloprid application in these years was 4,806 in 2005, 4,866 in 2006 and 1,536 in 2007. In 2005, trees on an east-west transect across the park north and south of 86th Street were sampled. In 2006 trees on the western boundary of the park along 8th Avenue north and south of the treatment demarcation line at 86th Street were sampled, and in 2007 trees along the eastern boundary along 5th Avenue north and south of the demarcation boundary were used in the experiment. Each year ten elms were sampled from treated and untreated populations. All elms used in the study were mature trees ranging in height from about 15 to 30 m. Both treated and untreated elms bordered roadways or paths. We used only elms whose foliage could be sampled from the ground by hand or pole pruners.

Elms were sampled five times in 2005, three times in 2006 and four times in 2007. In all years, four branches per tree were removed from each cardinal position. The excised foliage was brought back to the laboratory, where arthropods were counted using a dissecting microscope. All arthropods on the two most terminal leaves were counted, and natural enemies and their eggs were noted on three additional leaves occupying position 3 – 5 on the branch's terminus. To compute density of various taxa, leaf area was measured using LI-31100C area meter (Li-Cor® Biosciences, USA) and arthropod

abundance was expressed as the number per cm^2 of leaf area. Arthropod abundance was the dependent variable used for analyses.

University of Maryland, College Park, MD. Twenty elms in a managed landscape lining a roadway on the campus of the University of Maryland, College Park, MD were used. Trees were mature and were approximately 4.5 m tall. Trees received no supplemental water other than rain and no fertilizer was applied. The experiment was a completely randomized design with ten elm trees in each treatment. Ten elms were designated as untreated controls, and ten elms received a single soil drench application of imidacloprid (Merit® soluble powder formulation, 750g of imidacloprid/kg, Bayer, Kansas City, MO) at the label rate 2 g per 2.5 cm DBH dissolved in 1L of water. Imidacloprid was applied on 06/09/2005, 06/05/2006, and 05/11/2007. Foliage was collected from the elms and arthropod abundance was recorded as described above for elm samples from Central Park. In 2005, I sampled trees once prior to applying imidacloprid and five times following the applications. Arthropods were sampled three times over the course of the growing seasons in 2006 and 2007. In all years, arthropod abundance was used for analyses.

Structure of arthropod assemblage on boxwood shrubs treated with imidacloprid.

I compared the structure of the arthropod community between boxwoods treated with imidacloprid and those untreated in a managed landscape on campus of the University of Maryland, College Park, MD, USA. I used twenty boxwoods that were approximately 0.6 m tall, and grew in rows separated by about 0.3 m between individual plants. Boxwoods were not trimmed during the study and received no supplemental

water other than rain. The experiment was a completely randomized design with two boxwoods between shrubs assigned to treatments serving as buffers. Ten plants were assigned to receive imidacloprid and ten boxwoods were designated as untreated controls. Imidacloprid was administered to the plants on 05/17/2005 as a single soil drench application (Merit® soluble powder formulation, 750 g of imidacloprid/kg, Bayer, Kansas City, MO) at the label rate of 2 g per 0.3 m of height dissolved in 1 L of water.

Arthropods were passively sampled by pheromone-free sticky traps (Insect Trap and Monitor, EPA Est. #48377-NY-1, Model #288-I) placed throughout the boxwoods and collected after they had remained on the plants for one week. Traps' total adhesive area was 170 cm^2, and each individual trap was cut into three pieces that were wrapped around boxwood branches, sticky side out, and distributed throughout the canopy of each experimental shrub. I surveyed plants once before the imidacloprid was applied and five times post-application, from June to September 2005. Arthropods were surveyed every 3-4 weeks. Insects and arachnids were identified in the laboratory using a dissecting microscope. Insects were identified to family level where possible. Abundance of arthropods was the dependent variable used for analyses.

Statistical Analyses

To test and visualize how the community of arthropods responded to imidacloprid treatment through time, I utilized a multivariate approach based on redundancy analysis, a principle response curve (PRC) (Van den Brink and Ter Braak 1999, Dively 2005, Prasifka et al. 2005). PRC is a constrained form of principle components analysis. It performs weighted least-squares regression of values of inert and latent variables, referred to as axes, extracted from the species abundance data on treatment and time.

The weights are based on abundance of each taxon relative to its accumulation in the control treatment, therefore, response of the sampled arthropod fauna is expressed as deviation from the community in control treatment.

PRC yields canonical coefficients. Coefficients depict deviation of a sampled community from control. Values of the control treatments are graphed as zero and serve as a reference to treatment values. Treatment values are plotted against time. In addition to the PRC coefficients, the test specifies species scores for all taxa. These values illustrate how each group fits the curve of the entire community response. Species scores ≥ 0.5 and ≤ -0.5 are considered significant (Dively 2005, Prasifka et al. 2005). Taxonomic groups with a significant positive score follow the pattern of PRC of the sampled community, while groups with a significant negative score exhibit pattern opposite to that portrayed by the PRC. Taxa with scores near 0 (≤ 0.5 and ≥ -0.5) either do not respond to treatment, or their response is different from the one depicted by the PRC.

PRC also provides a quantitative test. Monte-Carlo permutations are used to test for significance of the response curve. An *F*-type test statistics is calculated and the permutations produce 1,000 new data sets that are equally likely under a null hypothesis of canonical coefficients equaled zero. Significance is then computed based on the proportion of *F* values greater or equal to the *F* value of the original data set (Dively 2005).

PRC analyses and corresponding Monte-Carlo tests were performed over all sampling dates in each year for elm trees and boxwood shrubs to examine how the arthropod community responded to imidacloprid applications. CANOCO software (Ter

Braak and Šmilauer 2002) was used for the analyses. For the elm experiments, which span over a three-year period, a separate PRC was generated for each sampling year. A single taxon weight was generated for each arthropod by combining data from all three years at each location. The species scores related the abundance pattern of each taxon to the PRCs in each sampling year (Prasifka et al. 2005). Mean number of arthropods per cm^2 of leaf tissue was used in analyses, and data were log-transformed. Permutations were configured to occur between treatments, and sampling dates were designated as blocks.

Following PRC analyses, I analyzed the abundance of each taxon with a significant species score at each site and year using repeated measures model of ANOVA with imidacloprid treatment as a fixed effect (SAS 2008, Statistix 2005). When significant time by treatment interaction was detected, ANOVA were performed at each date to determine effects of imidacloprid on arthropod abundance (simple effects). Mean abundance of arthropods per cm^2 of leaf area was compared between untreated and imidacloprid treated plants. Square root transformations of the data were performed, if assumptions of homogeneous variances and normal distribution were not met. Non-parametric Kruskal-Wallis tests were used if assumptions of ANOVA could not be achieved with transformations (Zarr 1999).

Results

Structure of arthropod assemblage on elm trees treated with imidacloprid.

Central Park, New York, NY. In each year, the abundance of arthropods on treated trees in Central Park differed significantly from the abundance of arthropods on control trees (Table 2.1). Abundance was consistently higher on elms treated with

imidacloprid (Figure 2.1). The first axes explained 10%-20% of variation, and the second axis explained additional 1%-4% of variance. Time accounted for 9%-29% of variance. The only 2 taxa that had significant species scores were the spider mite, *T. schoenei*, and tydeid mites *H. anconai* and *Lorryia sp.* The score of *T. schoenei* was positive. This indicates that its abundance increased on treated trees relative to untreated ones. Abundance of tydeid mites was lower on treated elms compared to untreated trees. While not significant, phytoseiids, *Galendromus* sp., had a positive species score, indicating a trend for higher abundance on treated elms. None of the other predators known to feed on spider mites such as lacewings (Neuroptera: Chrysopidae) and spider mite destroyers (Coleoptera: Coccinellidae) contributed to the response curve.

Abundance of *T. schoenei* was analyzed as a repeated measures analysis of variance for each sampling year. There was a significant interactive effect of imidacloprid treatment and time on abundance of spider mites in all sampling years ($F_{4, 72.2} = 24.14$; $P = 0.0001$; $F_{2,17} = 11.23$; $P = 0.0008$; $F_{3,16} = 4.52$; $P = 0.0177$ in 2005, 2006 and 2007, respectively) (Figure 2.2). Comparisons of spider mite numbers within sampling dates each year indicate that mites were more abundant on treated elm trees on one of the sampling dates in 2005 and 2 sampling dates in 2006 and 2007 (Table 2.2).

Imidacloprid applications and time did not have an interactive effect on tydeid mites in 2005 and 2006 ($F_{4, 71.9} = 0.99$; $P = 0.4173$; and $F_{2,17} = 0.13$; $P = 0.878$, respectively) (Figure 2.3). The insecticide did not significantly affect the mites in the first year of the study ($F_{1,17.8} = 0.37$; $P = 0.5499$). However, during the second year, tydeid mites were more abundant on untreated elm trees ($F_{1,18} = 42.16$; $P = 0.0001$) and this trend continued in 2007 ($F_{1,18} = 32.14$; $P = 0.0001$). Abundance of tydeids was time-

dependent in all sampling years ($F_{4, 71.9} = 34.62$; P = 0.0001; $F_{2,17} = 8.21$; P = 0.0032; and $F_{3,16} = 8.05$; P = 0.0017 in 2005, 2006 and 2007, respectively).

University of Maryland, College Park, MD. In each year, the abundance of arthropods on trees treated with imidacloprid was significantly higher than the community on untreated trees (Figure 2.4; Table 2.1). The first and second axes accounted for 6%-18% and 1%-5% of variance related to treatment, respectively, while 22%-37% of variance was explained by the effect of time. The significant and positive species score of *T. schoenei*, indicated that the mites' abundance followed the pattern depicted on the PRC graph. The eriophyid mite, *P. insolita*, also had significant and positive species scores. Taxa with negative scores, the scale insect, *Eriococcus spuria* Modeer (Hemiptera: Eriococcidae), and tydeid mites, *H. anconai* and *Lorryia sp.*, exhibited the opposite pattern and declined in numbers on trees that received applications of imidacloprid. In the case of *E. spuria* this is not surprising, as imidacloprid has been documented to kill this scale (Sclar and Cranshaw 1996). Similarly to the Central Park site, none of the key predators of spider mites had significant taxa weights. Phytoseiid mites (*G. herbertae* and *G. halvelous* Chant, with over 80% of phytoseiids sampled belonging to *G. herbertae*) had a negative species score at this site, which is opposite of the trend observed in the New York site. Phytoseiids on Maryland elms were less numerous on trees that were treated with imidacloprid.

Elm trees used in this experiment remained assigned to the same treatment for the duration of the study. Thus, repeated measures ANOVA was used to analyze the data as a continuous experiment that spanned a three-year period. There was a significant

interactive effect of imidacloprid applications and time on spider mite abundance ($F_{11, 7.5}$ = 4; P = 0.0335) (Figure 2.5). Spider mites were significantly more numerous on elms treated with imidacloprid at the last two sampling dates within each year (Table 2.3). Their abundance increased as the growing season progressed.

However, there was no detectable effect of imidacloprid application on the abundance of eriophyid mites ($F_{1,18}$ = 1.48; P = 0.2398) (Figure 2.6). Time was a significant factor in determining eriophyid abundance ($F_{11, 7.15}$ = 76.60; P = 0.0001), and it did not interact with imidacloprid treatment factor ($F_{11, 7.15}$ = 2.11; P = 0.1629). While there was no clear trend for these mites to prefer treated elms in the first sampling year, in the two latter years, *P. insolita* tended to be more numerous on trees that received imidacloprid applications

Another taxon that contributed to the response curve were the tydeid mites. They were significantly more abundant on untreated elms ($F_{1, 75}$ = 11.52; P = 0.0011) (Figure 2.7). Time also had a significant effect on the mites ($F_{11, 184}$ = 6.58; P = 0.0001), but there was no interaction between the two factors ($F_{11, 184}$ = 1.15; P = 0.3224). Whereas in 2005 tydeid abundance had no clear pattern, by 2006 and 2007 the mites were more numerous on untreated elms.

Lastly, there was a significant effect of imidacloprid treatment and time interaction on abundance of scale insects ($F_{11, 183}$ = 5.82; P = 0.0001) (Figure 2.8). Scales were significantly more abundant on untreated elm trees within each sampling date in 2006 and 2007 (Table 2.4).

Structure of arthropod assemblage on boxwood shrubs treated with imidacloprid.

Abundance of arthropods on boxwoods treated with imidacloprid was significantly higher than on untreated shrubs ($F = 4.940$; $P = 0.002$) (Figure 2.9). The first and second axes explained 29% and 6% of variation, while time accounted for 33% of variance. A total of 45 taxa were identified, and arthropods with significant species scores, that is a score ≥ 0.5 and ≤ -0.5, were reported and their abundance patterns further analyzed. We trapped many scale insects as males and airborne immature stages, called crawlers, and these could not be identified to family. Thus, the level of superfamily, Coccoidea, was used for this taxon. Arthropods that occurred on treated shrubs in higher numbers and had a significant positive scores included boxwood spider mites, *E. buxi*, scale insects (Hemiptera: Coccoidea), mymarid wasps (Hymenoptera: Mymaridae), and spiders (Aranea). Taxa with negative scores that were more abundant on untreated boxwoods were encyrtid wasps (Hymenoptera: Encyrtidae), ants (Hymenoptera: Formicidae), collembola (Collembola), and the boxwood psyllid, *Psylla buxi* Linn. (Hemiptera: Psyllidae). None of the arthropods that contributed to the response curve were spider mite predators.

Abundance of the dominant species, spider mites, was affected by a significant interaction between time and imidacloprid treatment ($F_{5, 90} = 2.46$; $P = 0.0390$). Numbers of *E. buxi* did not differ between treatments before application of the insecticide, while imidacloprid-treated boxwoods housed significantly greater abundance of mites in June and July (Figure 2.10, Table 2.5). Numbers of mites in both treatments decreased with time.

Despite having significant species scores and abundance patterns that contributed to the response curve, repeated measures analyses of the remaining taxa did not yield statistically significant differences (Table 2.6). Overall, scale insects tended to be more abundant on imidacloprid treated shrubs (Figure 2.11), and their numbers were significantly greater on one of the sampling dates. Mymarid wasps (Figure 2.12) also showed a tendency for greater abundance on treated shrubs. Spider abundance did not display a clear pattern for either treatment (Figure 2.13), but there were significantly greater numbers of spiders on boxwoods that received imidacloprid on the last sampling date. Another parasitoid, an encyrtid wasp, was more abundant on untreated plants on one of the dates (Figure 2.14), but was otherwise equally distributed among treated and untreated plants. Ants tended to be more abundant on untreated plants (Figure 2.15), whereas treated boxwoods housed significantly fewer boxwood psyllids, a hemipteran known to be susceptible to imidacloprid, on one of the sampling dates (Figure 2.16).

Discussion

Structure of arthropod assemblage on elm trees treated with imidacloprid.

In both New York and Maryland, the arthropod community on imidacloprid treated elms was significantly different from the community on untreated elms (Figures 2.1, 2.4). The primary taxon driving this response at both locations was *T. schoenei* and their populations reached levels significantly higher on treated elms every year. Maryland elms housed an assemblage of arthropods similar to the one in New York. However, in Maryland I found a greater number of arthropods with a significant response to imidacloprid treatment (Figure 2.4).

While there were several predatory and herbivorous arthropods collected, only a few taxa significantly contributed to the general response curve in both locations. Tetranychids were more numerous on treated elm trees in New York as the growing season progressed, and this trend was consistent in all sampling years (Figure 2.2). The magnitude of difference between treatments varied, and was largest in 2007. Similarly, in Maryland spider mites were consistently more abundant on treated elms, regardless of sampling year. Their numbers increased with time and the mites were most abundant in 2007 (Figure 2.5).

Notably, there was no indication of decreased abundance of any key predators of spider mites that could explain the explosion of mite numbers. Chrysopid larvae, coccinellids and phytoseiids were collected from imidacloprid treated and untreated elms at both locations, but their abundance patterns did not contribute to the general response curve, that is, they did not differ between treated and untreated elms (Figures 2.1, 2.4). A few other researchers found that applications of imidacloprid had little or no effect on predators of spider mites such as coccinellids, lacewing larvae, and predaceous mites (Sapute et al 2002, Kannan et al. 2004). However, in most studies imidacloprid was found to be toxic to a myriad of natural enemies that came in direct or indirect contact with the insecticide through residues on the surface or in plant tissues. Among these beneficial insects were coccinellid beetles, predatory bugs, and predatory mites (Mizell and Sconyers 1992, Sclar et al. 1998, Smith and Kirschik 1999, James and Coyle 2001, Studebaker and Kring 2003, James 2003a, James 2003b, Rebeck and Sadof 2003). Most of these studies were performed under laboratory conditions, and discrepancies between results of our field study and research done by others may stem from fundamental

differences in the nature of field and laboratory experiments. Natural enemies in the field have an option to leave a plant or habitat and move to a more favorable location. Moreover, alternative food sources uncontaminated by insecticide residues may provide beneficial arthropods with resources to sustain populations even on plants treated with imidacloprid. Arthropods in microcosms in the laboratory are usually confined to the experimental arena without alternative source of nutrition.

It is important that phytoseiid mites, which are key predators of spider mites, did not respond consistently to imidacloprid treatments and increased prey abundance. While phytoseiid abundance did not differ significantly between treatments, the predatory mites in New York tended to be more numerous on treated elms, while their abundance exhibited an opposite trend in the experiment conducted in Maryland. Elm trees at the Maryland site that received insecticidal treatments housed fewer phytoseiid mites than untreated trees suggesting that plants or prey present on the elms were toxic to the predator. Negative effects of imidacloprid to phytoseiid mites were reported previously (James and Coyle 2001, James 2003 (1), Stavrinides and Mills 2009). However, exposure to imidacloprid is not consistently detrimental to phytoseiids. James (1997) found females of *A. victoriensis* sprayed with imidacloprid to lay more eggs than their unsprayed counterparts. Additionally, a ten-fold increase in imidacloprid dose resulted in over 30% mortality of the mites, suggesting that the reproductive stimulation was caused by sublethal levels of the neonicotinoid.

It is difficult to link secondary outbreaks of *T. schoenei* to disruption of phytoseiids. Patterns of abundance of the predatory mites were variable and exhibited no clear trend in response to imidacloprid or numbers of *T. schoenei.* Other studies of mites

in urban habitats have suggested that predatory mites play and important role in suppressing populations of spider mites on trees along streets and in parks. Balder et al. (1999) found inverse density dependence for the spider mite *Eotetranychus tiliarum* and its phytoseiid predators on street trees in Berlin. On trees where predators were abundant, spider mites were relatively rare. Schneider et al. (2000) reported similar findings in a separate study involving *Eotetranychus tiliarum* along broad and narrow streets in Berlin. Along narrow streets where lindens were interplanted with other trees and shrubs, predatory mites were common and spider mites were rare, whereas along broad avenues where no trees other than hosts were present, predatory mites were rare and spider mites were extraordinarily abundant. In the Italian cities of Como and Milan, greater diversity and abundance of predatory mites in parks and woods reduced the abundance of spider mites on trees, while along avenues predators were rare and several species of spider mites reached outbreak status (Rigamonti and Lozzia 1999). It is noteworthy that phytoseiids do not always suppress populations of spider mites in urban settings. Ehler and Frankie (1979) found well developed communities of predatory mites in urban settings and suggested that mite outbreaks on oaks in cities were related to plant stress rather release from natural enemies. Kropczynska et al. (1986) reported a similar finding for populations of outbreaking spider mites on lindens in Warsaw, Poland. Here enhanced nutritional quality of lindens along city streets elevated fecundity of *E. tiliarum* and subsequent outbreaks of mites.

Abundance of tydeid mites on treated elms in New York (Figure 2.3) mirrored their abundance pattern in Maryland (Figure 2.7). Treated trees in 2006 and 2007 at both locations tended to house fewer tydeid mites. *H. anconai,* which was the most abundant

tydeid collected, is known to feed on eriophyid mites (Hessein and Perring 1986, Hessein and Perring 1988, Aguilar et al. 2001, Kawai 2002, Mainul and Kawai 2003), while *Lorryia sp* are plant and fungi feeders (Jeppson et al. 1975, Mendel and Gerson 1982, Badii et al. 2001). There is some evidence in the literature that imidacloprid-treated plants are less suitable for tydeids. Castagnoli et al. (2005) found that fecundity of *Tydeus californicus* (Acari: Tydeidae) decreased significantly when mites were exposed to a topical application of imidacloprid. Notably, tydeids were found to respond positively to imidacloprid in another study (Chapter 1, Figure 1.4), suggesting that additional factors may affect the response of this mite to imidacloprid applications.

Another taxon with a significant species scores at the study site in Maryland were eriophyid mites, *P. insolita*. Imidacloprid-treated elms housed significantly more eriophyids on two sampling dates (Figure 2.6). However, numbers of *P. insolita* did not show a consistent pattern of higher abundance on treated elms in Maryland. Harvey et al. (1998) reported that wheat curl mites, *Aceria tosichella* Keifer (Acari: Eriophyidae) exhibited increased incidence of infestation of wheat treated with imidacloprid, but there was no difference in the abundance of mites between imidacloprid-treated and untreated plants. Additionally, in an experiment conducted in a common garden described earlier (Chapter 1, Figure 1.3), *P. insolita* exhibited the same tendency for greater abundance on elms that received application of imidacloprid. Thus, I conclude that imidacloprid may exert the same effect on the eriophyid mites as it does on the tetranychids. However, inconsistent responses of *P. insolita* to imidacloprid applications warrant additional studies.

Lastly, imidacloprid had a significant effect on populations of the eriococcid scales, *E. spuria* in Maryland (Figure 2.8). This scale is known to be susceptible to applications of imidacloprid (Sclar and Cranshaw 1996), and these results confirm this finding. After a relatively low abundance on treated and untreated elms in 2005, scale numbers were significantly greater on untreated trees in 2006 and 2007, while imidacloprid treated plants were virtually scale-free. The low numbers on untreated trees in 2005, could be explained by the age of the trees, which were not colonized by the scales at the onset of the experiment.

Structure of arthropod assemblage on boxwood shrubs treated with imidacloprid.

As in studies of arthropod communities on elms, applications of imidacloprid had a significant effect on arthropod fauna on boxwood plants. Elevated numbers of *E. buxi* drove the shape of the response curve on imidacloprid treated shrubs. However, higher numbers of spider mites on treated plants did not result in a proportionate response of key predators of this mite. While predatory mites, ladybugs, dustywings (Coniopterygidae) and lacewing larvae were collected on treated and untreated boxwoods, their abundance did not significantly contribute to the shape of the response. Abundance of spider mites on boxwoods before treatment with imidacloprid was not significantly different among plants, and was highest at the first sampling date post treatment (Figure 2.10). On the three subsequent sampling dates, spider mites were significantly more abundant on treated shrubs and this pattern persisted from June until August. Notably, numbers of *E. buxi* were relatively high on all boxwoods before imidacloprid was applied, and their abundance declined on untreated boxwoods post-treatment, while it remained high on treated shrubs. This indicates that imidacloprid affected quality of the boxwoods as

hotter, more humid conditions of the season developed. Gonias et al. (2008) found that cotton plants treated with imidacloprid and exposed to temperatures ranging from 30-39 °C had higher levels of photosynthesis and chlorophyll fluorescence compared to untreated cotton. Additionally, the effect of imidacloprid on these parameters was greater in plants under higher temperatures. The authors linked the response of cotton to imidacloprid treatment to lower activity of glutathione reductase (GR), which is a detoxifying enzyme involved in plants' response to stress (Cakman and Marschner 1992, Foyer et al. 1995). Untreated cotton maintained at higher temperatures had higher levels of GR, suggesting that plants treated with imidacloprid experience less stress (Gonias et al. 2008). The pattern of abundance of *E. buxi* seems to suggest that this may be the case for boxwoods. Additionally, the second sample was taken only three weeks post-treatment, exemplifying the rapid response of spider mites to application of imidacloprid. Three weeks after imidacloprid was applied, boxwoods that received the insecticide application housed approximately 80% more spider mites than the untreated shrubs (Figure 2.10). Moreover, it appears that no predator in the boxwood – spider mite system was able to respond numerically to high populations of spider mites. This lack of top-down regulation has also been noted for other systems involving spider mites in urban habitats such as the one in this study (Ehler and Frankie 1979, Kropczynska et al. 1986, Balder et al. 1999, Schneider et al 2000)

Another taxon that was significantly affected by imidacloprid were scale insects. Due to the sampling method used, it was not possible to distinguish between the effects of treatment on different taxa of scales such as soft scales, which are known to be largely susceptible to imidacloprid (Sclar and Cranshaw 1996, Gill et al. 1999, Elbert et al.

2008), and armored scales, which are generally not affected by imidacloprid (Sadof and Sclar 2000). Negative effects of imidacloprid on encyrtids (Krischik et al. 2007) and other important parasitoid of scales, aphelinid wasp, (Rebek and Sadof 2003) were shown previously, suggesting that imidacloprid disrupts the dynamic between scales and their parasitoids. However, it is more likely that mature oak trees and other vegetation growing near the study site were the source of scales collected on the sticky traps.

Additionally, the effect of imidacloprid on psyllids, *P. buxi* may have had an important impact on ants (Figures 2.15 and 2.16). Lower numbers of psyllids, which are known to produce honeydew utilized by ants (Essig 1958, Basset 1991, Novak 1994), could explain decreased abundance of ants on treated boxwoods. Importantly, this does not indicate direct toxicity of imidacloprid-treated plants to formicids, and merely suggests that due to elimination of a food source, ants did not associate with treated boxwoods as much as with untreated ones. While there are known cases of negative effect of imidacloprid on ants (Rust et al. 2004), ants do not respond consistently to imidacloprid exposure (Kunkel et al. 1999, Zenger and Gibb 2001). Further investigation of indirect effect of imidacloprid on ants through elimination of a food source would be needed to conclusively assess ants' response to imidacloprid in this system. .

Surprisingly, psyllids were more abundant on boxwoods that received imidacloprid immediately following the application. Physiological effect of sublethal levels of imidacloprid on nervous system of insects, which manifests itself in increased excitability and movement (Thorne and Breisch 2001, Joost and Riley 2005) could provide a possible explanation for greater abundance of psyllids on treated boxwood shortly after applications were administered. Lambin et al. (2001) found that honeybees

exposed to low levels of imidacloprid exhibited increased motor activity, while their movement was impaired at higher concentrations of the insecticide. Thus, random movements of imidacloprid-intoxicated psyllids may have increased their occurrence on sticky traps in the first weeks after treatment when imidacloprid level was high enough to affect behavior, but too low too inflict mortality. Psyllids were significantly less numerous on treated boxwoods on the second post-treatment sampling, providing further evidence that imidacloprid successfully controls this key pest of boxwoods (Young 2002).

Conclusions

The use of insecticides to eliminate pests has frequently been shown to have far-reaching consequences to assemblages of arthropods (Roberts et al. 1973, Luck and Dahlsten 1975, DeBach and Rose 1977, McClure 1977, Merritt et al. 1983, Dreistadt and Dahlsten 1986, Morse et al. 1987, Amalin et al. 2001, Letourneau and Goldstein 2001, Raupp et al. 2001, Marquini et al. 2002, Devotto et al. 2006, Frampton et al. 2007, Liang et al. 2007). One of the most important of these consequences is an adverse effect on natural enemies, which often contributes to primary and secondary outbreaks of phytophagous arthropods (Roberts et al. 1973, Luck and Dahlsten 1975, DeBach and Rose 1977, McClure 1977, Merritt et al. 1983, Dreistadt and Dahlsten 1986). It has been argued that a systemic mode of action may mitigate negative effects of pesticides on beneficial arthropods by reducing exposure (Mullins 1993, Sclar and Cranshaw 1996, Gill et al. 1999). However, due to omnivory of some key predators, systemic insecticides present in plant tissues might place them at risk (Coll and Guershon 2002). A few of these omnivores, such as lacewing larvae (Neuroptera: Chrysopidae) (Limburg and

Rosenheim 2001, Patt et al. 2003), and minute pirate bug (Hemiptera: Anthocoridae) (Coll 1996, Armer et al. 1998, Coll and Guershon 2002, Ferkovich and Shapiro 2004) are also important predators of spider mites (Reddy 2001, Rosenheim et al. 2004).

I predicted that if applications of imidacloprid reduced the abundance of key predators of spider mites, then this could explain in part outbreaks of spider mites. The results of experiments described here do not support a change in the community of predators as the mechanism behind secondary outbreaks of spider mites following applications of imidacloprid.

In both elms and boxwoods, increased numbers of spider mites on plants treated with imidacloprid were driving the response curve of arthropod community. The survey confirmed the occurrence of outbreaks of tetranychids following imidacloprid applications. The overwhelming abundance of *T. schoenei* on treated plants did not seem to arise from elimination of a single key natural enemy or obvious disruption of the community of natural enemies. While it is well documented through laboratory studies that imidacloprid is toxic to and impairs foraging ability of many key predators, I did not find an absence or lower abundance of natural enemies to provide a satisfactory explanation for dramatically elevated abundance of spider mites on trees and shrubs treated with imidacloprid. Lack of effect of imidacloprid on key predators of *T. schoenei* and *E. buxi* as well as other natural enemies provides additional supporting evidence that mechanisms other than disruption of beneficial insects and arachnids is responsible for secondary outbreaks of spider mites. Results of the boxwood study suggest that imidacloprid affected quality of the plants as well. Moreover, the experiment exemplified how introduction of imidacloprid may result in cascading effects spanning

all trophic levels. Elimination of susceptible pests and increased abundance of other herbivores may lead to restructuring of the arthropod fauna on host plants treated with the neonicotinoid. Given what is known about the unusually long residual activity of imidacloprid in plants (Webb et al. 2003, Frank et al. 2007, Szczepaniec and Raupp 2007), imidacloprid's impacts on food webs may have far-reaching and long-lasting impacts.

Tables

Table 2.1. Results of quantitative comparisons between communities of arthropods on elms treated with imidacloprid and untreated elms.

Site	Year	*F*-value	*P*-value
Univ. of MD, College Park	2005	11.951	0.006
	2006	16.580	0.002
	2007	16.503	0.002
Central Park, New York	2005	11.274	0.01
	2006	16.103	0.002
	2007	18.888	0.004

Monte-Carlo permutations were used to generate 1,000 new data sets and resolve statistical differences between treatments. Sampling dates within each year were designated as blocks.

Table 2.2. Comparisons of abundance of *T. schoenei* on elms treated with imidacloprid and untreated elms in Central Park, New York.

Date	Test*	df	P-value
6/09/05	$F = 0.950$	1,18	0.3471
6/29/05	$X^2 = 0.052$	1	0.8193
8/03/05	$X^2 = 3.456$	1	0.0630
8/26/05	$F = 28.75$	1,18	<0.0001
9/16/05	$X^2 = 0.006$	1	0.9397
6/21/06	$F = 2.380$	1,18	0.1404
7/25/06	$X^2 = 4.080$	1	0.0009
9/08/06	$X^2 = 6.8961$	1	0.0086
6/15/07	$X^2 = 0.1570$	1	0.6919
7/03/07	$X^2 = 0.027$	1	0.8686
8/08/07	$X^2 = 11.071$	1	0.0009
9/26/07	$F = 2.60$	1,18	0.1246

ANOVA was used to compare means between treatments (F-value). If assumptions of normal distribution and homogeneous variances were violated and could not be corrected with transformations, a non-parametric test, Kruskal-Wallis, was used (X^2).

Table 2.3. Comparisons of abundance of *T. schoenei* on elms treated with imidacloprid and untreated elms in College Park, Maryland.

Date	Test*	df	P-value
6/02/05	$X^2 = 7.286$	1	0.0070
6/15/05	F = 1.060	1,18	0.3168
6/27/05	F = 0.240	1,18	0.6290
7/12/05	F = 2.370	1,18	0.1418
8/15/05	F = 18.730	1,18	0.0005
9/01/05	F = 14.100	1,18	0.0016
6/14/06	$X^2 = 0.082$	1	0.7749
7/31/06	$X^2 = 7.058$	1	0.0079
9/19/06	F = 5.430	1,18	0.0316
6/05/07	$X^2 = 0.197$	1	0.6569
6/27/07	$X^2 = 6.242$	1	0.0125
7/23/07	F = 13.760	1,18	0.0016

ANOVA was used to compare means between treatments (F-value). If assumptions of normal distribution and homogeneous variances were violated and could not be corrected with transformations, a non-parametric test, Kruskal-Wallis, was used (X^2).

Table 2.4. Comparisons of abundance of *E. spuria* on elms treated with imidacloprid and untreated elms in College Park, Maryland.

Date	Test*	df	P-value
6/02/05	$F = 0$	1,18	1.0000
6/15/05	$X^2 = 0.167$	1	0.6820
6/27/05	$F = 5.580$	1,18	0.0303
7/12/05	$F = 0.480$	1,18	0.4957
8/15/05	$X^2 = 3.717$	1	0.0538
9/01/05	$X^2 = 2.346$	1	0.1256
6/14/06	$X^2 = 10.185$	1	0.0014
7/31/06	$F = 74.55$	1,18	<0.0001
9/19/06	$X^2 = 15.249$	1	< 0.0001
6/05/07	$X^2 = 9.380$	1	0.0022
6/27/07	$X^2 = 9.170$	1	0.0025
7/23/07	$X^2 = 7.207$	1	0.0073

ANOVA was used to compare means between treatments (F-value). If assumptions of normal distribution and homogeneous variances were violated and could not be corrected with transformations, a non-parametric test, Kruskal-Wallis, was used (X^2).

Table 2.5. Comparisons of abundance of *E. buxi* on boxwoods treated with imidacloprid and untreated elms in College Park, Maryland.

Date	Test*	df	P-value
5/05/05	$F = 0.090$	1,18	0.7667
6/06/05	$X^2 = 6.858$	1	0.0088
7/06/05	$X^2 = 4.676$	1	0.0308
7/25/05	$X^2 = 8.371$	1	0.0038
8/19/05	$X^2 = 0.012$	1	0.9136
9/15/05	$X^2 = 0.002$	1	0.9696

ANOVA was used to compare means between treatments (F-value). If assumptions of normal distribution and homogeneous variances were violated and could not be corrected with transformations, a non-parametric test, Kruskal-Wallis, was used (X^2).

Table 2.6. Results of repeated measures analyses of most abundant arthropods on boxwoods treated with imidacloprid and untreated boxwoods.

Taxon	Main Effect	Time*Treatment
Tetranychidae	$F_{1, 18} = 16.97$; P = 0.0006	$F_{5, 90} = 2.46$; P = 0.0390
Coccoidea	$F_{1, 18} = 1.76$; P = 0.2016	$F_{5, 90} = 1.76$; P = 0.1298
Mymaridae	$F_{1, 18} = 1.00$; P = 0.3306	$F_{5, 90} = 1.00$; P = 0.4225
Aranea	$F_{1, 18} = 1.20$; P = 0.2878	$F_{5, 90} = 0.39$; P = 0.8565
Encyrtidae	$F_{1, 18} = 0.87$; P = 0.3648	$F_{5, 90} = 0.24$; P = 0.9484
Formicidae	$F_{1, 18} = 0.49$; P = 0.4913	$F_{5, 90} = 0.51$; P = 0.7680
Collembola	$F_{1, 18} = 0.28$; P = 0.6024	$F_{5, 90} = 1.10$; P = 0.3687
Psyllidae	$F_{1, 18} = 0.37$; P = 0.5528	$F_{5, 90} = 0.58$; P = 0.7156

Figures

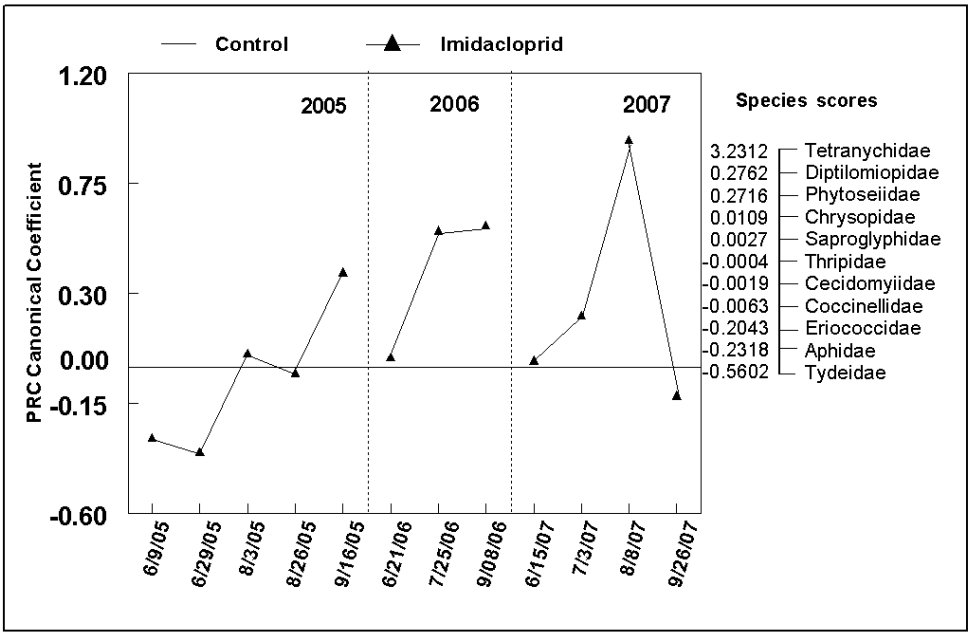

Figure 2.1. PRCs and species scores of arthropod community on elm trees treated with imidacloprid relative to untreated elms in Central Park, New York, NY. Control was graphed at 0 to distinguish it from sampled community. *P* values indicate significance level of comparisons between treatments within each year trees were sampled. Arthropods with positive species score ≥ 0.5 followed the response pattern shown in the PRCs, while groups with negative score ≤ -0.5 showed the opposite response pattern.

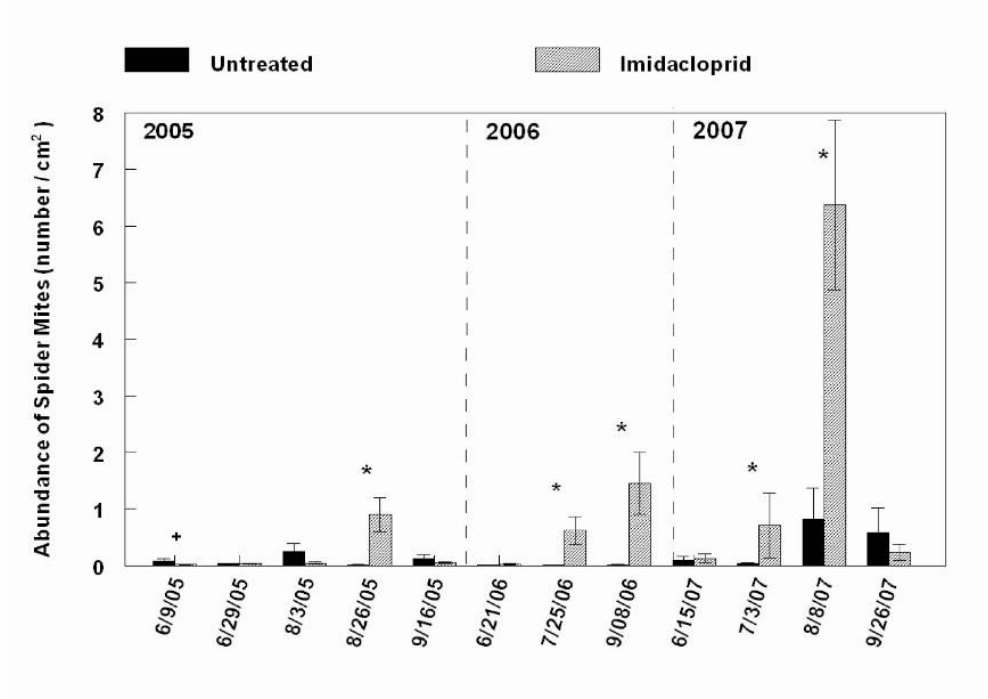

Figure 2.2. Comparisons of spider mite abundance between imidacloprid treated and untreated elms in Central Park, New York, NY from 2005 to 2007. Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Vertical lines represent standard errors. Asterisks mark means that were significantly different within each date at P = 0.05.

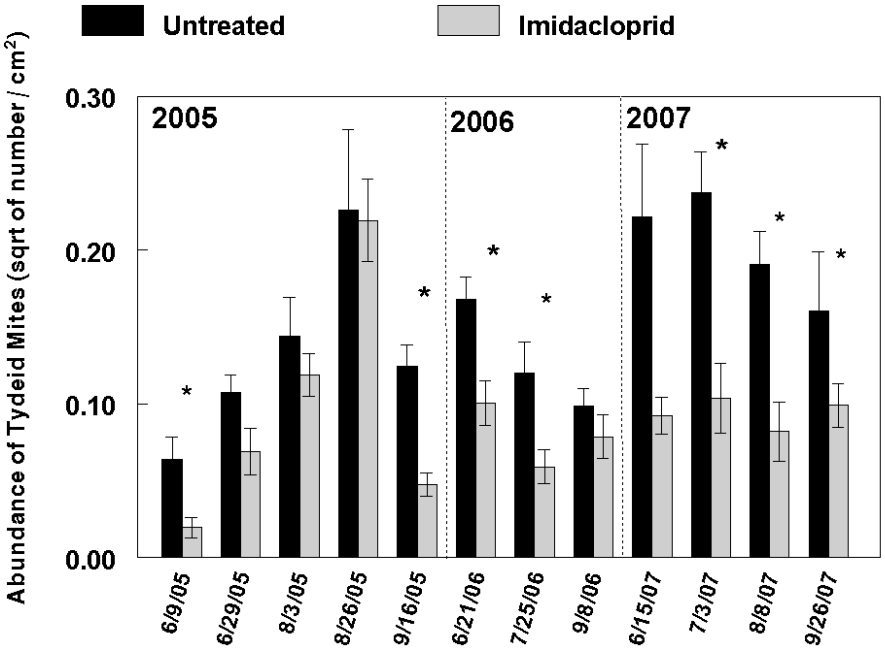

Figure 2.3. Repeated measures analysis of variance (ANOVA) and one-way ANOVA comparing abundance of tydeid mites between imidacloprid treated and untreated elms in Central Park, New York, NY from 2005 to 2007. Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Vertical lines represent standard errors. Asterisks indicate significant within-date difference in tydeid numbers P = 0.05.

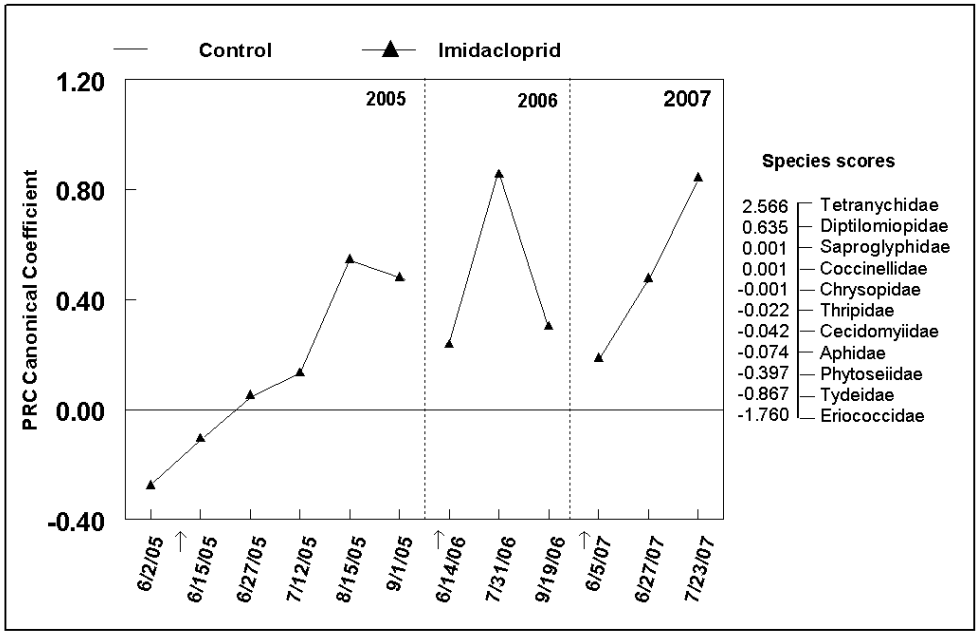

Figure 2.4. PRCs and species scores of arthropod community on elm trees treated with imidacloprid relative to untreated elms on campus of the University of Maryland, College Park, MD. Control was graphed at 0 to help distinguish it from sampled community. Arrows indicate dates on which imidacloprid was applied *P* values indicate significance level of comparisons between treatments within each year trees were sampled. Arthropods with positive species score ≥ 0.5 followed response pattern shown in the PRCs, while groups with negative score ≤ -0.5 showed the opposite response pattern.

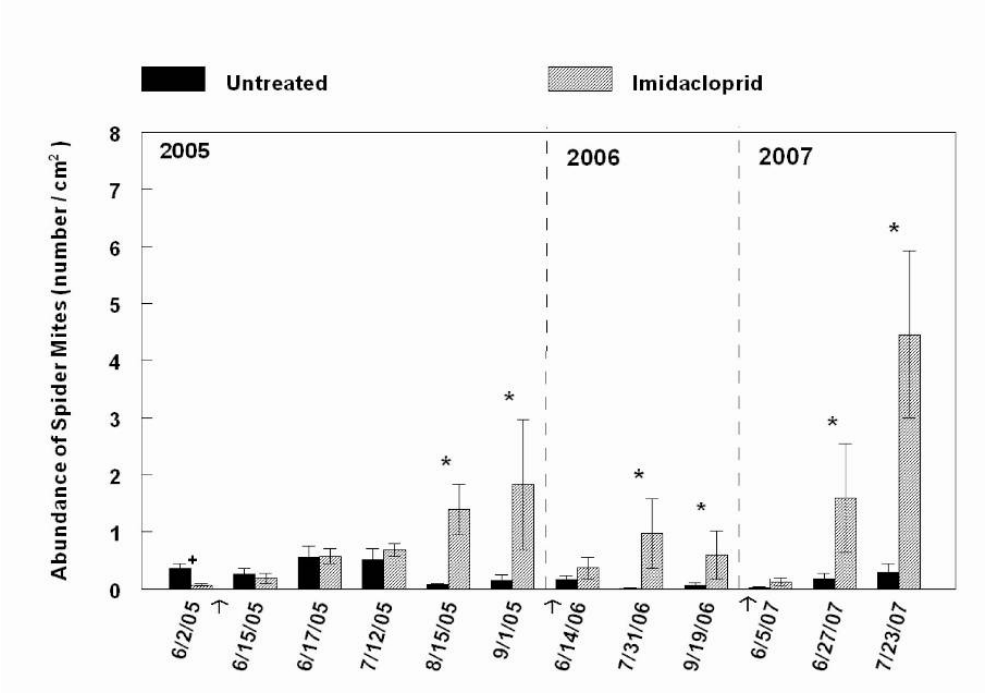

Figure 2.5. Comparisons of spider mite abundance between imidacloprid treated and untreated elms in a common garden at the University of Maryland, College Park, MD from 2005 to 2007. Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Vertical lines represent standard errors. Asterisks mark means that were significantly different on each
date at P = 0.05.

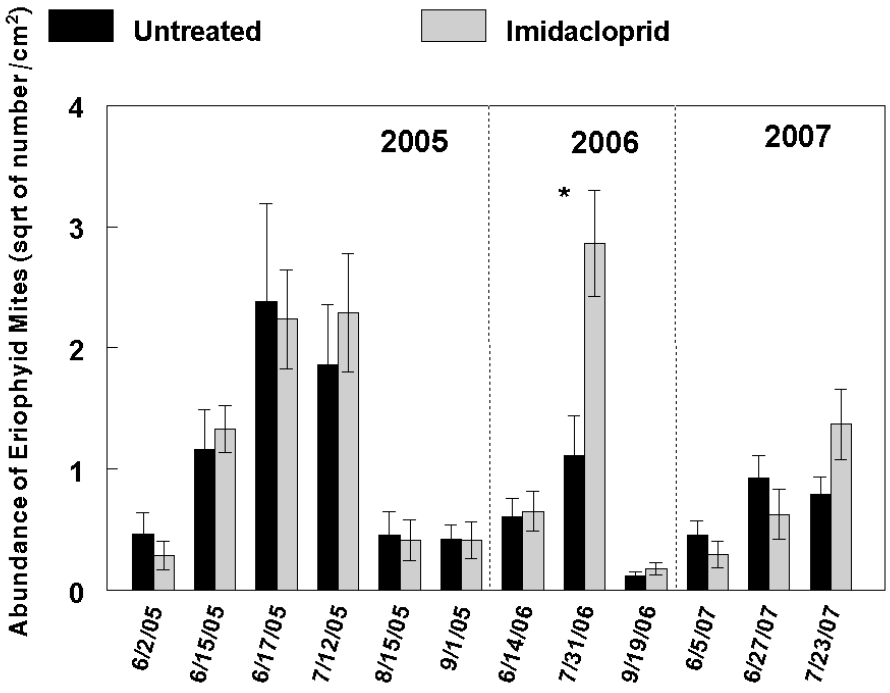

Figure 2.6. Repeated measures analysis of variance comparing abundance of eriophyid mites between imidacloprid treated and untreated elms in a common garden at the University of Maryland, College Park, MD from 2005 to 2007.Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Vertical lines represent standard errors. Asterisks mark means that were significantly different on each date at P = 0.05.

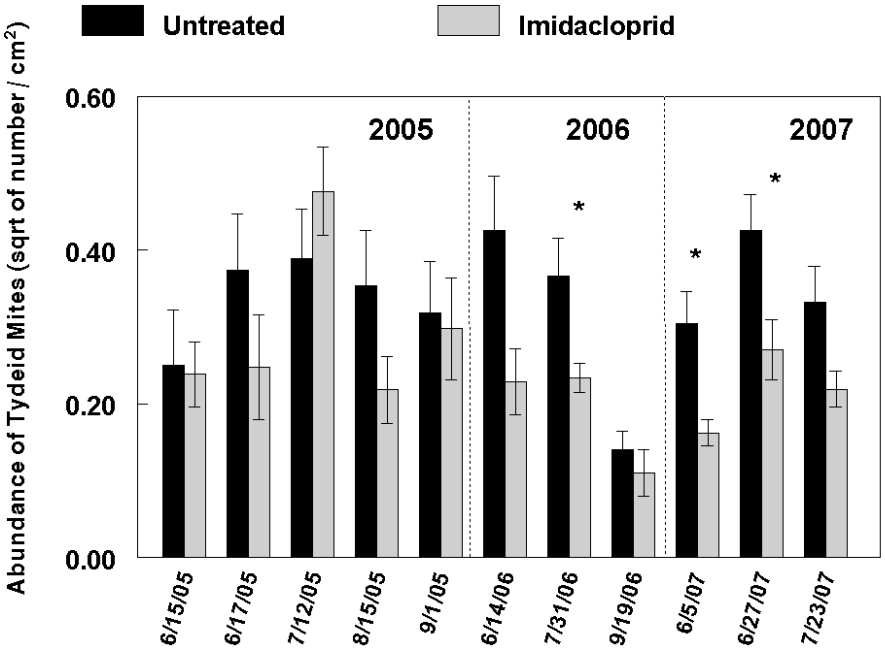

Figure 2.7. Repeated measures analysis of variance comparing abundance of tydeid mites between imidacloprid treated and untreated elms in a common garden at the University of Maryland, College Park, MD from 2005 to 2007.Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Vertical lines represent standard errors. Asterisks mark means that were significantly different on each date at P = 0.05.

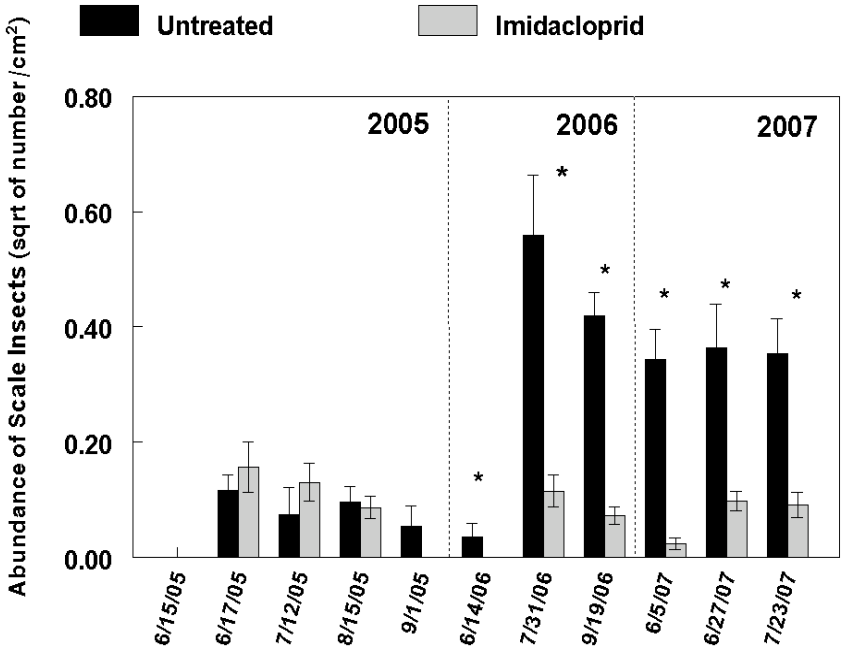

Figure 2.8. Comparison of the abundance of eriococcid scale insects between imidacloprid treated and untreated elms in a common garden at the University of Maryland, College Park, MD from 2005 to 2007. Black bars represent untreated controls while grey bars represent trees exposed to the insecticide. Asterisks mark means that were significantly different on each date at P = 0.05. Vertical lines represent standard errors. Asterisks mark means that were significantly different on each date at P = 0.05.

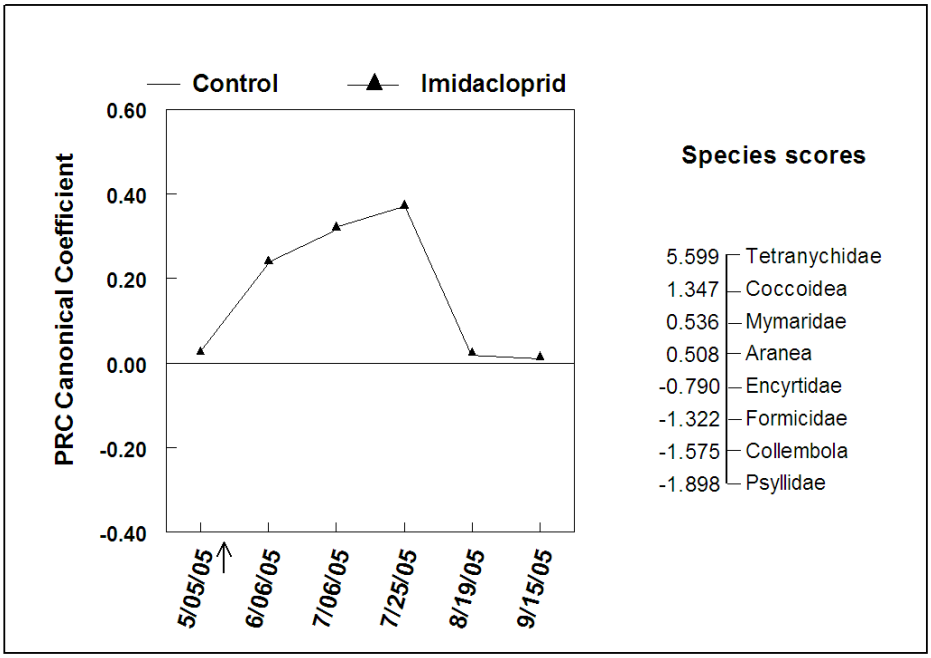

Figure 2.9. PRCs and species scores of arthropod community on boxwoods treated with imidacloprid relative to untreated boxwoods on campus of the University of Maryland, College Park, MD. Control was graphed at 0 to help distinguish it from sampled community. The arrow indicates the date on which imidacloprid was applied. Arthropods with positive species score ≥ 0.5 followed the response pattern shown in the PRCs, while groups with negative score ≤ -0.5 showed the opposite response pattern.

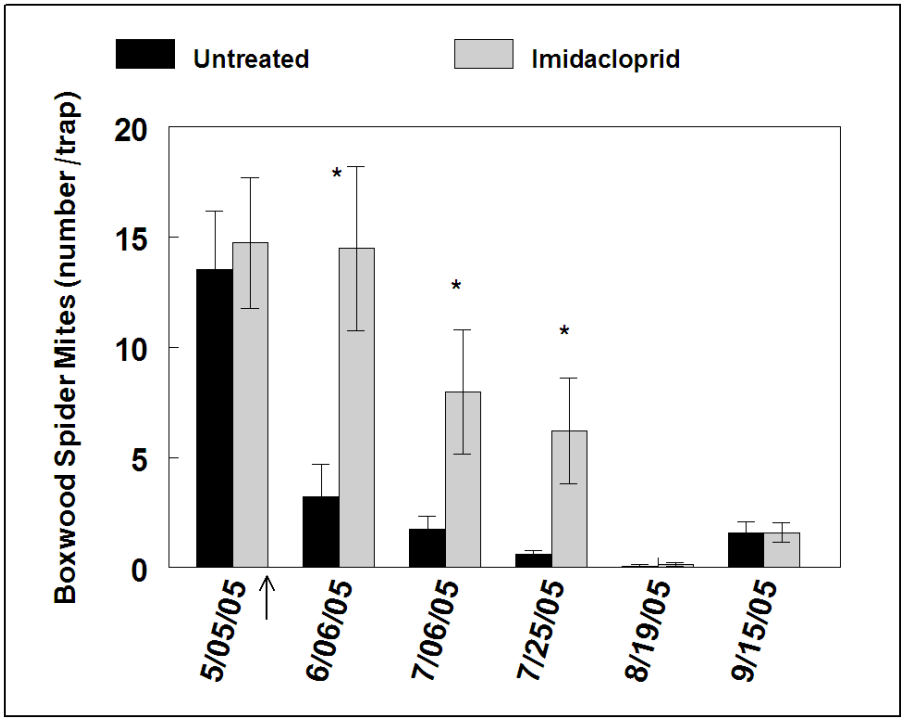

Figure 2.10. Abundance on *E. buxi* imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

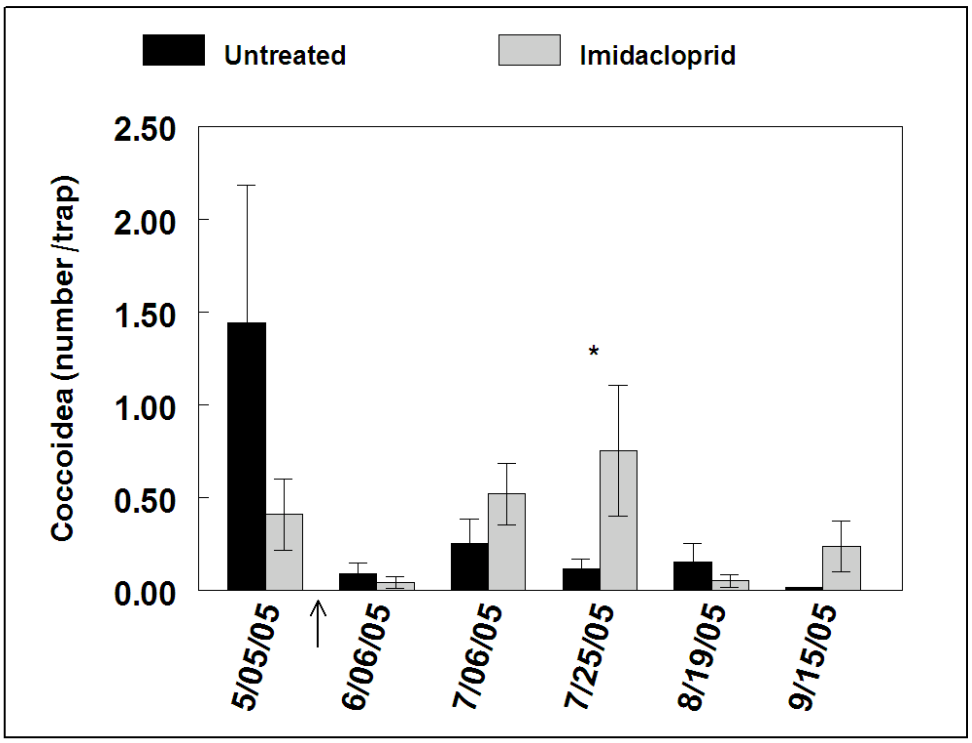

Figure 2.11. Abundance of scale insects on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

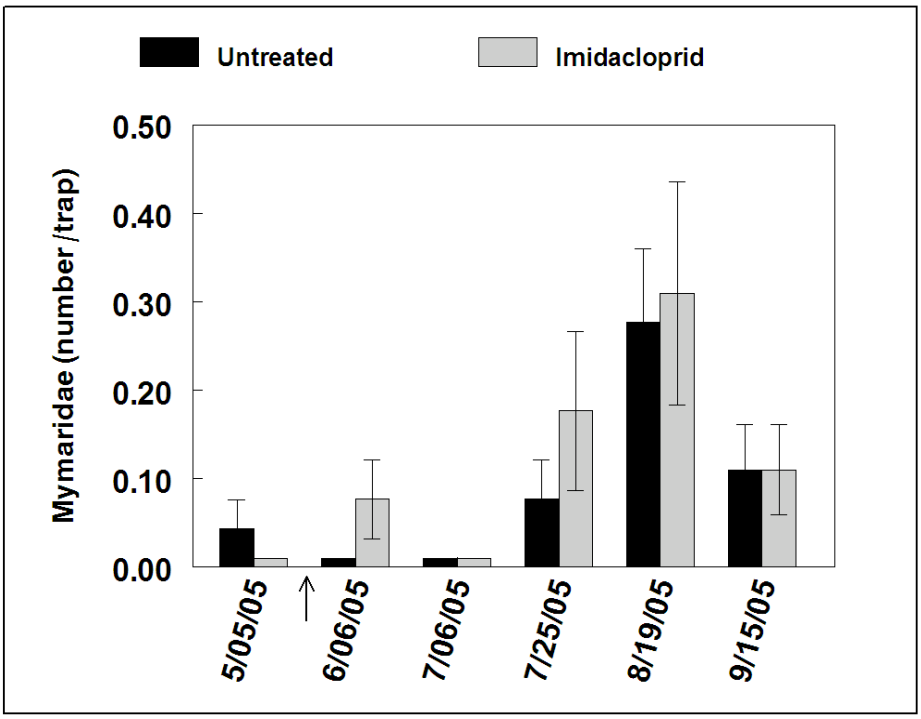

Figure 2.12 Abundance of a parasitoid wasp, Mymaridae, on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

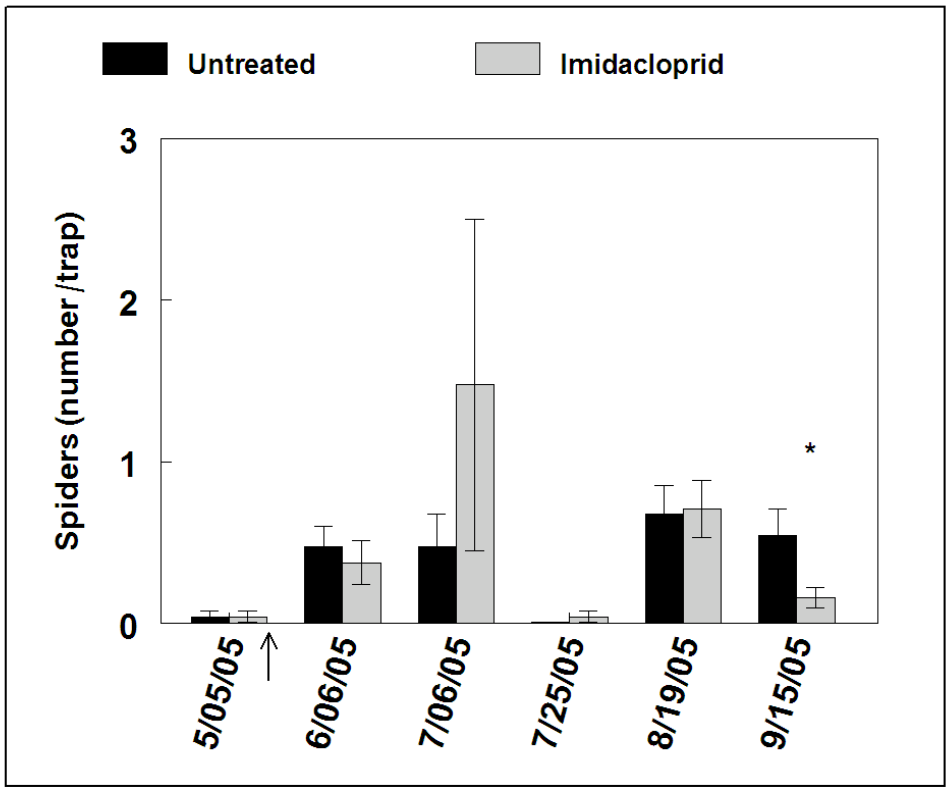

Figure 2.13. Abundance of spiders on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

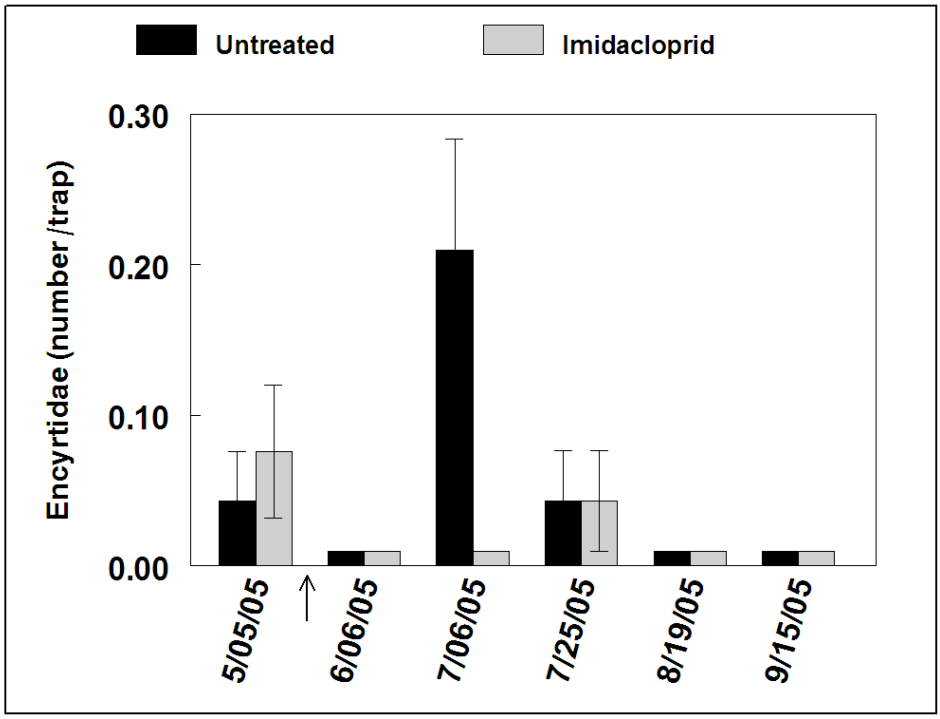

Figure 2.14. Abundance of an Encyrtid wasp on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

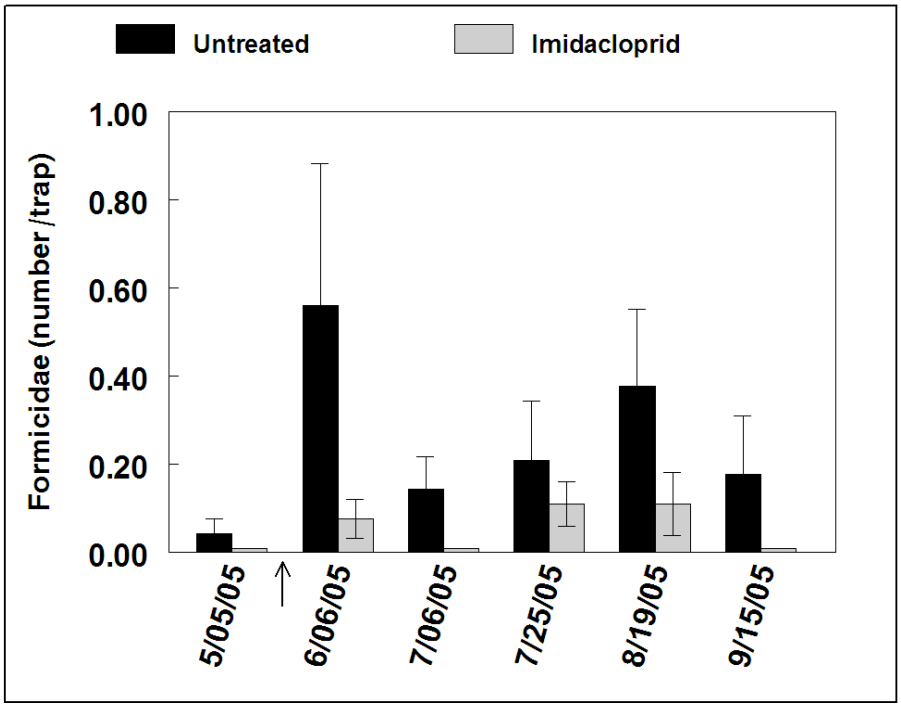

Figure 2.15. Abundance of ants on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

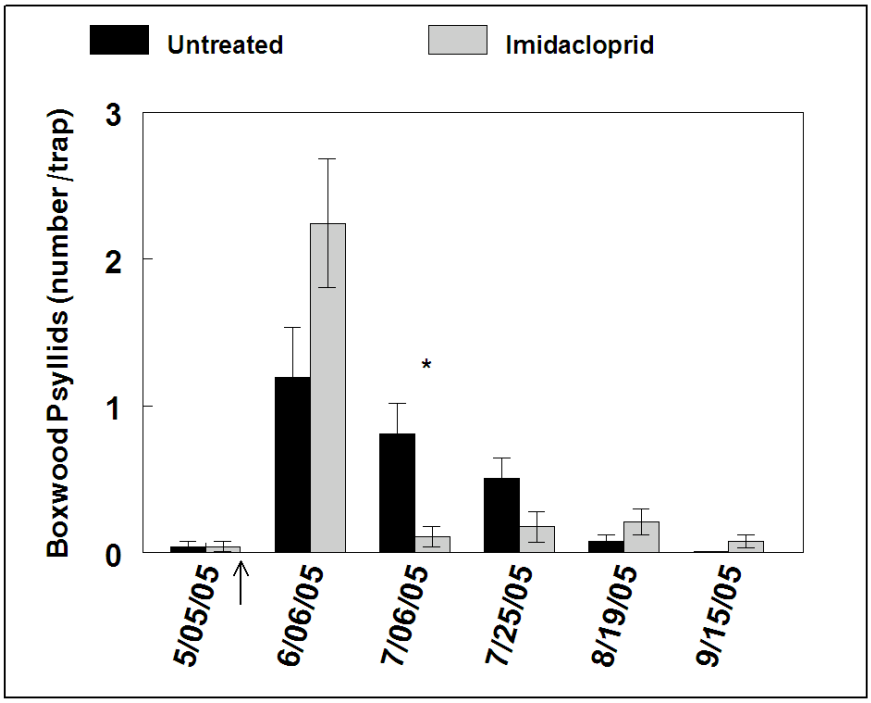

Figure 2.16. Abundance of *P. buxi* on imidacloprid treated and untreated boxwoods in College Park, MD. Black bars represent untreated controls while grey bars represent shrubs exposed to the insecticide. Vertical lines represent error bars. Asterisks mark means that were significantly different within each date at P = 0.05.

Chapter 3: Plant-mediated and direct effects of imidacloprid on reproductive performance of *Tetranychus schoenei* (Acari: Tetranychidae) and *Eurytetranychus buxi* (Acari: Tetranychidae).

Abstract

One of the hypothesized mechanism to explain outbreaks of spider mites that follow applications of imidacloprid was direct stimulation of mites' fecundity. Earlier studies provided evidence both supporting and refuting this hypothesis. To examine if imidacloprid changes reproductive performance of mites, I investigated how applications of the insecticide to plants and directly to mites affected their fecundity. *T. schoenei* and *E. buxi* were exposed to imidacloprid-treated elms and boxwoods, respectively. Additionally, the spider mites were sprayed with imidacloprid and then offered untreated foliage. Results of the experiments indicate consistently higher fecundity of both species of mites after consuming imidacloprid-treated plants, while their longevity remained the same between treatments. Spider mites sprayed with imidacloprid did not exhibit higher fecundity than their untreated counterparts, suggesting that the effect of imidacloprid on spider mite fecundity is mediated by changes in the quality of plants.

Introduction

Hormoligosis is a phenomenon in which sublethal amounts of a chemical or other stressor found harmful when administered at high dose stimulates growth, accelerates maturation, or increases reproductive abilities of an arthropod when present at a lower dose (Luckey 1968, Morse 1998). Previously, hormoligosis has been suggested as a key mechanism responsible for outbreaks of mites following the application of

imidacloprid (James and Price 2002). A response that is expected in the case of hormoligosis is a β-curve, where growth, fecundity or other fitness parameters of the arthropod increase at lower doses of the chemical, reach a peak and then gradually decrease with increasing levels of the agent (Calabrese and Baldwin 1998). The β-curve response of mites to increasing doses of imidacloprid has not been demonstrated and a level of imidacloprid detrimental to mites has not been identified. Thus, it seems more appropriate to use a different term to refer to enhanced fecundity of mites exposed to imidacloprid. Cohen (2006) suggested Pesticide-Induced Homeostatic Modulation (PIHM) as a more appropriate definition of direct stimulation of non-target organisms such as mites brought about by exposure to insecticides.

There are numerous reports of PIHM of mites in the literature. Saini and Cutkomp (1966) and Dittrich et al. (1974) reported that DDT increased oviposition and resulted in female-biased ratio in spider mites. Methyl carbamate applications had a stimulatory effect on spider mites as well (Dittrich et al. 1974, Boykin and Capmbell 1982, Costa et al. 1988, Calabrese 1999). Another insecticide class, pyrethroids, affected tetranychids in a similar way. Application of synthetic pyrethroids resulted in higher fecundity, female-biased ratio, decreased generation time and delayed diapause (Iftner and Hall 1984, Jones and Parella 1984, Costa et al. 1988, Gerson and Cohen 1989, Ayyappath 1997). McKnee and Knowles (1984) found that mites exposed to pyrethroid insecticide exhibited increased respiration and restlessness.

Little is known about the exact mechanism of direct stimulation of arthropod reproduction by pesticides. One of the possible pathways that could result in increased feeding rate and greater fecundity is a positive change in plant nutritional value following

insecticide application (Rodriguez et al. 1960, Saini and Cutkomp 1966, Boykin and Campbell 1982, Mellors et al. 1984). Gupta and Krischik (2007) found that rose plants treated with imidacloprid had elevated levels of chlorophyll and increased leaf area relative to untreated plants indicating a shift in the allocation of resources. Twospotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae), were more abundant on plants treated with imidacloprid. In another important study, tomato plants treated with imidacloprid were probed more often by thrips, *Frankliniella occidentalis*, (Thysanoptera: Thripidae), than untreated plants (Joost and Riley 2005). Another possible mechanism by which insecticides might elevate fecundity of arthropods is an increase in oocytes produced by the ovaries. Elevated oogenesis was suggested by Lemos et al. (2005) for stinkbugs exposed to sublethal levels of permethrin. However, there are no rigorous experiments that would uncouple cause-and-effect relationship between greater numbers of eggs from increased feeding rate. Increased respiration and enhanced locomotion were observed in spider mites exposed to a pyrethroid insecticide (McKnee and Knowles 1984)

Because of known cases of direct stimulation of reproduction of spider mites by various insecticides, a plausible mechanism underlying outbreaks of mites following imidacloprid applications was insecticide-induced increase in fecundity. James and Price (2002) suggested hormoligosis as the mechanism of outbreaks of twospotted spider mites in hops. James and Price (2002) presented evidence that imidacloprid administered as a foliar spray or soil drench affected mites directly by stimulating their fecundity and, in the case of treatments that involved soil applications of imidacloprid, also increasing mite longevity. More importantly, James and Price (2002) evaluated the effects of a topical

treatment of mites with the insecticide. Mites in this treatment were also found to produce more eggs than untreated females. Notably, in studies involving direct exposure, imidacloprid was applied to spider mites on leaf disks, and did not account for plant-mediated effects in this experiment. Another group of scientists, who repeated James and Price's study, did not find any effect of imidacloprid on spider mite fecundity (Ako et al. 2004). This result was consistent when different stains of *T. urticae* resistant and susceptible to acaricides were used in experiments as well (Ako et al. 2006).

I investigated direct and indirect (plant-mediated) effects of imidacloprid applications on fecundity of a spider mites found on elms, *T. schoenei* and boxwood spider mite, *E. buxi*. I exposed spider mites to plants that received treatments of imidacloprid through a soil drench. In addition, I administered topical application of the insecticide to the mites to account for indirect, plant-mediated effects of imidacloprid on the number of eggs laid by the mites. By eliminating the plant factor, I hoped to determine if a direct or plant-mediated PIHM was contributed to elevated populations of mites exposed to imidacloprid.

Methods

Fecundity of *T. schoenei* exposed to imidacloprid in elm trees and through a topical application.

Source of experimental mites. Each growing season, *T. schoenei* were collected from naturally infested elm trees in Central Park, New York, NY, and elms on the campus of the University of Maryland, College Park, MD. To obtain females of known age, I removed all adult females from the excised foliage using a fine paintbrush. Spider mites remaining on the leaves were kept in open Petri dishes (Falcon®, 150x15mm) with

moistened filter paper (Whatman®, 125mm), and covered with fine mesh held in place by a rubber band. These plates were placed in slightly closed plastic bags and stored in a Percival® growth chamber at 23 ± 2 °C and 16:8 L:D and 60 ± 10% relative humidity. The colony was inspected daily and mature females were used in subsequent experiments. This protocol was repeated before every bioassay to generate females of known age.

Plant-mediated effect of imidacloprid on mite fecundity. To evaluate how applications of imidacloprid through the soil affected spider mite fecundity, 18 elm trees, *U. americana,* were planted in a common garden at the University of Maryland Turf Research Farm at College Park, Maryland in May 2005. The trees were purchased from a nursery, were uniform in size and age, and had a trunk diameter at breast-height (DBH) of approximately 2.5 cm at the time of planting. They were hand-watered as needed throughout each season, and received applications of 15 g of a slow-release fertilizer Osmocote® (N:P:K of 17:7:12) once a year. In a completely randomized block design, nine elms were treated with imidacloprid (Merit® soluble powder formulation, 750 g of imidacloprid/kg, Bayer, Kansas City, MO) at the label rate of 1.4 tsp (~2 g) per 2.5 cm DBH dissolved in 1 L of water. Nine other elms were designated as untreated controls. Imidacloprid was administered on 06/05/2006, 05/11/2007, and 05/19/2008.

I evaluated fecundity of mites consuming leaves of elm trees treated with imidacloprid in late August and September 2007. A single female of known age was placed in a Petri plate (Falcon®, 60 x 15 mm) containing an excised leaf from an imidacloprid-treated or untreated elm. To ensure that spider mites did not escape from

the arena, I used the bottom of the 60 mm plate and used the top of a 35 mm plate as a lid. The two plates were kept tightly closed with 2 large-sized metal binder clips (Figure 3.1). Each plate contained a moistened filter paper (Whatman®, 42.5mm). The plates were kept in slightly closed plastic bags and maintained at 23 ± 2 °C and 16:8 L:D and 60 ± 30% relative humidity. Eggs were counted daily for each female until death using a dissecting microscope. Females were moved onto newly collected foliage in new microcosms every other day. The experiment consisted of nine replicates and nine subsamples. Subsamples consisted of females assigned to each replicate. Square root transformations were performed to correct for heteroskedasticity of data. Lifetime fecundity and longevity of mites were analyzed using ANOVA (Ott and Longnecker 2001, SAS 2008).

Direct effect of imidacloprid on fecundity of spider mites. To determine if imidacloprid affected fecundity of mites directly, I administered topical application of the insecticide to *T. schoenei* females in September 2007. To this end, I used methods described by James and Price (2002). A Potter Spray Tower was used to apply 2 ml of flowable formulation of Admire® (2 g of imidacloprid/L, Bayer, Kansas City, MO) at 50 kPa, resulting in a mean deposition of 1.6-1.8 mg of liquid per cm^2. Females of known age were collected from the colony maintained in the laboratory, moved onto fresh untreated leaves, and were then sprayed with imidacloprid or with distilled water. After treatment, the leaves and mites were allowed to dry for 20 min before mites were moved to new, untreated leaves for analysis of performance.

Performance of *T. schoenei* females that received sprays of imidacloprid or distilled water was assessed in the following way. Thirty treated and thirty untreated females were randomly assigned to arenas containing leaves from untreated elm trees growing in a common garden on campus of the University of Maryland, College Park, MD. Foliage from these untreated trees served as food for the mites, and was changed every other day. Using experimental arenas described above with temperatures, light cycles, and humidity of 23 ± 2 °C and 16:8 L:D and 60 ± 30% respectively, I counted mite eggs produced from the first day of exposure until death of the mite. The experiment was replicated 10 times for each of the three different untreated trees for a total of 30 replicates across blocks for each treatment. Square root transformations were performed to correct for heteroskedasticity of data. Lifetime fecundity and longevity of mites were analyzed using ANOVA (Ott and Longnecker 2001, SAS 2008).

Fecundity of *E. buxi* exposed to imidacloprid in boxwood shrubs and through a topical application.

Source of experimental mites. Boxwood spider mites were collected from naturally infested boxwoods with no history of insecticide exposure during the previous five years growing on the campus of the University of Maryland, College Park, MD. Mites were moved to containerized shrubs, *B. sempervirens*, var. Varder Valley in 3.7 L pots purchased from a commercial supplier and kept year-round at the University of Maryland Greenhouse facility located in College Park, MD, USA. Boxwoods were maintained at 22 °C and 18 °C during the day and night respectively, with ambient humidity and L:D cycle of 16:8 h Plants received approximately 200 ml of water once a

day through drip irrigation. Mite colonies sustained on these boxwoods were used in all subsequent experiments.

To obtain females of known age, I collected boxwood mites from plants in the greenhouse and removed all adult females from the excised foliage using fine paintbrushes. Spider mites remaining on the leaves were kept in open Petri dishes (Falcon®, 150x15 mm) with moistened filter paper (Whatman®, 125 mm), covered with fine mesh held in place by a rubber band. Plates were placed in slightly closed plastic bags and stored in a Percival® growth chamber at 23 ± 2 °C and 16:8 L:D and 60 ± 30% relative humidity. The colony was inspected daily and mature females were removed and used in subsequent experiments. This protocol was repeated before every bioassay to generate spider mite females of known age.

Plant-mediated effect of imidacloprid on mite fecundity. To evaluate how applications of imidacloprid applied to boxwoods as soil drenches affected spider mite fecundity, I treated ten boxwood plants free of spider mites with imidacloprid (Marathon® soluble powder formulation, 600 g of imidacloprid/kg, Bayer, Kansas City, MO) at the high label rate of 0.33 g per 3.7 L pot dissolved in 100 ml of water. Ten other boxwoods were designated as untreated controls. The insecticide was applied approximately six weeks prior to the onset of experiments.

I compared fecundity of mites consuming boxwood leaves treated with imidacloprid to those consuming untreated leaves by placing a single spider mite female of known age in a Petri plate (Falcon®, 60 x 15 mm) containing either an excised leaf from an imidacloprid-treated plant or a leaf from an untreated plant. Experimental arenas

described above were used in this experiment (Figure 3.1). The plates were kept in slightly closed plastic bags and maintained at 23 ± 2 °C and 16:8 L:D and 60 ± 30% relative humidity. Individual female fecundity was followed until death. Eggs were counted daily with the aid of a dissecting microscope. I changed Petri dishes and moved females onto newly collected foliage every other day. The experiment consisted of ten replicates with the experimental unit being a boxwood shrub that received imidacloprid or was an untreated control. Nine spider mite females were assayed on each plant and were considered subsamples. Square root transformations were performed to correct for heteroskedasticity of data. Lifetime fecundity and longevity of mites were analyzed using ANOVA (Ott and Longnecker 2001, SAS 2008).

Direct effect of imidacloprid on fecundity of spider mites. To determine direct effects of the insecticide on mite fecundity, I administered topical application of imidacloprid to *E. buxi* females as described above and in James and Price (2002). Females of known age were collected from the colony maintained in the laboratory and moved onto fresh leaves, which we sprayed with imidacloprid or with distilled water. After treatment, leaves and mites were allowed to dry for 20 min before mites were moved to untreated leaves for analysis of performance as described above. Thirty treated and 30 untreated females were randomly assigned to arenas containing leaves from three untreated boxwoods grown in containers in the greenhouse in conditions described previously. Three boxwoods were used as blocks to control for potential effects of plant variation. The experiment was replicated 10 times for each of the three untreated shrubs for a total of 30 replicates across blocks for each treatment. Square root transformations

were performed to correct for heteroskedasticity of data. Lifetime fecundity and longevity of mites were analyzed using ANOVA (Ott and Longnecker 2001, SAS 2008).

Results

Fecundity of *T. schoenei* exposed to imidacloprid in elm trees and through a topical application.

Plant-mediated effect of imidacloprid on mite fecundity. Spider mites that consumed foliage from imidacloprid treated elms laid significantly more eggs than females feeding on untreated plants ($F_{1,15} = 4.93$; $P = 0.042$) (Figure 3.2). Average lifetime fecundity increased from 21.12 (± 1.93) to 29.01 (± 2.69) when the spider mites were offered leaves from imidacloprid-treated elms. Mites also showed a trend for increased longevity when exposed to leaves from treated elms; however, the trend was not statistically significant ($F_{1,15} = 1.54$; $P = 0.2335$). *T. schoenei* exposed to the insecticide lived on average two days longer than mites feeding on leaves from untreated elms (16.18 ± 1.21 and 14.69 ± 1.14 on leaves from imidacloprid-treated trees and untreated controls, respectively). However, this difference was not significant.

Direct effect of imidacloprid on fecundity of spider mites. There was no interaction between the blocks (untreated plants used as food) and treatment ($F_{2,58} = 1.5821$; $P = 0.2073$). Thus, data across the three blocks were combined. When *T. schoenei* received topical application of imidacloprid or water and then were offered leaves from untreated elms, their average lifetime fecundity did not differ between treatments ($F_{1,58} = 0.5254$; $P = 0.4685$) (Figure 3.3). Spider mites that were sprayed with imidacloprid laid an average of 37.60 (± 4.20) eggs during their lifetime, while their

unsprayed counterparts produced 36.40 (± 3.63) eggs. As in the previous experiment, longevity of mites was not significantly enhanced when exposed to imidacloprid ($F_{1,58}$ = 1.45; *P* = 0.2285) and mites lived an average of 16.01 ± 1.86 and 15.17 ± 1.46 days for imidacloprid-treated mites and untreated females, respectively. Mites in this experiment were recorded to lay more eggs than females in the previous study that examined plant-mediated effects on fecundity (21.12 ± 1.93 to 29.01 ± 2.69 eggs per female). The previous study was conducted by multiple researchers due to a large sample size, while the direct effects of imidacloprid on mite fecundity were examined by an individual scientist, which could explain discrepancies between reproductive performances in the two experiments.

Fecundity of *E. buxi* exposed to imidacloprid in boxwood shrubs and through a topical application.

Plant-mediated effect of imidacloprid on mite fecundity. There was no interactive effect of time and treatment on spider mite fecundity ($F_{1,28}$ = 0.02; P = 0.915) or longevity ($F_{1,28}$ = 3.08; P = 0.0910). Thus, data from experiments conducted during the span of three months were combined. *E. buxi* that consumed boxwoods treated with imidacloprid laid significantly more eggs than mites that ate foliage of untreated plants ($F_{1,28}$ = 5.19; P = 0.0305) (Figure 3.4). Their average lifetime fecundity was 18.98 (± 1.023) on the insecticide-treated boxwoods, whereas mites on untreated foliage laid an average of 15.039 (± 1.39) eggs. Females' longevity, on the other hand, did not differ between the two treatments ($F_{1,28}$ = 0.11; P = 0.7398) (Figure 3.4). *E. buxi* exposed to imidacloprid through boxwoods lived for 13.42 (± 0.717) days, while females on untreated shrubs lived an average of 14.1 (± 0.9) days.

Direct effect of imidacloprid on fecundity of spider mites. There was no interactive effect of block and treatment ($F_{2,64} = 0.9471$; $P = 0.5813$), and data across the three blocks were combined. When *E. buxi* were exposed to imidacloprid through direct contact of an insecticide spray, their fecundity was not affected ($F_{1,64} = 0.01$; $P = 0.915$) (Figure 3.5). Females in both treatments laid approximately 18 to 19 eggs during their lives (18.27 ± 1.023 and 19.193 ± 1.9 for imidacloprid-sprayed mites and untreated mites, respectively). Boxwood spider mites that received topical application of imidacloprid lived for 12.72 (± 0.8) days, and their longevity was comparable to that of their counterparts sprayed with distilled water, who lived 13.761 (± 1.39) days ($X^2 = 0.0547$; df = 1; $P = 0.8151$) (Figure 3.5). Similar to the elm experiments, boxwood mites that were directly sprayed with imidacloprid were recorded to lay more eggs in both treatments than mites exposed to imidacloprid through foliage. Due to a large sample size, experiments examining plant-mediated effects of imidacloprid on fecundity of spider mites required employing a sizable group of researchers. Multiple technicians with varying degree of skill and experience could have been the source of the discrepancies in reproductive performance of mites in the two studies.

Discussion

Imidacloprid had a positive effect on mite fecundity when present in the plant tissue. Mites that consumed leaves from imidacloprid-treated elms laid almost 30% more eggs than their counterparts on untreated foliage. The increase in fecundity could not be attributed to a longer lifespan, since there was no difference in how long *T. schoenei* lived in either treatment. Similarly, foliage from boxwoods treated with imidacloprid

enhanced fecundity of the boxwood spider mite, while the insecticide had no effect on reproductive performance of mites when it was applied as a topical spray. Increased longevity did not account for higher lifetime fecundity in this case either, because mites in all treatments lived for a comparable number of days.

These parallel results provide support for imidacloprid's role as mediator of the nutritional quality of foliage as food for spider mites. This change ultimately translates to enhanced reproduction in spider mites that could explain dramatically higher numbers of mites on plants treated with imidacloprid.

This experiment provides evidence for imidacloprid-induced homeostatic modulation (Cohen 2006) of *T. schoenei* and *E. buxi*, and further supports conclusions drawn by James and Price (2002). However, in contrast to James and Price's (2002) study, when mites were directly exposed to imidacloprid, their fecundity remained the same as that of mites not sprayed with the insecticide. This outcome provides a clue to the mechanism through which imidacloprid affects mite fecundity. It appears that imidacloprid does not affect the mites directly, rather, it has an indirect, plant-mediated effect.

Our results are in accord with earlier reports of insecticides promoting changes in the quality of plants (Rodriguez et al. 1960, Saini and Cutkomp 1966, Boykin and Campbell 1982, Mellors et al. 1984). Changes in quality of plant associated with pesticides could result in greater feeding by herbivores and concomitant enhanced reproduction. Furthermore, insecticides are known to have positive effects on plant growth (Pless et al. 1971, Wheeler and Bass 1971, Chelliah and Heinsrich 1980, Mellors el al. 1984) suggesting a mechanism by which insecticides improve plant quality and

indirectly enhance fecundity of phytophagous arthropods. Another route by which insecticides may influence plant physiology and render plants a more suitable food source for herbivores is through disruption of defense pathways. Although hypothesized, this has not been extensively documented (Hardin et al. 1995). If imidacloprid exerts one or more of these effects on plants, then mites could feed more, lay more eggs, and increase in number dramatically.

There are a few reports in the literature connecting imidacloprid's influence on plant physiology to pest outbreaks. Imidacloprid decreased activity of the detoxification enzyme, glutathione S-transferase, in rice cultivar (Wu et al. 2003), which has been linked to increased fecundity and resurgence of a pyralid moth boring into rice stems, *Tryporyza incertulas* (Wang et al. 2005). Rice plants also showed higher content of soluble sugar (Wu et al. 2003), and this translated into higher sugar and lipid content of F1 generation of planthoppers, *Nilaparvata lugens* (Hemiptera: Delphacidae) feeding on imidacloprid-treated rice (Yin et al. 2008). Gupta and Krischik (2007) recently provided evidence that plants treated with imidacloprid may prove more nutritious to herbivores. In their experiment, roses that received granular and soil drench formulations of the insecticide at different doses had a significantly higher chlorophyll content, leaf area and nitrogen concentration than untreated plants. Additionally, outbreaks of twospotted spider mite, *T. urticae*, were observed on roses that received imidacloprid at concentration three times higher than the label rate. This report provides another documented case of secondary outbreaks of spider mite and exemplifies the importance of further examining the extent to which imidacloprid drives changes in plant physiology, resource allocation, and affects non-target herbivores. Moreover, extending research

beyond measuring the differences in plant growth and defense to include the mechanisms underlying these differences between imidacloprid treated and untreated plants will provide a more complete picture of factors that create outbreaks of mites.

I found evidence of imidacloprid-mediated increases in spider mite fecundity when the herbivore consumed leaves of plants treated with imidacloprid. However, the quest to find direct effects of imidacloprid on spider mite fecundity failed. It seems that changes in the quality of plants are the driving force behind secondary outbreaks of spider mites. Literature reports support the notion that imidacloprid has an effect on the physiology of plants, exemplified by increased growth rate (Tenczar and Krischik 2006), higher chlorophyll indices and leaf area (Gupta and Krischik 2007), and increased yield (Gonias et al. 2006, 2008). This stresses the importance of more in-depth investigations of the roles that imidacloprid may play in plant-herbivore interactions.

Figures

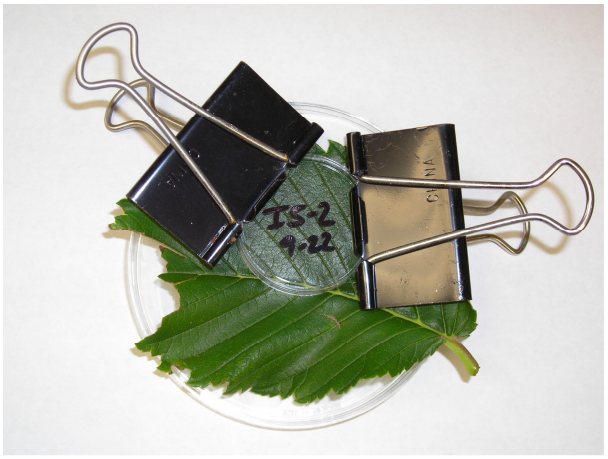

Figure 3.1. Experimental arena consisting of two plates held together by binder clips.

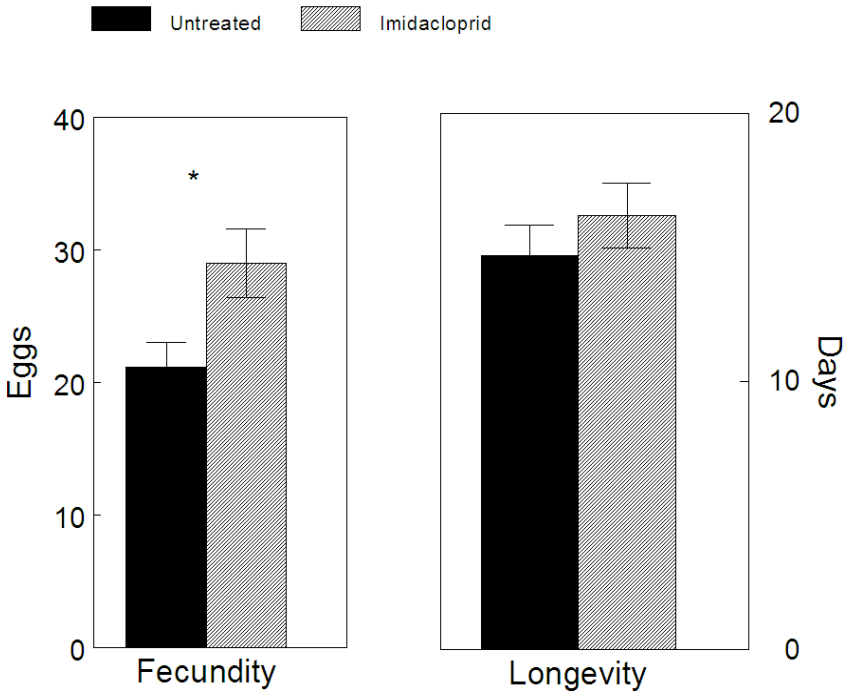

Figure 3.2. Average lifetime fecundity and longevity of *T. schoenei* feeding on foliage from imidacloprid treated and untreated elms. Bars represent means and vertical lines represent standard errors of the mean. Asterisk denotes means that are statistically significant at P = 0.05.

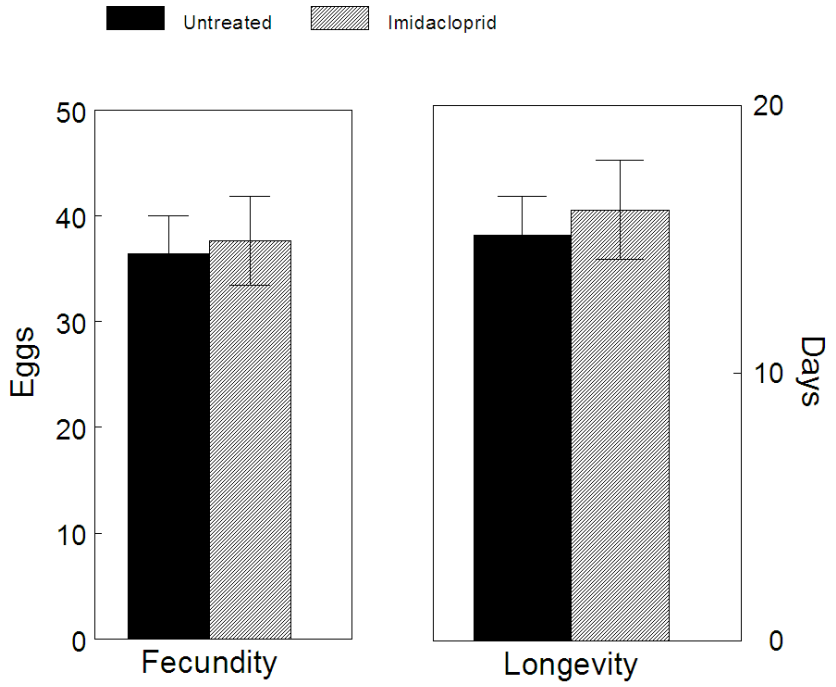

Figure 3.3. Average lifetime fecundity and longevity of *T. schoenei* that received imidacloprid or distilled water (control) topical spray. Bars represent means, and vertical lines represent standard errors of the mean.

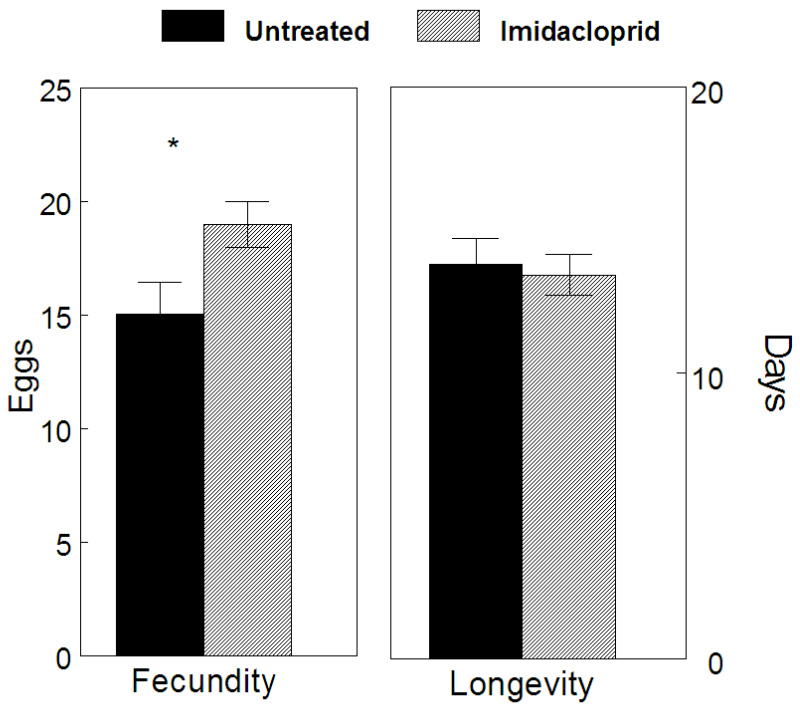

Figure 3.4. Average lifetime fecundity and longevity of *E. buxi* feeding on foliage from imidacloprid treated and untreated boxwoods. Bars represent means and vertical lines represent standard errors of the mean; star denotes means that are significantly different at P = 0.05.

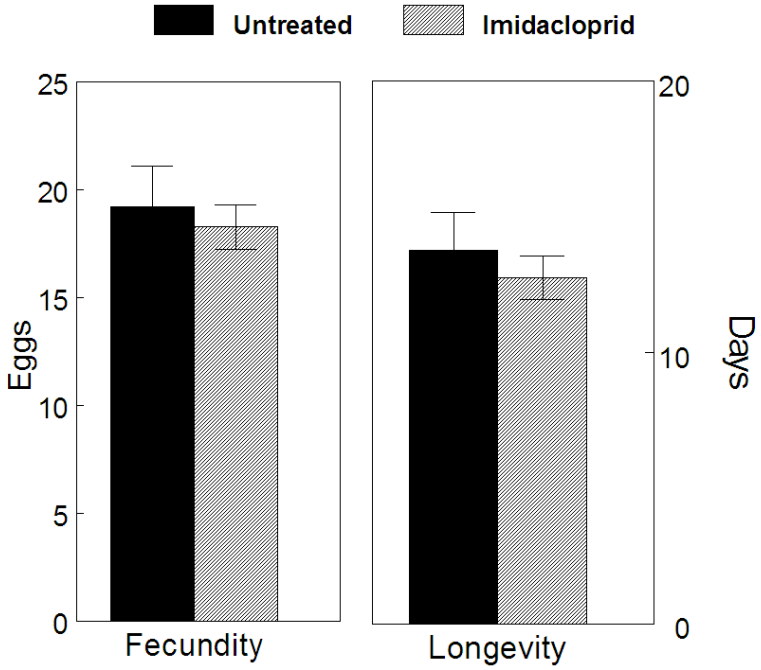

Figure 3.5. Average lifetime fecundity and longevity of *E. buxi* that received imidacloprid or distilled water (control) topical spray. Bars represent means and vertical lines represent standard errors of the mean.

Chapter 4: Differential expression of genes involved in inducible defense pathways in tomatoes treated with imidacloprid

Abstract

Effects of applications of imidacloprid and herbivore feeding (*Tetranychus urticae* Koch, Acari: Tetranychidae) on expression of selected genes regulated by jasmonic acid (JA) and salicylic acid (SA) were examined. Tomato plants, *Solanum lycopersicum*, were established as a model organism by confirming the occurrence of spider mite outbreaks and documenting differential reproductive performance of mites on tomatoes treated with imidacloprid. Expression patterns of jasmonic acid (JA)-regulated genes for cathepsin D inhibitor (CDI), proteinase inhibitor I and II (PI-I and PI-II) and salicylic acid (SA)-modulated genes for pathogenesis-related proteins P6 and P4 (PR-P6 and PR-P4) were compared using reverse-transcriptase polymerase chain reaction (RT-PCR). CDI and PR-P6 showed differential expression in plants treated with imidacloprid. Tomatoes that did not receive imidacloprid application expressed higher levels of CDI than imidacloprid-treated tomatoes, and this response was independent of the herbivore presence. Expression of PR-P6 was elevated when untreated tomatoes were exposed to *T. urticae*, but was suppressed in tomatoes that were exposed to imidacloprid and mites. Expression of the remaining genes was comparable among treatments. Significance of these results to understanding the mechanism underlying spider mite outbreaks following application of imidacloprid are discussed.

Introduction

Imidacloprid-driven changes in plant growth and yield. It has long been known that insecticides affect plant physiology (Pless et al. 1971, Wheeler and Bass 1971, Chelliah and Heinsrich 1980). A few recent reports suggest that new classes of insecticides such as neonicotinoids exert changes in plant physiology as well. Recently, Gupta and Krischik (2007) found rose plants that received three times the label dose of imidacloprid had a greater total chlorophyll index, content of leaf nitrogen, and leaf area than untreated plants. Additionally, Tenczar and Krischik (2006) described a case of poplar trees that exhibited an increased rate of growth between one and four months after applications of imidacloprid. In earlier studies, imidacloprid was reported to positively affect yield and growth rates of cotton. Gonias et al. (2006) found that cotton that received applications of imidacloprid had increased yield of 7% and elevated dry weight of 16%. In addition, imidacloprid-treated cotton had greater photosynthetic rates and chlorophyll indices than untreated plants (Gonias et al. 2008). This response was amplified when plants experienced elevated temperatures and limited water suggesting that imidacloprid enhanced tolerance of cotton to stress, a possibility alluded to by Thielert (2006). These findings illustrate that applications of imidacloprid may be linked to changes in plant physiology. Enhanced growth and yield suggest that applications of imidacloprid lead to differential allocation of resources, which could provide a clue to untangling the mechanisms leading to outbreaks of spider mites.

Plant defense theory. Theory of plant defense has evolved numerous times since its birth in 1950's (Stamp 2003). Many hypotheses that have emerged over the years

differ in some aspects, but all acknowledge that investment in defense comes as a cost to other plant functions (Stamp 2003). One favored hypothesis, Growth-Differentiation Balance (GDB) hypothesis, recognizes that plants must balance resources used for competition and those used for defense against herbivores. These important selective forces shape the evolution of a plant's patterns of resource allocation (Herms and Mattson 1992). The hypothesis states that since growth is highly nitrogen-demanding, and plants need to grow in order to compete for resources, when faced with high availability of this limiting resource plants choose to grow. If photosynthesis increases in an environment rich in resources, then both growth and secondary metabolism benefit. However, when photosynthesis remains constant with increasing availability of nutrients, growth is favored over differentiation. As a result, fewer resources are allocated to synthesis of defense compounds that are expressed constitutively (Herms and Mattson 1992, Stamp 2003).

GDB theory has been well supported for constitutive defenses (Herms and Mattson 1992, Glynn et al. 2003, Stamp 2003). It is not clear, however, if changes in availability of limiting nutrients also govern the expression of inducible defenses, which are less costly to produce. Herms and Mattson (1992) suggested that induced responses should be highest in fast-growing plants and plant tissues, for they are the strongest photosynthesis sinks and rich in photosynthetic products. However, literature offers mixed support for this theory. A study by Glynn et al. (2003) illustrates the dichotomy in expression of inducible defenses by plants exposed to a range of resources. Glynn et al. (2003) investigated whether high levels of fertilization affected rapid induced response (RIR) of poplar trees against two lepidopteran pests. They found that plants in the high

nutrient treatment elicited a strong RIR in response to herbivory by the gypsy moth. This follows a prediction by Herms and Mattson (1992) regarding inducible defenses in plants. By contrast, trees in a low nutrient environment had high levels of RIR when exposed to tussock moth (Glynn et al. 2003). In this case, inducible response followed a pattern predicted for constitutive defenses by the GDB theory. The fact that responses of plants depended on the type of herbivore rather than on nutrient regimen suggested a large range of adaptive phenotypic plasticity of plants with respect to expression of inducible defenses (Baldwin 1999, Agrawal et al. 2002). The literature offers examples of studies where fertilization resulted in both strong and poor elicitation of RIR. Hunter and Schultz (1995) found that applications of fertilizer to oak trees decreased their inducible defenses to sap-sucking and gall-forming insects. Mutikainen et al. (2000), however, reported that fertilized silver birches exhibited strong RIR to a geometrid moth.

While it is not entirely clear if the expression of inducible pathways parallels the predictions of GDB theory for constitutive defenses, there are examples that support this. It is thus possible to hypothesize that if plants treated with imidacloprid grow at a faster rate than untreated plants, according to the GDB theory fewer resources are available to direct to constitutive and possibly inducible defenses.

Jasmonic acid: its effect on spider mite performance and role in plants. Patterns of expression of jasmonic acid (JA), a defense hormone induced by cell-content feeding herbivores such as mites is a plausible route to explore potential effects of imidacloprid on plant defenses. Importantly, there are several reports of JA affecting the performance of mites in a way similar to imidacloprid. For instance, *T. urticae* performed better on

tomato plants genetically engineered to be deficient in their ability to synthesize JA (Walling 2000). Moreover, Thaler et al. (2002) reported a decrease in the number of spider mite eggs on tomato plants following foliar applications of JA, while Omer et al. (2000) was able to induce resistance to a spider mite with foliar application of JA. The application of exogenous JA decreased spider mite fecundity in this experiment. Li et al. (2002) and Ament et al. (2004) investigated the impact of the JA-mediated response on spider mite performance further. Performance of the spider mite, *T. urticae*, which consumed tomato plants lacking genes required for expression of JA was enhanced when compared to plants expressing the phytohormone. However, there is a discrepancy between the two studies: Li et al. (2002) found that spider mite feeding and fecundity were increased on mutant plants, while Ament et al. (2004) found that fecundity did not differ between the treatments. However, egg viability was higher when mites consumed mutant plants. Nonetheless, both research groups present reports of a direct correlation between JA deficiency and increased mite performance that in turn could affect population size.

Jasmonic acid and its derivatives are ubiquitous in plant tissues (Taiz and Zeiger 2002). Jasmonate, a cyclic oxygenated fatty acid, is synthesized through the octadecanoid pathway. Membrane-bound linolenic acid is released into cytoplasm and converted to 12-oxo-phytodienoic acid in the chloroplasts though a multi step enzymatic process involving lipoxygenase, allene oxide synthase, and allene oxide cyclase (Leon and Sanchez-Serrano 1999). 12-oxo-phytodienoic is then converted to JA through a series of oxidations in peroxisomes (Leon and Sanchez-Serrano 1999). JA signaling can be induced by developmental cues, osmotic stress, wounding, elicitors such as wounding,

and potassium levels (Turner et al. 2002, Armengaud et al. 2004). Proteins, whose expression is enhanced by JA are thionin, a fungal defense protein (Bohlmann et al. 1998), plant defensins 1 and 2, which are involved in antimicrobial defense (Gfeller and Farmer 2004), and proteinase inhibitor, which is involved in inhibition of herbivore feeding (Bergey et al. 1996). Another result of JA signaling was elucidated by applying exogenous jasmonate. Foliar applications to tomato and potato plants were found to increase levels of polyphenol oxidase, an enzyme thought to promote plant resistance against insect herbivores (Thaler 1999, Thaler et al. 2002). Furthermore, methyl jasmonate applied to tobacco plants stimulated expression of nicotine, a powerful defensive chemical (Baldwin 1998).

The phytochemical JA is involved in regulation of plant growth and development and leaf senescence (Bell et al. 1995, Reymond and Farmer 1998, Gfeller and Farmer 2004, Lorenzo et al. 2004). Initially, JA was described as a senescence-promoting substance (Leon and Sanchez-Serrano 1999, Thaler 1999). This view was supported by reports of the hormone's involvement in promoting leaf senescence in rice and *Arabidopsis thaliana* plants (Hung and Kao 1997, and He et al. 2002). The molecular bases of JA-related leaf senescence was investigated in potato and tobacco plants, and it was shown that JA inhibits growth by affecting one of the checkpoints of mitosis (Ulloa et al. 2002, and Swiatek et al. 2004). In case of potato plants, the activity and expression of cyclin-dependent kinases was found to be down-regulated by JA (Ulloa et al. 2002). The fact that JA interferes with activity of cyclin-dependent kinases implies its direct and crucial role in controlling growth of plants.

Involvement of JA in defense pathways, has been widely studied (Baldwin et al. 1998, Baldwin 1999, Thaler et al. 2004). Expression of 67-84% of genes involved in defense is mediated by jasmonates, and activation of these genes is not dependent on whether the feeding herbivore is a specialist or a generalist (Gfeller and Farmer 2004, Reymond et al. 2004). A large pool of knowledge of JA's role in defense came from experiments that involved applications of exogenous jasmonate and using transgenic plants unable to synthesize JA. Defense-inducing properties of foliar applications of the phytohormone were observed to reduce herbivory of leaf miners (Black et al. 2003), thrips, aphids, caterpillars and flea beetles (Thaler 1999).

Salicylic acid (SA) and SA and JA cross-talk. JA pathways are known to interact with salicylic acid (SA), another phytochemical vital to a plants' response to herbivores. SA is synthesized in plants from phenylalanine that is converted to trans-cinnamic acid through decarboxylation and then hydroxylated to SA (Lee et al. 1995, Shah 2003). Accumulation of SA induces systemic acquired response (SAR) in plants that is directed against pathogens (Lee et al. 1995, Datta and Muthukrishnan 1999, Walling 2000, Shah 2003). SAR regulates expression of pathogenesis-related (PR) proteins. SAR is involved in countering fungal, viral, and bacterial attack, but it is also elicited in response to herbivore wounding (Datta and Muthukrishnan 1999, Walling 2000). Exposure to feeding by spider mites for example, has been shown to elicit expression of PR proteins (Walling 2000, Li et al. 2002, Ament et al. 2004).

Evidence of cross-talk between the two phytochemicals is well-supported (Doares et al. 1995, Walling 2000, Gatehouse 2002, Heidel and Baldwin 2004). SA has been

shown to down-regulate expression of PIs regulated by JA (Doares et al. 1995, Walling 2000, Gatehouse 2002, Heidel and Baldwin 2004), while JA is known to elicit accumulation of PR proteins independently of SA (Penninckx et al. 1996, Pieterse and van Loon 1999, Schaller et al. 1999, Walling 2000, Choh 2004).

The complicated interactions between the two pathways and the fact that wounding by spider mites stimulates expression of defensive proteins regulated by both JA and SA underlined the need to examine differential expression of genes regulated by both phytochemicals. To this end, I investigated the effect of imidacloprid application on expression of selected genes, whose transcription is known to be up-regulated by spider mites (Li et al. 2002, Ament et al. 2004). Genes regulated by JA, Cathepsin D Inhibitor (CDI), Protease Inhibitor I (PI-I) and Protease Inhibitor II (PI-II), and genes coding for proteins in the SA pathway, Pathogenesis-related Protein P6 (PR-P6) and Pathogenesis-related Protein P4 (PR-P4) were chosen.

CDI, PI-I and PI-II are protease inhibitors, and interfere with herbivores' digestion of plant tissues (Gatehouse 2002, Lizon et al. 2006). They belong to the group of proteins inhibiting serine protease that have been widely studied (Sanches-Serrano et al. 1986, Clevelend et al. 1987, Ryan 1990, Ritonja et al. 1990, Ryan 2000, Walling 2000, Lizon et al. 2006). PI-I and -II are active against chymotrypsin and trypsin and chymotrypsin respectively (Ryan 1990, Datta and Muthukrishnan 1999), while in addition to inhibiting serine proteases, CDI also inhibits an aspartic protease, cathepsin D (Ritonja 1990, Lizon et al. 2006).

While serine proteases have been well-studied, relatively less is known about the PR family of proteins. Proteins are included in the PR family if their expression is

induced by SAR pathway (Datta and Muthukrishnan 1999). The PR-P4 are chitinase proteins I and II without lysozomal activity and are thought to be employed by plants as they attack fungal and bacterial walls (Datta and Muthukrishnan 1999). They are also expressed in tomatoes attacked by spider mites (Li et al. 2002, Ament et al. 2004). Lastly, PR-P6 is a family of PR proteins related to tomato protease inhibitor I and inhibits serine proteases (Datta and Muthukrishnan 1999).

Tomato as the model system for imidacloprid-mediated disruption of defenses. There are a few crucial similarities in the effects that both imidacloprid and jasmonic acid have on spider mites. Responses of tetranychids to plants treated with imidacloprid resemble responses seen in plants deficient in JA. A model organism, *Solanum lycopersicum*, was used to investigate changes in JA mediated plant defense associated with applications of imidacloprid. The objectives of this research were twofold. First, I attempted to establish that the same phenomena illustrated for woody ornamental plants, namely outbreaks of mites and differential fecundity, hold true for the tomato system. Finding the link between the model system and woody ornamentals was essential to extrapolate possible disruption of defenses in tomato back to trees and shrubs growing in landscapes. Second, I wanted to examine if expression of selected genes from JA and a related, salicylic acid (SA) pathway were affected by imidacloprid application. Genes involved in SA were used in the experiment as well because of known cross-talk between the two pathways (Traw and Bergelson 2003, Thaler et al. 2002, Heidel and Baldwin 2004).

Methods

Study system: *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Solanum lycopersicum (*Solanales: Solanaceae)

Herbivore and its biology. *T. urticae* is a generalist pest attacking a wide variety of plants. These spider mites' hosts include over 900 species of plants (Walter and Proctor 1999), and damage inflicted by these mites can cause significant economic loss to agriculture and ornamental plant production (Huffaker et al. 1969, Helle and Sabelis 1985). The mites overwinter as adult females, and go through four developmental stages before becoming a fully mature mite: egg, larva, characterized by three pairs of legs, protonymph and deutonymph (Helle and Sabelis 1985, Evans 1992, Walter and Proctor 1999). Development from egg to adult is correlated to temperature and humidity with higher temperatures and lower humidity resulting in shorter development time (Helle and Sabelis 1985). Eggs are deposited on the surface of the leaf, usually the underside, or on and inside webbing produced by mites at high densities.

T. urticae feeds by puncturing mesophyll cells and sucking out the cell contents (Helle and Sabelis 1985). Their feeding damages spongy mesophyll cells, although damage in the palisade layer has been observed at high mite densities as well (Evans 1992). There have been reports of toxic properties of mite saliva as early as 1964 (Simon 1964), and other reports supporting this claim followed (Avery and Briggs 1968, Andrews and LaPre 1979). More recently, it has been shown that elicitors in mite saliva stimulate the cascade of plant defense responses (Dicke et al. 1993, Takabayashi et al. 2000, Walling 2000, Janssen et al. 2002). Feeding by spider mites elicits release of

kairomones such as methyl salicilate, linapool, and (3E)-4-8-dimethyl-1,3,7-nonatriene (Evans 1992).

Spider mites feed mainly on the underside of leaves, and symptoms of their damage can range from stippling and irregular blotches of discoloration to yellowing or bronzing of the leaf (Helle and Sabelis 1985, Evans 1992). Defoliation is associated with heavy infestations. High mite populations can also lead to mite dispersal, although other factors such as plant host quality and desiccation also play a role in dispersal (Helle and Sabelis 1985, Evans 1992). Female spider mites, sometimes immatures but rarely males, assume a distinct dispersal position with their forelegs raised, and are carried off the leaf by wind (Evans 1992).

Host plant. Tomato (*Solanum lycopersicum*, formerly: *Lycopersicon esculentum)* is one of the more extensively studied agricultural plants due to its high cultural and economic importance. It evolved in South America, and belongs to the "night shade" family, Solanaceae (USDA 2009). The genome of tomato is being sequenced as a part of the 'International Solanaceae Genome Project (SOL): Systems Approach to Diversity and Adaptation' (Mueller et al. 2005). Many crucial aspects of plant physiology such as plant development, fitness, yield, plant defense pathways and pathogen resistance mechanisms have been studied by utilization of tomato genome (Bennett and Leyser 2006, Bian et al. 2006, Manning et al. 2006, Quinet et al. 2006, Schaefer et al. 2006, Semel et al. 2006).

Effects of imidacloprid on abundance, fecundity and longevity of *T. urticae* on tomato plants treated with imidacloprid.

Source of experimental mites. A colony of spider mites, *T. urticae*, was maintained on potted tomato plants, *S. esculentum* var. Castlemart. Tomatoes were grown in 18 cm (diameter) pots in Sunshine® All-Purpose soil mix (Bellevue, WA, U.S.A.). Tomatoes were maintained in a walk-in growth chamber at the Greenhouse Complex at the University of Maryland, College Park, MD, U.S.A. Plants were maintained at a 12:12 light: dark cycle, with relative humidity of 60% and received light intensity of 596.3 $\mu mol/m^2/s$. The plants were fertilized every other week with Miracle-Gro® Liquid Plant Food (24:8:16 ratio of N:P:K) at label rate of 0.5 g dissolved in 3.7 L of water. Tomatoes were watered by hand every other day. Spider mites were obtained from infested greenhouse plants never treated with insecticide and moved onto leaves of tomatoes used to maintain colonies of mites. New plants grown in the conditions described above were introduced to the colony of mites as needed.

For experiments requiring females of known age, tomato leaves with a high density of spider mite were cleared of all adult females using a fine paintbrush. Leaves were inspected daily for new females, which were removed and used in subsequent studies.

Abundance of mites on imidacloprid-treated tomato plants. To determine if spider mite populations varied between plants treated with imidacloprid and untreated plants, six-week old tomato plants in 18 cm pots maintained as described previously received the following treatments. Five of the plants received an application of

imidacloprid according to the label of Marathon® 60 WP (soluble powder formulation, 600 g of imidacloprid/kg, Bayer, Kansas City, MO) with 0.0235 g/pot dissolved in 100 ml of water applied directly to soil. Five tomato plants were assigned as untreated controls. Two weeks after imidacloprid was administered, 500 female spider mites obtained from the colony were moved onto each of the five imidacloprid-treated and five untreated tomato plants. Ten leaves from each plant were randomly selected, and five females were placed on each leaf using a fine paintbrush. Abundance of spider mites was evaluated four and eight weeks following commencement of the experiment. Tomatoes were left intact, and leaves were not excised from the plants. A hand lens (10X, Hasting Triplet, Bausch & Lomb®) was used to count spider mites and their eggs on both sides of each leaf. The response variable was the abundance of mites per tomato leaf. Results were analyzed using one-way analysis of variance (ANOVA) following tests for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) (Ott and Longnecker 2001, SAS 2008).

Fecundity of mites raised on tomato plants. To examine reproductive performance of *T. urticae* on tomatoes that received an application of imidacloprid, ten six-week old tomato plants potted in 18 cm pots and maintained in conditions described above were used. Five of the plants were treated with imidacloprid as stated previously, and five were left untreated. Two weeks after the insecticide was administered, a single female was moved onto one of nine leaves of each of the ten experimental plants. Leaves were not removed from tomatoes during the experiment. Eggs were counted using a hand lens and subsequently removed using a fine paintbrush. Fecundity of each mite was

followed every other day until their death. Number of eggs laid during lifetime of each female and their longevity were the response variables analyzed. The entire study was repeated twice and the results were analyzed using ANOVA when data exhibited a normal distribution (Shapiro-Wilk tests) and homogeneity of variance (Levene's test) (Ott and Longnecker 2001, SAS 2008). Data from the two trials were combined if block by treatment interaction was found to be insignificant (Ott and Longnecker 2001).

Expression of selected genes involved in JA and SA pathways in tomatoes exposed to imidacloprid and herbivore feeding.

Tomatoes used in this experiment were placed in 10 cm (diameter) pots and maintained as described previously. The experiment had a 2x2 factorial design with two levels of insecticide (imidacloprid present and absent) and two levels of herbivory (mites present and absent). Imidacloprid (Marathon® 60WP) was applied to four-week old plants at label rate (0.013g dissolved in 50 ml of water applied directly to the soil). Six tomatoes received the application while six additional plants were designated as untreated controls. Two weeks after imidacloprid was administered, five female spider mites were moved to a single leaf of three imidacloprid-treated tomatoes and three untreated plants. Mites were placed on one of the youngest, but fully expanded leaves on each of the six plants assigned to herbivore treatment, and were allowed to feed on the foliage for 72 h prior to RNA extraction.

Leaves exposed to mite feeding and corresponding foliage on herbivore-free plants were used for the extraction. A single leaf was removed from each of the 12 plants, ground in liquid nitrogen using mortar and pestle and RNA was extracted from the leaves using RN-Easy Mini Kit from Qiagen® (Valencia, CA). DNA contamination was

removed using RNase-Free DNase Set (Qiagen®,Valencia, CA) during RNA extraction. Quality of RNA was confirmed according to the kit instructions (Qiagen® Valencia, CA). Concentration of the RNA was measured using a spectrophotometer (Eppendorf® BioPhotometer Plus) and brought to an equal concentration of 0.2 µg among all samples using RNase-free water dilutions. Extracted mRNA was stored at -80° C until it was used for the RT-PCR.

GenBank accession numbers for the six genes induced by mite feeding (Ament et al. 2004) are listed in Table[primers]. Primers were designed using PrimerQuest℠ software (Integrated DNA Techn.®) and later ordered from Sigma-Adrich® (St. Louis, MO). Elongation factor (EF1- α) was used as a positive control. Reverse transcription of mRNA to cDNA and amplification of the fragments was performed using Qiagen® One-Step RT-PCR Kit in a Lightcycler480 (Roche, Indianapolis, IN). The PCR protocol consisted of a 1 min denaturation process at 94° C, followed by annealing of primers at 60-65° C, and 35 cycles at 72° C. Products of the PCR were visualized using ethidium bromide in agarose gel electrophoresis. The relative intensity of bands was obtained using ImageJ (ImageJ 1.41, National Institute of Health, USA) software. Measurements from each band were standardized by the control, EF1- α. The quantitative measures of differences in expression were compared with a redundancy analysis, using Canoco software (Ter Braak and Smilauer 2002, Prasifka et al. 2005). A 2x2 factorial ANOVA was performed to test for main and interactive effects of the two levels of imidacloprid and herbivore treatments on expression of each gene (Ott and Longnecker 2001, SAS 2008). Multiple pairwise comparisons made with Tukey's procedure were used to compare expression of each gene among treatments if assumptions of normal distribution

(Shapiro-Wilk test) and homogeneity of variance (Levene's test) were met (Ott and Longnecker 2001).

Results

Effects of imidacloprid on abundance, fecundity and longevity of *T. urticae* on tomato plants treated with imidacloprid.

Outbreaks of spider mites on tomatoes treated with imidacloprid. Abundance of spider mites on tomatoes treated with imidacloprid did not differ from their numbers on untreated plants four weeks after the onset of the study ($F_{1,8}$=1.44, P =0.316). However, tomatoes that received imidacloprid application housed significantly greater population of mites than untreated ones eight weeks after mites were introduced to the plants ($F_{1,8}$=7.43, P=0.026) (Figure 4.1). There were 50% fewer mites on untreated tomatoes than on imidacloprid-treated plants (22.20 (±3.75) and 10.57 (±1.6045) mites on treated tomato plants and untreated tomatoes, respectively.

Fecundity of mites raised on tomato plants. Reproductive performance of mites was significantly greater when the females consumed tomatoes treated with imidacloprid compared to foliage from untreated plants ($F_{1,18}$ = 7.53, P = 0.0207) (Figure 4.2). Spider mites on tomatoes exposed to imidacloprid produced 85.18 (±6.78) eggs in their lifetime, and mites on untreated tomatoes laid 66.29 (±5.66) eggs. However, imidacloprid applications did not have an effect on longevity of spider mites ($F_{1,18}$ = 0.24, P = 0.6325, Figure 4.2). In both treatments, the mites lived an average of about 22 or 21 days (21.95 (±1.91) and 20.73 (±2.20) on imidacloprid exposed tomatoes and untreated plants, respectively).

Expression of selected genes involved in JA and SA pathways in tomatoes exposed to imidacloprid and herbivore feeding.

Gel electrophoresis was performed to visualize the differences in expression of selected genes (Figure 4.3). Based on Monte-Carlo permutations, relative intensity of bands standardized by the positive control was significantly different among treatments (*F* ratio = 6.918; P = 0.014). Comparisons of treatment effects within each gene are presented in Figure 4.4. There was no interactive effect of imidacloprid and herbivore treatments on levels of CDI transcripts ($F_{1,8}$ = 0.01; *P* = 0.93). Expression of CDI was significantly lower in tomatoes that received imidacloprid application compared to untreated plants ($F_{1,8}$ = 20.1; *P* = 0.002), while presence of herbivore did not affect expression of CDI ($F_{1,8}$ = 0.71; *P* = 0.424). There was no effect of imidacloprid treatment ($F_{1,8}$ = 0.69; *P* = 0.429) and herbivore feeding ($F_{1,8}$ = 3.17; *P* = 0.113) on expression of PI-I, and the two factors had no interactive effect on expression of this gene ($F_{1,8}$ = 2.7; *P* = 0.139). Similarly, there was no interactive effect of imidacloprid application and presence of spider mite on expression of the gene coding for PI-II ($F_{1,8}$ = 0.23; *P* = 0.645). This gene also exhibited no difference in expression due to imidacloprid presence ($F_{1,8}$ = 4.17; *P* = 0.075) or herbivore feeding ($F_{1,8}$ = 0.35; *P* = 0.571). There was an interaction between the two factors for PR-P6 ($F_{1,8}$ = 7.55; *P* = 0.025). Untreated plants exposed to feeding by *T. urticae* had increased in levels of PR-P6 transcripts. Presence of the herbivore did not elicit expression of this gene in imidacloprid-treated tomatoes in comparable levels. Neither the insecticide nor feeding by spider mites elicited differential expression of PR-P4 ($F_{1,8}$ = 0.9; *P* = 0.371 and $F_{1,8}$ = 0.28; *P* = 0.614 for imidacloprid effect and herbivore effect, respectively).

Discussion

Tomato plants treated with imidacloprid elicited the same population and individual response from the tetranychid mites as woody ornamental plants treated with imidacloprid (Chapters 1, 2, and 3). Spider mite outbreaks that occurred on tomatoes receiving imidacloprid applications confirm that the phenomenon widely documented on the trees and shrubs in the field takes place on a vegetable as well (Sclar et al. 1998, Raupp et al. 2004, Gupta and Krischik 2007, Raupp et al. 2008). In addition to increased abundance, spider mites laid more eggs when tomatoes were treated with imidacloprid. These two pieces of evidence suggest that the effect exerted on mites by imidacloprid is general, and occurs on plants as vastly different as the woody perennials and annual plants.

Imidacloprid application to tomatoes resulted in differential expression of genes coding for two serine protease inhibitors, CDI and PR-P6. Untreated tomato plants expressed CDI regardless of mite presence, while imidacloprid applications suppressed transcription of these genes to mRNA. The fact that untreated tomatoes expressed this gene in comparable levels in both herbivore treatments could be caused by two factors. Firstly, PI's are known to be expressed constitutively (Jongsma 1997, Gatehouse 2002) and it is possible that this gene is expressed in plant tissues that are not under attack. Additionally, the duration of feeding by spider mites, number of mites that the leaves were exposed to, and time elapsed from the onset of herbivore attack may all explain the apparent lack of difference in level of expression of CDI in tomatoes with and without the mites. More importantly, tomatoes that were treated with imidacloprid did not

express this gene, and expression was not elicited even in the presence of spider mites. This suggests that imidacloprid interferes with expression of this PI.

PR-P6 was expressed at a comparable level in plants treated with imidacloprid and untreated ones not subjected to feeding by spider mites. When plants were exposed to spider mites, however, transcription levels of PR-P6 were significantly higher in tomatoes that were not treated with imidacloprid compared to treated plants. As with CDI, there seems to be a strong inhibition of transcription of this gene to mRNA.

Both of the genes that were differentially expressed in the presence of imidacloprid encode proteins known to be serine protease inhibitors. Expression of CDI is modulated by the JA pathway, and while most PR proteins are generally regulated by SA, there are numerous reports in the literature of PR proteins induced by JA (Penninckx et al. 1996, Pieterse and van Loon 1999, Schaller et al. 1999, Walling 2000, Choh 2004). Additionally, PR-P6 has protease inhibiting property, and its function in plant defenses against herbivore attack has been demonstrated (Datta and Muthukrishnan 1999, Li et al. 2002, Ament et al. 2004). Both genes may have been regulated by JA in plants that were not treated with imidacloprid, while their down-regulation in tomatoes exposed to imidacloprid was related to disruption of JA signaling.

Imidacloprid's negative effect on expression of protease inhibitors could translate into significant advantages for spider mites. Proteases produced by mites brake down proteins in plant tissues and aid in assimilation of amino acids by the herbivore (Datta and Methukrishnan 1999). Inhibitors of these proteins are crucial for plant defenses, and decrease in their quantity and diversity would allow mites easier access to plants' resources.

While it is not possible to discern directly from this experiment how exactly imidacloprid applications alter expression of these two genes, previous studies offer possible mechanisms. Imidacloprid breaks down into several metabolites in plants. One of them is a 6-chloronicotinic acid (6-CNA) (Suchail et al. 2001) (Figure 4.5). This stable metabolite resembles in structure an important phytochemical involved in plants response to pathogen attack, isonicotinic acid (INA) (Figure 4.5) (Francis et al. 2008). Thielert (2006) suggested structural similarity between 6-CNA and an inducer of systemic resistance of plants earlier. INA plays an important role in the response of plants to pathogens (Silverman et al. 1995, Mauchi-Mani and Metraux 1998, Dmitriev et al. 2003) and is thought to serve as SA analogue as well, eliciting expression of the same genes that are elicited by SA (Silverman et al. 1995, Friedrich et al. 1996). INA is efficiently translocated through the plant, and induces SAR most likely by its translocation rather than a release of a systemic signal (Friedrich et al. 1996). Because INA is involved in defense pathways of plants under attack by pathogens and may stimulate the same pathway regulated by SA, the accumulation of INA mimic, 6-CNA, could result in switching on a plant's responses typical for pathogen attack. Given that SA is known to down-regulate expression of PI's (Doares et al. 1995, Heidel and Baldwin 2004), this could result in differential expression of these genes in plants treated with imidacloprid.

Expression of genes coding for other PIs, PI-I and PI–II was not affected by imidacloprid. Plants treated with imidacloprid and untreated controls generally did not express PI-I in absence of spider mites, while feeding by spider mites elicited accumulation of transcripts of PI-I. Additionally, PI-II had comparable expression in

tomatoes in all four treatments. This could be caused by constitutive expression of this gene, which has been shown for PIs in general (Jongsma 1997, Gatehouse 2002), duration of feeding by spider mites, number of mites that the leaves were exposed to, and time elapsed from the onset of herbivore attack. It is not clear why patterns of expression of PI-I and PI–II differed from CDI and PR-P6. It is important to note, however, that these proteins may have unrelated structures and are grouped into the family of PI's because of their common ability to bind proteases (Datta and Methukrishnan 1999). There may be other roles that these proteins play in plants other than defense. (Datta and Methukrishnan 1999).

Lastly, expression of PR-P4 also did not differ among treatments. PR-P4 proteins are chitinases, and while their expression is activated by wounding inflicted by spider mites (Li et al. 2002, Ament et al. 2004), they are thought be directed mainly against cell walls of fungi and bacteria (Datta and Methukrishnan 1999). The exact function of this family of proteins is unknown in tomato (Walling 2000). It is possible that the duration of mites' feeding on tomatoes and their low numbers were not sufficient to elicit expression of this protein.

Cross-talk between the two phytohormones and negative feedback of SA on JA-regulated pathways has been documented (Doares et al. 1995, Walling 2000, Gatehouse 2002, Heidel and Baldwin 2004). They provide support for a hypothesis that the imidacloprid metabolite accumulated and mimicked INA, thereby, influencing regulation of the JA pathway. If imidacloprid exerts an SA-like impact on of the plants' pathways, then inhibition of genes regulated by JA, such as PI's, could take place. This would in turn eliminate a component of plant's defenses employed during spider mite feeding and

provide a plausible mechanism of spider mite outbreaks. Even though disruption in expression of tomato genes involved in plant defense cannot be related directly as a mechanism driving outbreaks of spider mites on woody ornamental plants, these results suggest that imidacloprid affects general and conserved defenses of plants and as such creates conditions conducive to spider mite outbreaks.

Variation in expression of the selected genes in tomatoes treated with imidacloprid and exposed to spider mites may stem from a complex relationship between JA and SA. A study examining the effect of feeding of mirid bugs on expression of PIs found them up-regulated 24 h after attack (Doares et al. 1995). In contrast, Heidel and Baldwin (2004) reported that feeding by mirids down-regulated expression of PIs after 72 h. In both cases concentrations of SA were high, implying that an unknown signal plays a role in how SA affects PIs' (Heidel and Baldwin 2004).

This study examined transcription of the genes to mRNA at a single point in time during defensive response of imidacloprid-treated and untreated tomatoes to the mites. This prevents us from making inferences on how changes in expression translated to differences in concentration of these defense compounds. Post-transcriptional modifications and effects of imidacloprid on transcription of other genes could affect the total responses of tomatoes to spider mites. Further research on the full extent of physiological changes in plants driven by imidacloprid is needed before the complete mechanism of spider mite outbreaks is discerned.

Figures

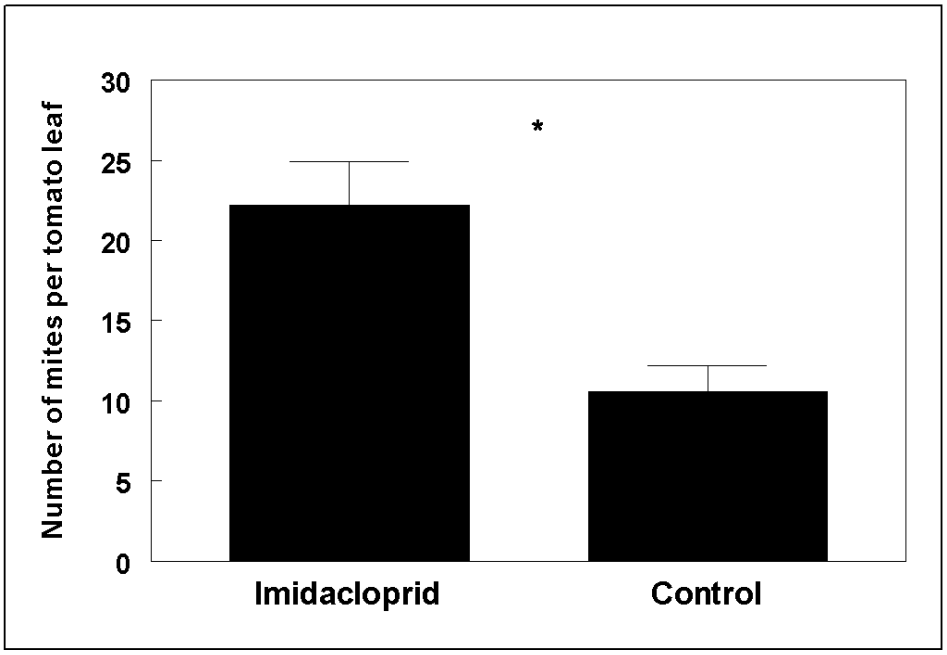

Figure 4.1. Abundance of *T. urticae* on imidacloprid treated and untreated tomatoes. Bars represent means and vertical lines represent standard errors. The asterisk marks means that were significantly different at P = 0.05.

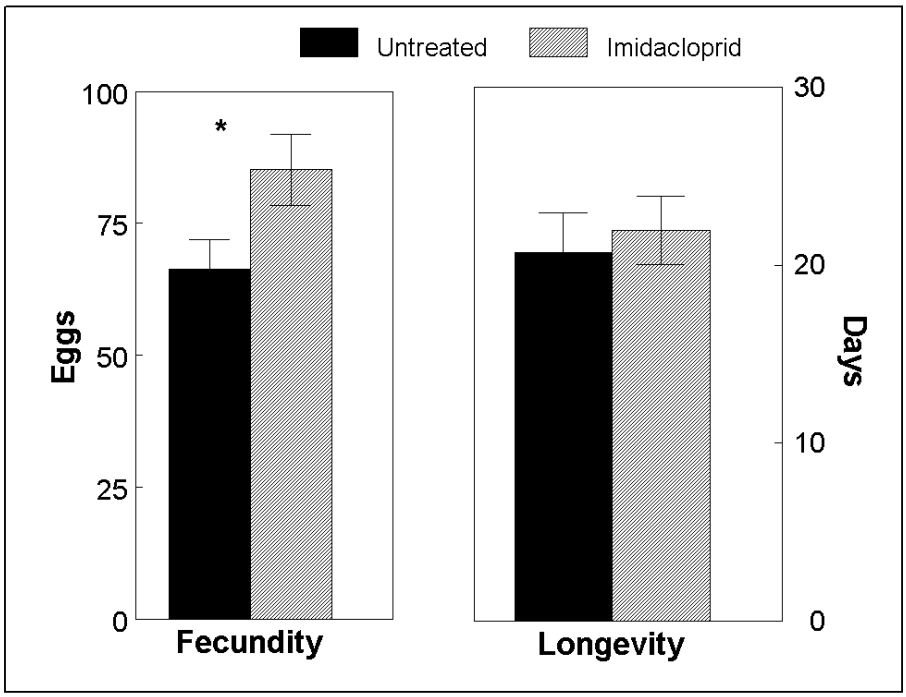

Figure 4.2. Fecundity and longevity *T. urticae* on imidacloprid treated and untreated tomatoes. Black bars represent untreated controls while grey bars represent plants exposed to the insecticide. Vertical lines represent standard errors. The asterisk marks means that were significantly different at P = 0.05.

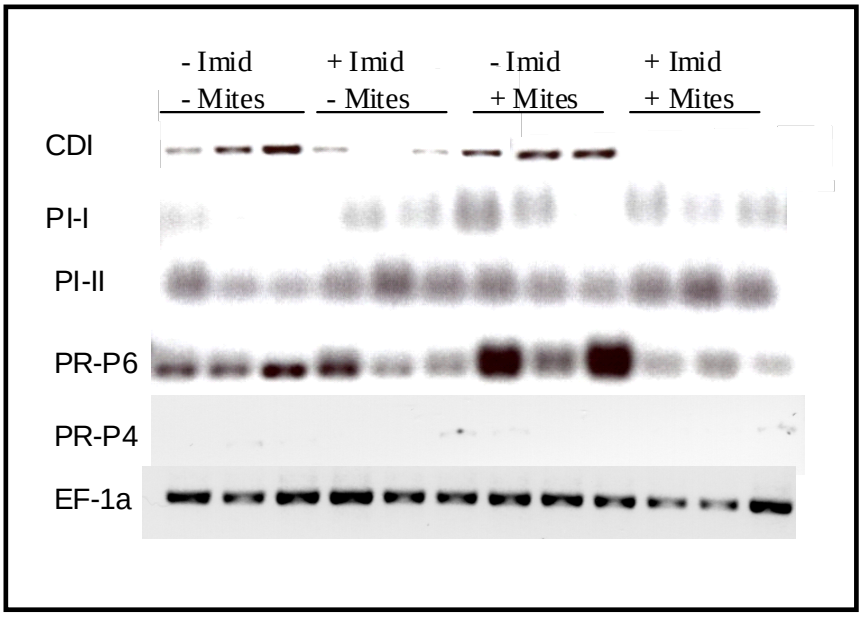

Figure 4.3. Gel electrophoresis of RT-PCR products from tomatoes exposed to imidacloprid (+ Imid) and untreated tomatoes (- Imid) in presence (+ Mites) and absence (- Mites) of herbivory. Expression patterns of cathepsin D inhibitor (CDI), proteinase inhibitor I (PI-I), proteinase inhibitor II (PI-II), pathogenesis-related protein P6 (PR-P6) and pathogenesis-related protein P4 (PR-P4) are presented. Elongation Factor - 1α (EF-1α) was used as a positive control.

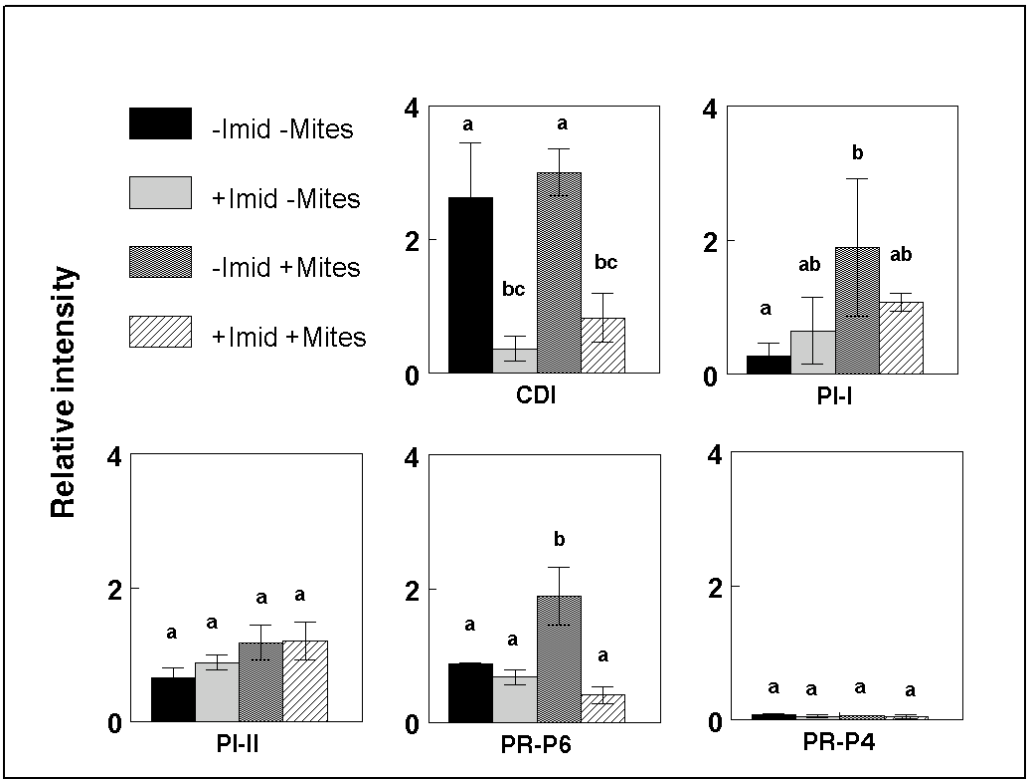

Figure 4.4. Comparisons of expression of selected genes in tomatoes subjected to two levels of imidacloprid (-Imid, +Imid) and two levels of herbivore feeding (-Mites, +Mites). Expression patterns of cathepsin D inhibitor (CDI), proteinase inhibitor I (PI-I), proteinase inhibitor II (PI-II), pathogenesis-related protein P6 (PR-P6) and pathogenesis-related protein P4 (PR-P4) are presented. Relative intensity of each band was standardized by a corresponding expression of a positive control, EF 1- α. Bars represent means while vertical lines represent standard errors. Bars that share a letter are not significant at P = 0.05.

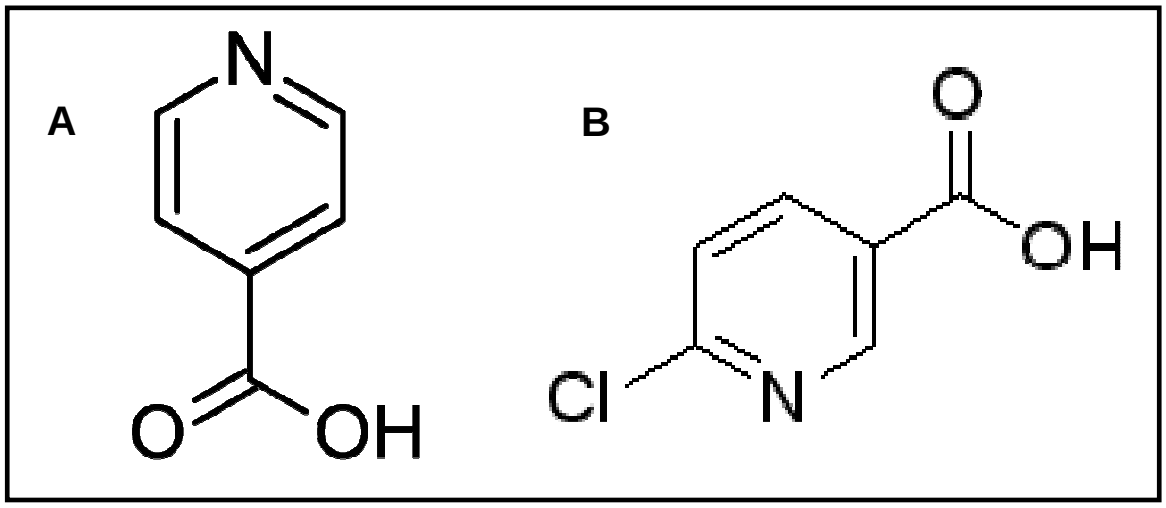

Figure 4.5. Chemical structures of isonicotinic acid (A) and 6-chloronicotinic acid (B).

Conclusions and future directions

The goal of this work was to document outbreaks of spider mites following applications of imidacloprid, and to examine the three main mechanisms, elimination of natural enemies, direct effect of imidacloprid on mite fecundity and disruption of plant defenses thought to cause the outbreaks. The findings of this research further support earlier reports of consistent outbreaks of spider mites on woody ornamental plants treated with imidacloprid in landscapes and greenhouse. Notably, abrupt increases in abundance of mites in relative absence of natural enemies in the greenhouse provided evidence that elimination of natural enemies may not be the leading cause of the outbreaks. Furthermore, surveys of arthropods on elms and boxwoods treated with imidacloprid did not unveil significant differences in composition or numbers of natural enemies of spider mites. Phytoseiid mites, a key predator of spider mites, displayed a variable pattern of abundance that deserves a closer, more rigorous examination. Thus, while it is not possible to dismiss the possibility of negative impact of imidacloprid on communities of natural enemies of spider mites, additional factors seemed to contribute to increased abundance of mites on imidacloprid-treated plants.

These additional factors were identified in experiments that investigated changes in fecundity of mites and expression of plant defense pathways in response to imidacloprid presence. Exposure to the neonicotinoid through plant tissues resulted in higher fecundity that could significantly influence population levels of spider mites. This provided evidence that imidacloprid's impact on mite fecundity is plant-mediated, which was confirmed through differential expression of two proteinase inhibitors in tomatoes treated with imidacloprid. Lower concentrations or complete absence of these protease

inhibitors could benefit the mites by enhancing their ability to assimilate plant nutrients. Similarities in structure of imidacloprid metabolite and salicylic acid analogue could provide the possible mechanism of imidacloprid's impact on expression of these protease inhibitors.

While the results of this work suggest that imidacloprid applications result in outbreaks of spider mites through disruption of important components of plant defenses, it is conceivable that the outbreaks are an outcome of interactive effects of all three mechanisms. Additional studies would contribute to our knowledge of the extent of imidacloprid's effect on plants, primary and secondary pests, non-target organisms and the interactions between the communities of arthropods in general.

To better understand the effects of imidacloprid applications on structure and composition of arthropod communities, different sampling methods than ones described in this study should be used. Excising foliage and transporting it to the laboratory could have led to a biased account of pterous insects such as lacewing adults that would be less likely to remain on foliage while it was removed from the plant. Using vacuum sampling would ameliorate this bias, and allow for a more comprehensive assessment of arthropod fauna. Additionally, abiotic factors such as temperature and rainfall should be examined as covariates that could affect the interactions between imidacloprid-treated plants and arthropod herbivores.

In terms of physiological responses of plants exposed to imidacloprid, comparing concentrations of simple sugars and proteins would provide information on changes in nutritional quality of plants that could in turn affect spider mites. Moreover, examining the effect of imidacloprid on expression of genes involved in plant growth and

differentiation would show if plants treated with the neonicotinoid exhibit patters of allocation characteristic to high resource environment, which according to GDB theory of plant defense would mean diverting resources to primary metabolism. Notably, establishing such pattern of allocation in addition to inhibited defenses would provide important empirical evidence that plants' investment in induced defenses follows predictions outlined by the GDB theory.

Additionally, the proposed hypothesis that imidacloprid metabolite mimics salicylic acid analogue, isonicotinic acid (INA), could be confirmed through treatments of plants with synthetic INA and measurement of the response of spider mites. Comparing abundance of mites and expression of protease inhibitors on plants treated with INA to plants exposed to imidacloprid would shed light on the parallel effects of the two chemicals on plants and their herbivores. It would also be very informative to create high output of information on the effect on the two compounds on general genetic expression of plants through microarray analysis. In addition to a wealth of information on how INA and imidacloprid affect plant's genome, it would also allow for direct comparisons between INA's and imidacloprid's impact on genetic expression in plants.

Lastly, jasmonic acid and salicylic acid are involved in plants' recruitment of the third trophic level, natural enemies, through production of volatiles. If imidacloprid applications affect expression of genes modulated by jasmonic acid, the volatiles regulated by JA may be affected as well. This could have far-reaching consequences for the plants, other herbivores and the structure of arthropod fauna. Examining if production of volatiles is disrupted by imidacloprid applications would shed light on another, indirect aspect of imidacloprid's effect on natural enemies.

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