ABSTRACT

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With a growing number of reports showing secondary outbreaks of spider mites following systemic applications of imidacloprid, a study was designed to test the indirect effects of this insecticide on natural enemies of spider mites. We exposed two predators, *Chrysoperla rufilabris* and *Stethorus punctillum*, to mites that were reared on boxwoods and elms treated with imidacloprid and examined prey consumption, predator mobility and mortality. Mites reared on treated foliage were extremely toxic to predators, eliciting sharp reductions in feeding, locomotion, and longevity. These findings document that non-target pests feeding on plants treated with imidacloprid cause lethal and sublethal responses in natural enemies. We discuss implications on the compatibility of imidacloprid with IPM practices and infer impacts on generalist and specialist natural enemies.

INDIRECT EFFECTS OF IMIDACLOPRID ON NATURAL ENEMIES OF
SPIDER MITES IN TWO SYSTEMS.

By

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Dedication

This thesis is dedicated to my mom, who has shown me—and exemplified—that it's alright to be a space cadet sometimes and that there is always light at the end of the tunnel.

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Abstract

With a growing number of reports showing secondary outbreaks of spider mites following systemic applications of imidacloprid, a study was designed to test the indirect effects of this insecticide on natural enemies of spider mites. We exposed two predators, *Chrysoperla rufilabris* and *Stethorus punctillum*, to mites that were reared on boxwoods and elms treated with imidacloprid and examined prey consumption, predator mobility and mortality. Mites reared on treated foliage were extremely toxic to predators, eliciting sharp reductions in feeding, locomotion, and longevity. These findings document that non-target pests feeding on plants treated with imidacloprid cause lethal and sublethal responses in natural enemies. We discuss implications on the compatibility of imidacloprid with IPM practices and infer impacts on generalist and specialist natural enemies.

Introduction

Literature review

An ecological outbreak is defined as an explosive increase in the abundance of a particular species over a relatively short period of time (Berryman 1987). A pest outbreak then is a marked increase in an organism that has a negative impact on human health or well-being, be that economic, ecologic, or emotional (Berryman 1987). Even in naturalized systems, Nothangle and Schultz (1987) provide extensive evidence suggesting that many of the present-day pest species have only become pests after the advent of anthropogenic habitat alterations. For instance, gypsy moth could only become a major pest after chestnut blight allowed favorable host trees to become dominant in the eastern hardwood forest (in Barbosa and Schultz 1987). Other studies illustrate that even within forest ecosystems, outbreaks are more likely near points of extensive human influence, like margins and logging sites (Battisti et al. 2000).

Nowhere is human influence on habitats more extreme than the urban environment. Not surprisingly, pest outbreaks are common occurrences in urban landscapes (Raupp et al. 1992, Raupp et al. in press). A number of factors in these managed systems contribute to allow arthropod pests to reach economic and aesthetically damaging levels. These factors include both bottom-up (plant-mediated) and top-down (natural enemy-mediated) processes (Daugherty et al. 2007, Raupp et al. in press). While few ecological studies examine mechanisms underlying pest outbreaks in urban environments, the fast pace of urban development and the

potential for significant losses to pests predicates that we gain more insight into ecological processes in urban settings (McIntyre 2000).

A number of bottom-up processes contribute to pest outbreaks in urban landscapes. Given that landscape plants did not evolve in man-altered habitats several studies suggest that resistance to herbivores will be lost in these plants due to selection for traits advantageous in the introduced range (drought and hypoxia tolerance, etc.) in lieu of energetically costly defensive chemicals (Blossey and Noetzold 1995, Maron et al. 2004). Herms (2002) and Ghandi and Herms (in press) also suggested that many landscape plants do not share an evolutionary history with their pests and growing conditions, which can lead to damaging population levels of these pests. Other plant-mediated factors contributing to outbreaks can be as simple as the inherent susceptibility of the host to a particular pest, as some plant species and cultivars are more resistant than others (Reinert et al. 2006, Chappell and Robacker 2006). Planting of resistant plants is a largely underutilized pest management technique (Morgan et al. 1978, Nielsen 1989, Raupp et al. 1992).

Plants in the urban landscape are typically exposed to elevated levels of abiotic stress. That may predispose them to pest attack (Mattson and Haack 1987, Herms and Mattson 1992, Herms 2002, Raupp et al. in press) When compared to a forest setting, urban habitats are considerably more stressful (Beran et al. 1985, Krizek and Dubik 1987, Whitlow and Bassuk 1988, Whitlow et al. 1992). Possibly the most prevalent stress factor in the landscape is drought. While technically a lack of available water, drought is especially common in urban and suburban environments due to soil compaction, loss of organic matter, poor soil structure,

reflected heat, restricted or severed root space, lack of infiltration, competition with grass and other and groundcovers. Excess salinity due to the deposition of de-icing road salt may compound these problems (Whitcomb 2006). White (1969) and Mattson and Haack (1987) provide a number of mechanisms by which drought can increase insect attack on landscape plants. They also document 14 different insect pest genera that outbreak on drought-stressed plants. Air pollution, common in many urban environments, has been linked to gypsy moth and bark beetle infestations (Endress et al. 1991, Koehler et al. 1978, respectively). Mechanical damage, unavoidable in urban settings, leads to increased activity of lepidopteran and coleopteran borers (Koehler et al. 1983, Koehler 1991). Furthermore, Mattson and Haack (1987) argue that because plant responses to stress are similar regardless of the stress type, most stresses will predispose plants to pest outbreaks. Indeed, numerous studies have shown outbreaks of pests in urban environments that rarely reach high densities in naturalized settings as a result of stress (Frankie and Ehler 1978, Nielsen 1989, Driestadt et al. 1990, McIntyre 2000). However, several studies suggest that the relationship between plant stress and herbivore abundance is non-linear (Herms 2002, Huberty and Denno 2004).

In addition to planting susceptible hosts and stress in urban habitats, design elements may increase pest abundance. Vegetational and structural complexity impact arthropod communities immensely (Rypstra et al. 1999, Shrewsbury and Raupp 2000, Langellotto and Denno 2004). Homogeneity, both structurally and in the plant palette, often act in tandem to facilitate pest outbreaks of managed systems (Raupp et al. 1992, Brown et al. 2006, Shrewsbury and Raupp, 2006). Shrewsbury

and Raupp (2000, 2006) link this increased pest activity to a loss of natural enemy abundance and diversity in response to alternate prey items. Tooker and Hanks (2000) referred to urban landscapes as "impoverished areas" in regards to biodiversity of natural enemies.

In addition to design, improper cultural practices may lead to outbreaks as well. Excessive fertilization is often associated with inflated numbers of sucking insects due to the higher nitrogen content in sap (McClure 1991, Herms 2002, Davies et al. 2005, Lu et al. 2005). Similarly, excessive fertilization has been linked to infestations of sawflies and borers (Schoeneweiss 1975). Pruning, if performed improperly or at incorrect times, can also increase pest abundance (Svihra and Koehler 1989, Byers et al. 1980).

Perhaps the most notable and counterintuitive examples of pest outbreaks resulting from landscape management practices are outbreaks associated with pesticide application. Pesticides often negatively disrupt natural enemy communities either through non-target toxicity or elimination of food sources, leading to a loss of top-down control of many pests (Stern et al. 1959, Smith and van den Bosch 1967, Croft 1990). A mosquito fogging program in a resort community resulted in decimation of parasitoids, allowing pine needle scale (*Chionaspis pinifoliae* (Fitch)) to reach outbreak proportions. When the fogging program was modified, the natural enemy communities rebounded, causing the scale population to decline (Luck and Dahlstein 1975). Similarly, weekly application of dimethoate to a community in Michigan to control filth flies, resulted in outbreaks of fruit lecanium (*Parthenolecanium corni* [Bouche']). Again, when the program was adjusted to IPM

standards, the scales were brought under control (Merritt et al. 1983). When a localized population of Japanese beetle (*Popillia japonica* Newman) was discovered in California, the control measures proved to be more toxic to natural enemies than the target pest, resulting in secondary pest outbreaks of whiteflies, mealybugs, scales, and mites (DeBach et al. 1977). In addition to outbreaks resulting from large-scale insecticide applications, Raupp et al. (2001) showed that twelve species of armored scales became more abundant in landscapes that received weekly sprays of residual pesticides.

A new class of insecticides, neonicotinoids, were originally believed to obviate non-target effects and concomitant pest outbreaks. Owing to their broad host range, systemic mode of action, and low mammalian toxicity, neonicotinoids are among the most widely-used pesticides in the urban landscape (Sclar and Cranshaw 1996; Gill et al. 1999). Among this relatively new class of agrochemicals, imidacloprid *(N*-[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide), first registered for use in the U.S. in 1994, is the most popular. Imidacloprid is the most heavily applied, in terms of volume, insecticide in the world (Ware 2000). Bayer CropScience holds over 80 brands of imidaclroprid-containing products labeled for use in over 140 different crops (Thielert 2006). Imidacloprid, like other neonicotinoid insecticides, acts upon the central nervous system of the insect, binding to acetylcholine receptors on the nerve cells, causing nerve impulses to fire constantly (Ware 2000). Mammalian acetylcholine receptors, in addition to being less sensitive than insect receptors to imidacloprid, are located in the muscles

rather than the nerves, which causes imidacloprid's low mammalian toxicity (Zwart et al. 1994, Ware 2000).

Imidacloprid can be absorbed through plant roots when applied as a soil drench or in granular form, or are injected into trunk or root flares. Imidacloprid is highly water-soluble and very mobile in the xylem, where it is transmitted to the shoots and leaves (Sur and Stork 2003). It then moves through the plant's water supply via xylem and the apoplast (Isaacs 1999, Sur and Stork 2003). When applied as a soil drench, roughly 5% of the active ingredient is taken up by the plant, varying between species (Sur and Stork 2003). This systemic mode of application eliminates the complications associated with drift of insecticides applied as a traditional spray. As they can remain in plant tissues for up to three years, neonicotinoids are often applied less frequently than foliar pesticides (Webb et al. 2003, Raupp et al. 2005). Less frequent treatments, combined with reduced direct contact with non-target arthropods, originally contributed to imidacloprid's designation as relatively benign to natural enemies (Mizell and Sconyers 1992; Kaakeh et al. 1996).

Imidacloprid is labeled for use against a broad range of key pest species, primarily sucking insects, but it is effective against some coleopteran, dipteran, and lepidopteran pests (Elbert et al. 1991). Many of these target insects, such as Asian longhorn beetle, emerald ash borer, and hemlock wooly adelgid, are invasive, exotic pests that threaten profitability of nurserymen or ecological sustainability of natural forest stands and urban forests. In some instances mortality is severe enough to alter the native range of plants and push them toward extinction altogether (Nowak et al. 2001; MacFarlane and Meyer 2005; Preisser et al. 2008). In some instances, systemic

insecticides are one of the most effective control methods for these invasive insect pests (Webb et al 2003; Herms 2003) and are likely to see continued, if not increased use.

Soon after its introduction in 1994, arborists and landscapers began to notice that mite abundance and injury became more prevalent on plants treated with imidacloprid. Sclar et al. (1998) first documented an outbreak of two phytophagous mite species on honeylocust trees and bedding marigolds. James et al. (2001) reported an increase in two-spotted spider mites, *Tetranychus urticae* (Koch), on hop plants that had been treated with imidacloprid. Furthermore, Canadian hemlocks, *Tsuga canadensis* (L.), treated with imidacloprid produced vastly elevated populations of spruce spider mite, *Oligonychus ununguis* (Jacobi), and hemlock rust mite, *Nalepella tsugifolia* Keifer, when compared with untreated control hemlocks (Raupp et al. 2004).

We propose two categories underlying secondary outbreaks of spider mites: bottom-up processes and top-down processes. Bottom-up processes are mechanisms mediated by resources increasing fitness of the mites and a top-down effect, and top-down processes are mechanisms mediated by altered natural enemy activity. Hormoligosis, a sublethal effect of a pesticide leading to increased fecundity in an organism, is one example of a bottom-up effect (Luckey 1968). James and Price (2002) demonstrated a hormoligotic effect with twospotted spider mites sprayed with imidacloprid and those reared on bean leaves treated systemically with imidacloprid. Fecundity and longevity was increased in the mites exposed to imidacloprid through either avenue. Another unexplored hypothesis that would contribute to a bottom-up

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increase in mite populations is a change in plant quality. This idea, proposed by Bayer Corporation, implicates imidacloprid as a plant growth regulator, thereby enhancing the quality of treated leaves as food for non-target herbivores (Thielert 2006). Thielert (2006) claimed that imidacloprid application leads to increased biomass accumulation, increased yield, increased stress tolerance, and increased disease tolerance. These claims are corroborated in part by Weston (2008), who found an increase in V*iburnum dentatum* L. height after application of imidacloprid. However, the study failed to control for greater levels of herbivory on the untreated plants. Elevated nitrogen levels are a strong factor inducing herbivorous pest outbreaks, as described previously, and Mote et al. (1995) found that seed treatments of imidacloprid resulted in cotton plants with higher nitrogen and chlorophyll content in the leaves. Either hormoligosis, plant quality or a combination of both factors could contribute to a bottom-up inflation of mite levels.

A second category of processes that could explain secondary outbreaks of mites on plants treated with imidacloprid is the loss or impairment of top-down regulation by natural enemies. Imidacloprid is toxic to a number of natural enemies, such as lady beetles, pirate bugs, big eyed bugs, and lacewing larvae when applied as a spray (Mizell and Sconyers 1992). Mullins (1993) conjectured that lethal effects of imidacloprid on natural enemies should be eliminated for seed, soil or trunk applications as the pesticide should not contact the organism. However, a growing body of literature suggests that omnivorous natural enemies can be exposed to toxic levels of systemic imidacloprid through plant residues, nectar, or sap feeding (Sclar et al. 1998, Smith and Krischik 1999, James and Vogele 2001, James and Coyle 2001,

Rebek and Sadof 2003). Smith and Krischik (1999) noted a reduction in survival, an increase in preovipositional time, and impaired coordination in *Coleomigilla maculate* (DeGeer), a facultative pollen feeding lady beetle following exposure to imidacloprid. Several studies have demonstrated the presence of imidacloprid in the nectar of treated flowering plants and its toxicity to parasitoids and green lacewing adults resulting in reduced survivorship and impaired foraging behavior (Stapel et al. 2000, Rebek and Sadof 2003, Krischik et al. 2007, Rongers et al. 2007). Kunkel et al. (2001) reported impaired locomotion, excessive grooming, and an inability to escape from ant predation in *Harpalus pennsylvanicus* (DeGeer), a commonly occurring carabid beetle, after several methods of exposure to imidacloprid, including consumption of contaminated food items. Poletti et al. (2007) documented a reduction in functional responses of two predatory mite species following exposure to imidacloprid. They showed a 55% and 87% reduction in spider mite eggs consumed by *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) if the eggs were sprayed with imidacloprid. All of these studies examined only direct exposure to imidacloprid: exposure to spray, spray residues, or plant products.

Only recently have scientists begun to understand that systemic insecticides may indirectly affect natural enemies through exposure of prey that have ingested pesticides. In the first study of this kind, lettuce seedlings were treated with a soil drench of imidacloprid, commonly used to control the lettuce aphid, *Nasnovia ribisnigri* (Mosley), and potato aphids, *Macrosiphum euphorbiae* (Thomas). Imidacloprid was applied and aphids allowed to feed for 48 hours. These intoxicated prey items were then presented to larvae of the Tasmanian Brown Lacewing,

Micromus tasminiae (Walker), over 8 days. The imidacloprid-exposed aphids killed

96% of exposed lacewing larvae, and the lettuce seedlings continued to produce toxic

aphids up to 40 days after treatment (Cole and Horne 2006). Walker et al. (2007)

later used lettuce aphids to explore the lethal and sublethal effects of varying doses of

imidicloprid on the Tasmanian brown lacewing larvae. They found that low doses of

systemic imidacloprid, when vectored through aphids, slowed the development rate of

the lacewing larvae. The majority of the aphids used in these studies were dead, but

the studies showed that predators fed on prey items that have been killed by ingesting

imidacloprid (Cole and Horne 2006, Walker et al. 2007). More recently, Papachristos

and Milonas (2008) demonstrated that doses of imidacloprid insufficient to affect pea

aphids, *Aphis fabae* Scopoli, lowered overall survivorship of the coccinellid beetle

Hippodamia undecimnotata en Grace when raised on aphids from treated plants.

Adult longevity and egg production was also adversely affected in beetles fed aphids

reared on treated bean plants. While aphid resistance to imidacloprid has not been

documented yet, the pesticide's wide usage has already given rise to resistance in

leafhoppers (Gorman et al. 2008, Matsumura et al. 2008).

    The aforementioned studies utilized aphids which are target pests for

imidacloprid. In a nontarget pest system, James (2003) reported that spider mites

reared on leaf discs sprayed with imidacloprid were toxic to a predatory mite,

Galendromus occidentalis Nesbitt.

    Herbivorous mites are not adversely affected by imidacloprid, and in many

cases, seem to benefit from its application (James and Price 2002). Previous studies

demonstrate that imidacloprid can be retained in tissues or the gut of aphids despite

the fact that imidacloprid has been shown to produce an antifeeding response in
aphids, and those that do feed on treated plants are killed after feeding (Nauen 1995).
A similar phenomenon may occur in mites, which appear to show no aversion to
imidacloprid and may well be aided by its application (Sclar et al. 1998, James et al.
2001, Raupp et al. 2004).

Objectives

The purpose of this research was to evaluate lethal and sublethal effects of
indirect exposure to imidacloprid on natural enemies of spider mites. The central
hypothesis is that if mites can store or sequester imidacloprid in their bodies, then
natural enemies feeding on these mites will be exposed to a dose of imidacloprid that
cause lethal or sublethal responses. The hypothesized avenue of exposure in our
study systems is the following: plants are treated systemically with imidacloprid,
mites feed on these treated plants, sequestering or storing imidacloprid, and a predator
is exposed to imidacloprid upon consuming the mite. I hypothesize that exposure to
imidacloprid intoxication will manifest itself as a reduction in feeding, impairment of
locomotion, and a shorter lifespan. Furthermore, I predict that plants treated with soil
drenches of imidacloprid will not cause detrimental effects to predators through
exposure to foliage alone. Predators, if not feeding on any plant products, will only
exhibit pesticide intoxication after eating prey reared on systemically treated plants.

The two study systems utilized in this series of experiments, American elms
and boxwoods have shown vastly elevated and damaging mite abundances following
the application of imidacloprid. Szczepaniec (2009) have documented that boxwood
spider mites, *Eurytetranychus buxi* (family: Tetranychidae), become six times more

abundant on plants treated with soil drenches of imidacloprid as compared to untreated plants. After the detection of the Asian Longhorned Beetle in New York City's Central Park in 2002, thousands of trees were treated with trunk injections of imidacloprid in 2002-2007 as part of an eradication program. Elms treated with imidacloprid hosted six to 200 times more spider mites, *Tetranychus schoenei* (family: Tetranychidae), than untreated trees (Raupp et al. 2008, Szczepaniec 2009). These mites are occasional pests of fruit trees and other woody ornamentals (Johnson and Lyons 1991), but reached levels high enough to defoliate the Central Park elms treated with imidacloprid prematurely (personal observation).

Methods

Boxwood system

Due to their evergreen habit and limited palatability to deer, boxwoods are one of the most popular plants in Maryland and cold hardy cultivars are planted increasingly frequently in the northern states (Raupp et al. 1985, Bachtell pers. comm. 2008). Intense pressure from the boxwood leafminer, *Monarthropalpus flavus*, and boxwood psyllids, *Cacopsylla buxi*, whose damaging stages are protected from conventional sprays by feeding inside leaves or cupped terminal foliage, makes systemic insecticides invaluable for control of these pests (d'Eustachio and Raupp 2001). Twenty 'Winter Gem' boxwoods (*Buxus microphylla* [Siebold & Zucc.] var. *koreana* [Nakai ex Rehder]) were obtained from Conard Pyle Co. nursery in Centreville, MD. The plants had a roughly spherical, 50cm high canopy. No systemic insecticides were applied to the plants before the onset of the study. Plants were in 3 gallon pots, and potting medium consisted of a soilless bark mixture enriched with a time release fertilizer. Plants were housed at the University of Maryland Research Greenhouses (College Park, MD) under drip irrigation and a temperature of $23° \pm 4°C$. Supplemental HID lighting was provided at 14:10h (L:D).

Eurytetranychus buxi (Garman) is a common pest of cultivated boxwoods, particularly those treated with imidacloprid (Johnson and Lyon, 1991). Mites were reared on boxwoods that had never been treated with insecticides before utilization in this study.   Clippings from these infested plants were then transferred to the study replicates 3 weeks prior to treatment. Positive identification of the mites was

provided by Ronald Ochoa at the Systemic Entomology Lab (BARC, USDA, Beltsville, MD).

Boxwoods infested with mites were randomly assigned to two treatments. Ten were treated with imidacloprid (Merit 75WP, Bayer Corporation, Kansas City, MO) at a rate of 0.225g AI per pot three months prior to bioassays. Ten plants served as untreated controls. Irrigation was halted two days preceding the application of imidacloprid. A 100 mL solution of Merit 75WP was applied to ten pots, and 500 mL of water was poured onto the pots 15 minutes later. Untreated controls received 600mL of water. Plants were returned to drip irrigation two days after the insecticide was administered. Mites were allowed to feed and reproduce under exposure to imidacloprid.

Elm system

While once among the most widely planted street trees in America, American elms, *Ulmus americana* (L.), were virtually eliminated in the urban landscape after the introduction of Dutch elm disease, *Ophiostoma novo-ulmi* (Townsend and Douglass 2004). The introduction of disease resistant cultivars has created a renewed interest in these stress-tolerant trees. Fourteen Two-year-old *Ulmus americana* 'Valley Forge' were grown under ambient greenhouse conditions (24±4°C) in five gallon containers and given drip irrigation. Plants were roughly 1m tall from the soil line, and the canopy was pruned to ensure uniformity. No artificial lighting or fertilizer was provided. Potting medium was a peat based soilless mixture. Whips were received from Bruce Carley (Acton, MA) and were grown in the greenhouse for one year before the beginning of the study.

Tetranychus schoenei (McGregor) is an occasional pest of fruit and ornamental trees (Johnson and Lyon 1991). Leaves infested with mites were collected from the field and placed on the trees in the greenhouse six weeks before the onset of the first experiment. Positive identification of the mites was provided by Ronald Ochoa at the Systemic Entomology Lab (BARC, USDA, Beltsville, MD).

The fourteen plants were randomly assigned to two treatments. Plants in the treated group were administered a labeled dose of imidacloprid two months before the onset of the experiments. Preceding insecticide treatment, water was withheld for two days. Formulated imidacloprid (Merit 75WP, Bayer Corporation, Kansas City, MO) was applied at 0.3g AI /pot dissolved in 100mL water to seven plants. 500mL water was provided 15 minutes after imidacloprid application. Control plants were given 600mL water. Plants were returned to drip irrigation two days after treatment. Mites were later placed onto the plants and allowed to feed and reproduce.

Predators

The two predators used in the experiments were selected on the grounds of that they or their congeners were observed on plants treated with imidacloprid, their importance in spider mite control as reported in the literature, and their ready availability from commercial retailers. *Stethorus punctillum* (Weise), and its congeners (Coccinellidae) are effective spider mite predators both as a larvae and adults (Congdon et al. 1993, Raworth 2001). Britto et al. (2007) showed that *S. tridens* adults can eat up to 30 spider mites in one day. From personal observations, we found *Stethorus* adults and larvae in association with large mite populations. Larvae of the green lacewing, *Chrysoperla rufilabris* (Burmeister) and its congeners

(Chrysopidae) are generalist predators of soft-bodied prey such as aphids, thrips, whiteflies and spider mites (Huffaker and Messenger 1976, Rongers et al. 2007). Field observations of *Chrysoperla spp.* were frequent on mite-infested elm trees, and a coniopterygid lacewing was commonly found in association with outbreaks of spider mites on boxwood (Szczepaniec 2009).   Predators were obtained from a commercial insectary (IPM Labs: Locke, NY). Upon receipt, the insects were transferred to a growth chamber set to 11°C with a photoperiod of 12:12h (L:D) and RH of 70-75%. *Stethorus punctillum* adults were stored in a plastic vial misted with water and supplied with a 4cm square of cloth soaked in sugar-water. *Chrysoperla rufilabris* larvae were stored in a plastic vial filled with rice hulls to provide refuge and reduce cannibalism. The vial was misted with water and supplied with previously frozen eggs of *Ephestia keuhniella* (Zeller). Only second instar larvae were used in this study. Any larvae that molted during the course of the assay were excluded from the results.

Prey consumption bioassay (boxwood)

        To determine the effect of indirect exposure to imidacloprid through prey on the feeding rate of predators, the following prey consumption assay was conducted in the spring of 2007. Mites were removed from each of twenty boxwood plants assigned to the two treatments described above and placed in an arena. The arena consisted of a 55 cm filter paper (Whatman International Ltd. Maidstone, England) placed on top of a Petri dish (5.9 x 0.9cm)   Mites were transferred individually with a paintbrush to avoid injury. Five adult female mites were placed on the filter paper and a smaller Petri dish (3.9 x 1.1cm) was affixed to the filter paper and larger dish

with a rubber band.   A single adult *S. punctillum* or larva *C. rufilabris* was introduced into each arena. The arenas were held under lighted ambient laboratory conditions (23±2°C) while the assays were underway. The number of mites eaten was recorded at intervals of 0.5, 1.5, 2.5 and 3.5 h after the predator was introduced. Two cohorts of five mites were assayed from each plant and the number of mites eaten was averaged.

Predator mobility bioassay (boxwood)

        To determine the affect of exposure of imidacloprid indirectly through prey on the mobility of predators, the following assay was conducted in the spring of 2007. An arena was constructed of a 118mL Solo Cup (Solo Cup Company, Urbana, IL). A water source was included which consisted of a trimmed micropipette tip stuffed with saturated cotton gauze. Each arena was supplied with 6-8 excised leaves from treated or untreated plants heavily infested with mites (all life stages) reared on the same plant. The arenas were held under lighted ambient laboratory conditions (23±2°C) while the assays were being conducted. A single adult of *S. punctilum* or larva of *C. rufilabris* was introduced into each arena and allowed to feed. To test its mobility, the predator was moved with a paintbrush from the arena to the center of a 4cm circle drawn on paper. A timer was actuated once the insect began moving and was stopped once the insect reached the edge of the circle or until 40s had elapsed. Unresponsive insects were assigned a time of 40 s. Observations were made at intervals of 0.5, 1.5, 2.5, and 3.5 h of exposure to contaminated prey. After the observation, the insect was placed back into its respective arena. As insects were observed to move in a linear fashion, mobility was calculated as time to leave the circle divided by 2cm,

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representing the radius of the circle. Three predators were assayed after exposure to mites from each of the twenty treated and untreated plant replicates. An additional assay was performed to ascertain any effects of insecticide residues on the leaf surface on the mobility of predators. Methods were identical to those described above, except that leaves were cleared of all mites and mite eggs. These assays were analyzed as a 2x2 factorial design with mites present or absent as one factor and treated or untreated foliage as another factor.

Prey consumption and predator mobility bioassay (elm)

To determine the effect of exposure to imidacloprid indirectly through prey on the feeding rate of predators, the following assay was performed in the summer of 2008.   Seven plants were assigned as replicates to each treatment. Leaf disks 22mm in diameter were taken from each tree using an apple corer (Progressive International: Kent, WA). The disks were cleaned of mites and placed lower side down in a Petri dish (3.9 x 1.1cm) filled with saturated cotton gauze (Personna Medical: Staunton, VA) (James and Price, 2002). Ten adult female mites were transferred from each plant and placed on the respective leaf disk. A single adult of *Stethorus punctillum* or a larva of *Chrysoperla rufilabris* was then introduced to the leaf disk. Conditions and treatment of the assay were the same as that of the boxwood assays, however, both consumption and mobility observations were made with the same predator. Predators that drowned during the course of the experiment were excluded from analysis. As insects were observed to move in a linear fashion, mobility was calculated as time to leave the circle divided by 2 cm, representing the radius of the circle. Three cohorts of ten mites were assayed from each plant. The number of mites eaten and mobility

rate was averaged by treatment. An additional assay was performed to ascertain any effects that pesticide residue present on the leaf surface might have on predator mobility. Methods are identical to those presented above, but the leaf surface was cleared of all mites and mite eggs.

Predator mortality bioassay (elm)

To determine the effect of imidacloprid on predator mortality, the following assay was performed. *S. punctillum* adults or the *C. rufilabris* larvae were placed in a 118mL Solo Cup (Solo Cup Company, Urbana, IL) and supplied with a trimmed micropipette tip filled with cotton saturated with a sugar-water solution (10mg sugar: 100mg water). Leaves infested with spider mites from treated trees described in the previous study or from untreated trees were provided at the beginning of the experiment and every two days after. The arenas were held under lighted ambient laboratory conditions (23±2°C) while the assay was conducted. Every 24 hours, predators were observed and considered dead if they were completely unresponsive to the touch of a probe and not making any movements. Predators found dead at the first observation were given a longevity of zero days, predators found dead at the second observation were given a longevity of one day, and so on. .

Statistical analyses

Prey consumption bioassays were analyzed with a repeated measures analysis using Statistix software (Statistics v. 5.1). Pairwise comparisons were made at each individual observation using Tukey's Honestly Significant Difference (HSD) test.

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Mobility bioassays were analyzed with a repeated measures analysis using SAS with a 2x2 factorial model, where treatment was one factor and mites present/absent was another factor (SAS v. 9.1). Pairwise comparisons at each individual observation were made using Tukey's HSD test.

Mortality bioassays were compared using a censored mortality analysis using R (R Development Core Team 2008). This analysis was chosen as data were censored at 10 days for *Stethorus* and 20 days for *Chrysoperla*. Because data observations ended before all predators had expired, a simple analysis of existing data would not be accurate for only the observed data. The curves were modeled using an exponential distribution for *Chrysoperla* and using an extreme distribution for *Stethorus*. Longevities of both predators were averaged between treatments and analyzed using a two sample T test with equal variances (Statistics v. 5.1).

Results

Prey consumption bioassay (boxwood)

Figure 1 illustrates that both the *C. rufilabris* larvae and *S. punctillum* adults ate markedly fewer *E. buxi* when mites were reared on boxwoods treated with imidacloprid than those reared on untreated plants. Repeated measures analysis revealed that treatment had a significant effect over the course of the experiment for *C. rufilabris* (Table 1). *C. rufilabris* consumed approximately twice as many mites from treated compared to untreated plants. For *S. punctillum*, the interaction of time and treatment was significant, so only simple means at each observation were analyzed. After 3.5 hours almost twice as many mites from untreated plants were consumed.

Lacewing larvae consumed mites faster than did the *S. punctillum*s in the untreated samples. At the first observation, the lacewing larvae had eaten 3.5 mites on average, as opposed to the 1.25 mites eaten by the *S. punctillum*. This trend continued at 1.5 hours, in which the *C. rufilabris* ate 4.25 mites compared to *S. punctillum*'s 3.10. By 3.5 hours, however the *S. punctillum*s ate more total mites (4.85) than the lacewing larvae (4.45)

Predator mobility bioassay (boxwood)

Predators exposed to mites on treated leaves showed significantly less mobility rate than either the predators exposed to mites reared on untreated foliage or predators exposed to treated or untreated leaves without mites (Figure 2). The interaction of treatment and the presence of mites was significant in *C. rufilabris*,

allowing us to infer that only contaminated mites had a significant impact on the performance of the predator (Table 1, Figure 2). Average mobility of 1.74 mm/s at 3.5 hours for predators exposed to imidacloprid through prey was less that half of that of predators that consumed untainted mites. Similarly, the interaction of treatment and the presence of mites was highly significant for *S. punctillum*, and the average mobility of 0.52 mm/s at 3.5 hours for lady beetles exposed to contaminated mites was only about 20% of that observed in lady beetles exposed to mites from untreated plants (Table 1, Figure 2). Predators on both treated and untreated leaves, when mites were removed, did not differ in mobility from the predators fed mites reared on untreated foliage (4.09 mm/s, 4.12 mm/s, and 3.99, respectively at 3.5 hours for *C. rufilabris;* 3.40 mm/s, 3.70 mm/s, and 3.70 mm/s, respectively at 2.5 hours for *S. punctillum*). *S. punctillum* showed a slightly general negative trend in mobility over time in the treatments without mites exposed to imidacloprid while *C. rufilabris* maintained a steady mobility over time.

Predators also showed signs of imidacloprid intoxication, which seemed more severe in *S. punctillum* than *C. rufilabris*. Qualitative signs of intoxication included tremors, regurgitation, lack of directional response or complete incapacitation.

Elm study system

Prey consumption and predator mobility bioassay (elm)

There was a significant time by treatment interaction with respect to the number of mites eaten by predators from treated and untreated elms by *T. schoeni* (Table 2, Figure 3). *T. schoeni* from elms treated with imidacloprid wee consumed far less by both predators, an effect significantly different by 0.5 hours (Table 2,

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Figure 3). The difference between consumption of mites from treated and untreated plants became larger as the experiment progressed. At the last observation, *C. rufilabris* and *S. punctillum* both ate 80% fewer mites when mites originated from trees treated with imidacloprid.

As with the boxwood system, lacewing larvae fed less than did lady beetles. By the first observation, *C. rufilabris* had eaten nearly 6 mites on average, and *S. punctillum* had eaten only 2.9 mites. This trend continued throughout all the observations, with *C. rufilabris* eating 8.9 mites by 3.5 hours compared to 6.7 eaten by *S. punctillum*.

There was a significant interaction between time of exposure and mobility of both predators (Table 2, Figure 4). Both predators became intoxicated when exposed to mites feeding on treated foliage, and their mobility was hampered by 0.5 hours (Figure 4). While the effect did not increase over time, differences were pronounced and significant at both beginning and end observations. Lacewing larvae moved 70% slower after eating mites from treated leaves, and coccinellids were 80% slower on average at the last observation (2.80 mm/s and 0.87 mm/s for *C. rufilabris* fed mites reared on untreated or treated plants, respectively and 2.44 mm/s and 0.57 mm/s for *S. punctillum*). The repeated measures analysis showed that the interaction of treatment and mites present/absent was highly significant, allowing us to ascertain that only mites on treated foliages elicited a significant impairment in the predators (Table 2).

Unlike the boxwood system, *S. punctillum* did not seem to have any obvious negative trend in the treatments without mites exposed to imidacloprid. However,

both predators had slightly lower mobility by the end of the experiment than at the beginning in the treatments without treated mites.

In addition to impaired mobility, predators exhibited clear signs of imidacloprid intoxication. These included complete lack or lack of directional response to external stimuli, tremors, regurgitation, excessive grooming of the antennae, and inability to right itself when placed on its back. In some instances, the predator became incapacitated while a mite was still impaled on its mandibles.

Mortality bioassay (elm)

Predators had significantly shorter longevity when fed mites from treated plants. In *C. rufilabris*, larvae lived 12.65 days on average, out of a potential 20 days when fed mites from untreated plants compared to 2.60 days when fed mites from treated plants (X^2=29.88, DF=1, p<0.0001). While the experiment ended at day 20, we continued to rear the few live lacewing larvae, and 4 larvae developed into pupae. The deleterious indirect effect of imidacloprid was similar for *S. punctillum*, in which adults that consumed mites from untreated elm plants lived 9.37 days, out of a potential 10. Beetles presented with mites on treated foliage lived only 1.00 day on average and differed significantly from the control (X^2=85.15, DF=1, p<0.0001).

Discussion

Comparison to previous studies

Several previous studies focused on direct effects of insecticides on natural enemies, but only a few examined effects of indirect exposure of predators through prey that were targets of pesticidal applications. This study is novel in that the route of exposure was through a nontarget pest, one that is not adversely affected by the systemic insecticide, imidacloprid. With regard to sublethal effects, indirect exposure to imidacloprid via spider mites caused significant reduction in the mobility of lady beetle adults and lacewing larvae. This in turn likely explains significant reductions in prey consumption observed in both predators following indirect exposure to imidacloprid through the prey. Furthermore, it appears that mites are the only avenue of imidacloprid reaching the predators as enclosures with treated foliage elicited no adverse reaction in either predator. Moreover, predators that consumed mites exposed to imidacloprid through treated plants, experienced dramatic reductions in longevity thus confirming the lethality of this product to lacewing larvae and lady beetle adults. These findings support our hypothesis that indirect exposure to imidacloprid disrupts the natural enemy community, which in turn promotes secondary outbreaks of phytophagous mites.

We have documented two sublethal effects and eventual lethal effects of indirect imidacloprid exposure, and several papers illustrated lethal and sublethal effects of imidacloprid on beneficial arthropods when predators or parasitoids are directly exposed to the pesticide through plant products or spray residues (Sclar et al. 1998, Smith and Krischik 1999, Stapel et al. 2000, James and Vogele 2001, James

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and Coyle 2001, Rebek and Sadof 2003, Torres and Ruberson 2004, Krischik et al. 2007, Rongers et al. 2007). Comparatively, there are few studies implicating deleterious effects of indirect pesticide exposure through prey. James (2003) suggested that mites raised on plants treated with systemic imidacloprid could be toxic to predatory mites. However, this supposition is not well supported by the literature, as several other studies found that exposure to foliar imidacloprid produced minor or no negative responses in predatory mites (Mizell and Sconyers 1992, Leicht 1993, James and Vogele 2001, Sangsoo et al. 2005). In fact, James (1997) earlier described a hormoligotic response to imidacloprid in another phytoseiid mite (James 1997). Poletti et al. (2007) documented a reduction in functional responses of predatory mites when presented spider mite eggs sprayed with imidacloprid. Poletti et al. (2007) showed that direct exposure to surface residues was a possible route of exposure to predators that consumed nontarget pests such as mites and their eggs.

Papachristos and Milonas (2008) examined the effects of low doses of systemic imidacloprid on the aphid predator, *Hippodamia undecimnotatta* when fed lettuce aphids, *Nasonovia ribisnigri* that were reared on treated lettuce seedlings. Low doses of imidacloprid applied to lettuce have no detectable effects on the performance of aphids reared on treated plants (Papachristos and Milonas 2008), However, reductions in larval survival, extended pre-ovipositional period, and reduced per capita fecundity was observed when aphids from treated plants were consumed by *H. undecimnotatta.* While depicting novel examples of indirect pesticide exposure, these three studies revolve around a target pest unlikely to

increase dramatically following the application of imidacloprid as aphids are highly

susceptible to imidacloprid (Elbert 1991).

Bottom-up versus top-down mechanisms

Phytophagous mites are not controlled by systemic applications of

imidacloprid. In fact, several species benefit from its application and attain much

higher densities on treated compared to untreated plants (Sclar et al. 1998, James et

al. 2001, Raupp et al. 2004, 2008). The mechanisms for bottom-up effects of

imidacloprid on pest mite populations remain unclear in most cases. One hypothesis,

termed hormoligosis by Luckey (1968), poses a sublethal dose of an insecticide as the

cause for increased fecundity of herbivores. James and Price (2002) reported

significant increases in the fecundity of *Tetranychus urticae* females sprayed with

imidacloprid. However, similar studies failed to support the hypothesis of

hormoligosis (Sclar et al. 1998, Ako 2004).

While bottom-up mechanisms of underlying mite outbreaks are not yet fully

understood, findings reported here strongly support the hypothesis that systemic

imidacloprid contributes to mite outbreaks by disrupting top-down control by natural

enemies. This is in concordance with Shrewsbury and Raupp (2006) who found that

influences affecting predator abundance were crucial in determining the severity of

the outbreak. Damaging outbreaks of spider mites are relatively uncommon on

untreated boxwoods and elms under field conditions (Creary, personal observation).

To produce colonies of pests useful for these experiments, we needed to move

nursery plants into greenhouses, suggesting that natural enemies were keeping the

mites in check while outside.

A large proportion of pesticide research concerns lethal effects of pesticides, but knowledge of the sublethal effects of insecticide on beneficial arthropods is crucial toward gaining a complete understanding of an insecticide's impact (Desneux et al. 2006). For example, Poletti et al. (2007) noted that the functional response was notably altered in two predatory mites, *Neoseiulus californicus* and *Phytoseiulus macropilis,* treated with imidacloprid. Smith and Krischik 1999 reported dramatic reductions in the mobility of lady beetles exposed to imidacloprid. This study demonstrated that imidacloprid remains in sufficiently high concentrations in prey to reduce mobility, reduce prey consumption, and kill predators exposed to imidaclorpid or its metabolites. Due to combined sublethal and lethal effects, predators may be consuming few mites on plants treated with imidacloprid, thereby allowing pest populations to continually increase.

Differential implications for specialist and generalist predators

Another novel aspect of this work is that it investigates responses of both a specialist predator, *Stethorus punctillum*, and a generalist predator, *Chrysoperla rufilabris* to potentially toxic prey. Although lacewings are noted as predators of spider mites, few studies illustrate the specific role of *C. rufilabris* in spider mite control. In addition to occurring commonly in the field, *S. punctillum* are also widely used in augmentative biological control for crops in protected culture (Congdon et al. 1993, Rott and Ponsonby 2000, Roy et al. 2005, Creary, personal observation). Under field conditions, *S. punctillum* are thought to seek areas of high mite densities in mid to late summer, when mite populations are highest and most damaging (Roy et al. 2005, Creary, personal observation). Congden et al. (1993) showed that *Stethorus*

served as capable biological control agents due to their effective searching and dispersal habits. As they are drawn to large mite populations, they would likely aggregate on plants treated with imidacloprid as these host higher densities of mites than untreated plants (Sclar et al. 1998, Raupp et al. 2004). This is corroborated by Shrewsbury and Raupp (2006) who suggest that specialist predators show a numerical response to increasing prey densities. We have shown that mites reared on treated plants are highly toxic to *S. punctillum*, thereby reducing its capacity for prey consumption, mobility, and survival. It seems probable that these mite specialists would be drawn to treated plants and would consequently become intoxicated by ingesting mites, thereby exacerbating rapidly rising populations of mites found there. Further studies need to be conducted to test this hypothesis.

Generalist predators are important natural enemies that mitigate pest outbreaks in managed and natural (Hanks and Denno 1993, Shrewsbury and Raupp 2006). Shrewsbury and Raupp (2000) found that population densities of the azalea lace bug, *Stephanitis pyrioides* (Scott) decreased as shade and structural complexity increased. A likely reason for this decrease in abundance was an increase in the abundance of generalist predators. Raupp et al. (2001) found that overall diversity of arthropods increase as plant diversity increases. A later study by Shrewbury and Raupp (2006) conclusively documented an increase in generalist predators in structurally diverse landscapes (Shrewsbury and Raupp 2006). A number of other studies also show increased predator abundance in diverse habitats (see reviews by Russell 1989, Gurr et al. 2000, Langellotto and Denno 2004). Shrewsbury and Raupp (2006) also suggested that generalist predator abundance increases as abundance of

alternate prey items increases. The implications of these findings upon this study system are twofold.

First, generalist predators, like *C. rufilabris*, may suppress mite populations on untreated plants. Mite populations on untreated plants rarely, if ever, reach the high damaging levels that populations on plants treated with imidacloprid do (Sclar et al. 1998, Raupp et al. 2004, Creary personal observation). Generalist predators could provide an acceptable degree of mite control on untreated plants as alternate food sources not killed by imidacloprid, such as aphids, soft scales, and leafhoppers, are abundant (Creary, personal observation).

Second, we would expect to find fewer generalist natural enemies on plants treated with imidacloprid for the following reasons. The abundance of alternate prey items would be low on treated plants, as imidacloprid is toxic to many commonly occurring herbivores (Mullins 1993). Additionally, imidacloprid contained in pollen, sap and nectar of treated plants may be toxic to omnivorous natural enemies, thereby reducing their incidence (Sclar et al. 1998, Smith and Krischik 1999, Stapel et al. 2000, James and Vogele 2001, James and Coyle 2001, Rebek and Sadof 2003, Torres and Ruberson 2004, Krischik et al. 2007, Rongers et al. 2007). Due to the cost of treatment with imidacloprid, especially for large trees, typically only specimen plantings or plants with severe pest infestations are treated (Creary, personal observation). As specimen plantings are often isolated, and large key pest populations indicate lack of natural enemies, one would suspect that generalist predators would be rarer in plantings treated with imidacloprid. However, we have shown that indirect exposure to imidacloprid is highly toxic to *C. rufilabris*, so the

generalist predators drawn to plants treated with imidacloprid are likely to become intoxicated shortly after their arrival. In order to conclusively assess the effects of imidacloprid on generalist predators, one would need to test immigration of these predators to both areas of high prey density and plants treated with imidacloprid. If they are drawn to high mite densities and cannot discriminate between treated and untreated plants, then treated plants may act as sinks for these natural enemies as well. Thus, application of systemic imidacloprid is likely to disrupt both specialist and generalist predators of spider mites, contributing to damaging outbreaks, but specialist predators are more prone to severe repercussions as they are drawn to high densities of spider mites.

Comparison of study systems

Another interesting finding from this study is that *E. buxi* seems to elicit a weaker response in predators than *T. schoenei* when the mites were reared on their respective host plants treated with imidacloprid. Both predators were able to eat more mites from treated boxwoods than from treated elms (Figures 1 and 3). Similarly, mobility was not reduced to as great an extent when predators were fed mites from treated boxwood as opposed to treated elms. Elms treated with imidacloprid evoked a severe, immediate response in both predators. *S. punctillum* adults and *C. rufilabris* larvae ate on average one mite from a treated elm and did not eat any more throughout the course of the experiment. Ingestion of just one mite was sufficient to completely intoxicate the insect for the next three hours. This differential response is contrary to our expectations. Boxwood mites are considerably larger than elm mites, so we expected the boxwood mites to contain a larger dose of imidacloprid.

Differences in physiology between the two plants could account for the mites being more or less toxic when reared on either plant. Elms are considerably more succulent and have greater water content in their leaves than do boxwoods by virtue of being deciduous (Larcher 2004). As imidacloprid is soluble and transmitted in water, this would result in a greater concentration of the insecticide in areas of greater water content. Sur and Stork (2003) noted that different plant species absorb different percentages of imidacloprid from soil drenches, which could also explain the difference in mite toxicity. Additionally, boxwoods were grown in a bark-based medium, and elms were grown in a peat-based soil-less mixture, which retains more water and perhaps more imidacloprid.

Biological control and imidacloprid

This study has numerous ramifications for biological control of mites on plants in protected culture with augmentative releases of predators. When using mites from untreated plants, we found that *S. punctillum* was able to find and completely eradicate small mite populations, such as those within the arenas used in the boxwood prey consumption trials. While they did not eat proportionally as many mites in the elm system over the study period, the increase in mites eaten was constant until the end. This is in concordance with Congden et al. (1993) who found that *S. punctillum* were able to find and control small and medium mite infestations due to their effective dispersal and searching habits as specialist mite predators. While *C. rufilabris* ate a smaller proportion of the mites provided in the boxwood system, these insects had a higher feeding rate. Similarly, in the elm system, lacewing larvae reached an upper limit fairly early in the experiment. Pappas et al.

(2007) showed that spider mites were not an ideal prey type for a closely related lacewing species, *Dichochrysa prasina* Burmeister, as development time and adult weight were adversely affected when fed mites in comparison to several aphid species and eggs of the moth, *Ephestia kuehniella*. For augmentative releases of natural enemies, development and pupation of the biocontrol agent is secondary to its efficacy at controlling the target pest. *Stethorus* continued to prove an effective biological control agent for spider mite infestations. We found that *C. rufilabris* was able to eat a large number of mites in a short period of time and could be used for control of high density populations of spider mites.

Mizell and Sconyers (1993) suggested that systemic applications of imidacloprid will not contact predators, and concluded that toxicity to natural enemies should be negligible. These attributes enhance imidacloprid's compatibility with IPM programs. However, the growing body of literature illustrating the deleterious effects of imidacloprid on beneficial insects, especially honey bees has led France and Germany to ban seed treatments of sunflowers with imidacloprid (Suchail et al. 2000, Halm et al. 2006, Karnatak et al. 2006). A similar body of evidence showing that systemic applications of imidacloprid do in fact intoxicate natural enemies through ingestion rather than contact (Sclar et al. 1998, Smith and Krischik 1999, Stapel et al. 2000, James and Vogele 2001, Rebek and Sadof 2003, Torres and Ruberson 2004, Krischik et al. 2007, Rongers et al. 2007). Meanwhile, this study joins Cole and Horne (2006), Walker et al. (2007) and Pappachristos and Milonas (2008) in proving that in addition to direct or residue contact, and ingestion of plant products, natural enemies are negatively impacted by feeding on prey exposed to imidacloprid.

Other studies have shown that imidacloprid causes secondary outbreaks of spider mites (Sclar et al. 1998, Raupp et al. 2004). This study supports the hypothesis that disruptions in the natural enemy community contributes to these secondary pest outbreaks. Predators fed mites from plants treated with imidacloprid exhibited a stark impairment in locomotion, feeding ability, and longevity. If natural enemies are attracted to high densities of spider mites, such as those found on treated plants, it seems likely that those plants would act as a trap for beneficial insects in the urban landscape. This reduction in overall natural enemy abundance could then spur further outbreaks in the landscape, even on untreated plants. As the mite outbreaks caused by imidacloprid often cause aesthetic damage to the plants, landscape owners may turn to miticide applications, causing even further disruption of natural enemy communities. However, systemic imidacloprid is invaluable to manage many key pests, such as hemlock wooly adelgid and emerald ash borer—pests that will ultimately kill the host plant. This study does not contradict all the benefits of using imidacloprid. However, the costs to the natural enemy community need to be taken into account before any application of imidacloprid as it is clear that top-down regulation provided by natural enemies is severely curtailed by indirect exposure to imidacloprid through contaminated prey.
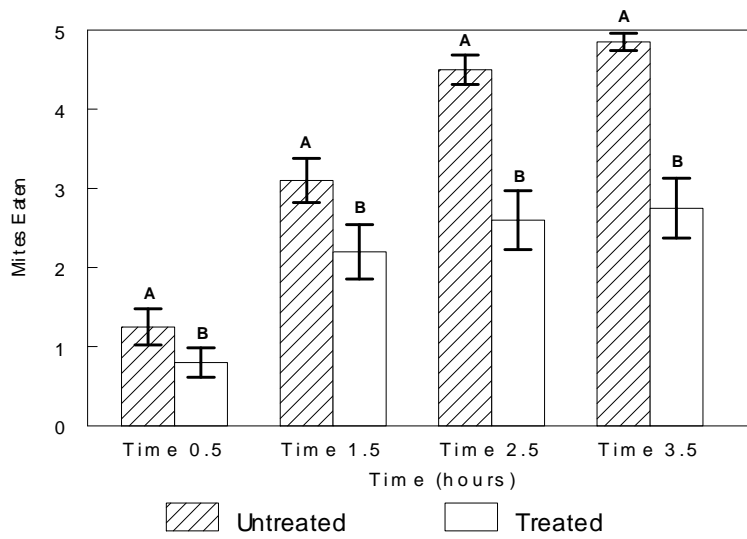
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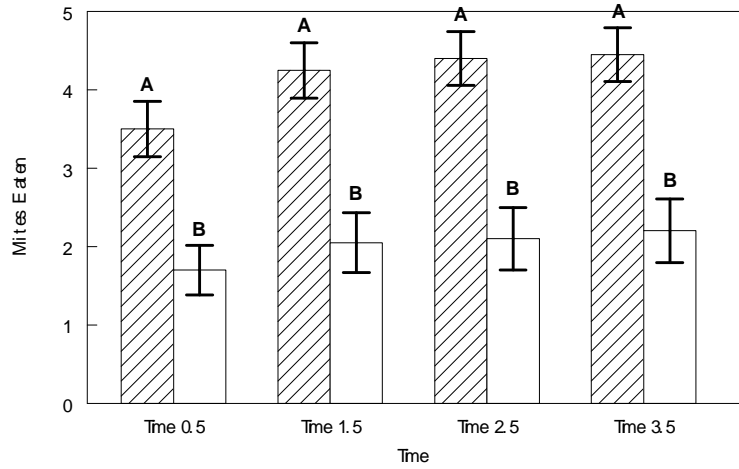
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Figure 1. Mean number of *Eurytetranychus buxi* females from untreated boxwoods and boxwoods treated with systemic imidacloprid consumed by (A) *Chrysoperla rufilabris* larvae and (B) *Stethorus punctillum* adults at four observations over time. Bars represent means and vertical lines represent respective standard errors. Means that share a letter did not differ by a Tukey HSD pairwise comparisons made at each observation with experimentwise α=0.05

A
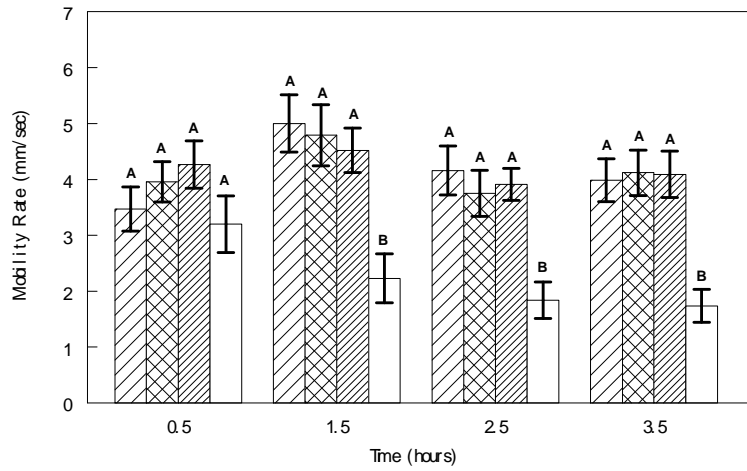
B

Figure 2. Mean mobility of (A) *Chrysoperla rufilabris* larvae and (B) *Stethorus punctillum* adults after exposure to four treatments: Untreated boxwood foliage with and without *Eurytetranychus buxi* and boxwood foliage treated with systemic imidacloprid with and without *E. buxi* at three observations over time. Bars represent means and vertical lines represent standard errors. Means that share a letter did not differ by a Tukey HSD pairwise comparisons made at each observation at experiment wise α=0.05

A

B
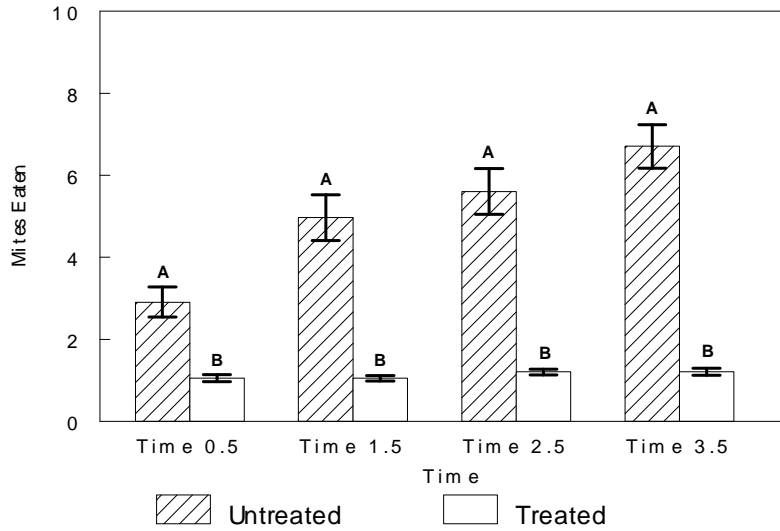
Figure 3. Mean number of *Tetranychus shoenei* adult females eaten from untreated American elms and elms treated with systemic imidacloprid consumed by (A) *Chrysoperla rufilabris* larvae and (B) *Stethorus punctillum* adults at four observations over time. Bars represent means and vertical lines represent respective standard errors. Means that share a letter did not differ by a Tukey HSD pairwise comparisons made at each observation with experimentwise $\alpha=0.05$
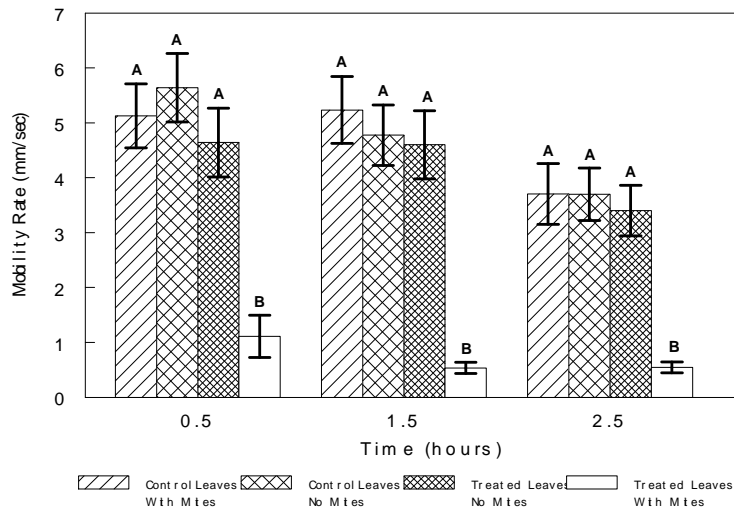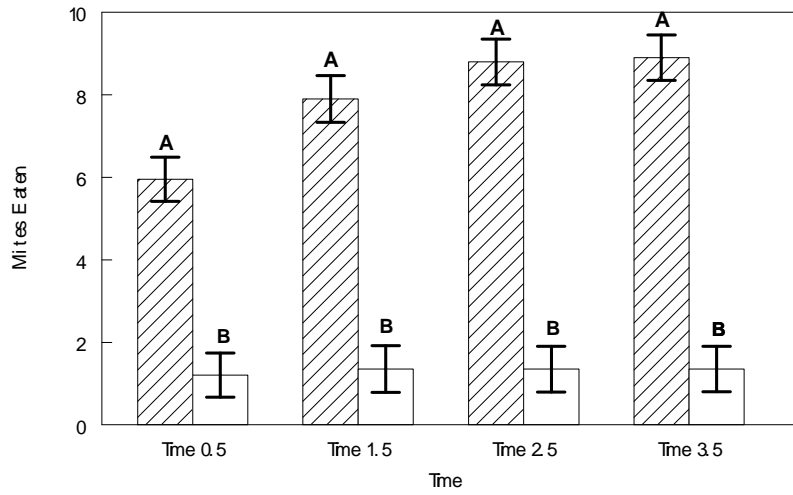
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A

B

Figure 4. Mean mobility of (A) *Chrysoperla rufilabris* larvae and (B) *Stethorus punctillum* adults after exposure to various treatments: Untreated elm foliage with and without T*etranychus schoenei* and elm foliage treated with systemic imidacloprid with and without *T. schoenei* at four observations over time. Bars represent means and vertical lines represent standard errors. Means that share a letter did not differ by a Tukey HSD pairwise comparisons made at each observation at experimentwise α=0.05
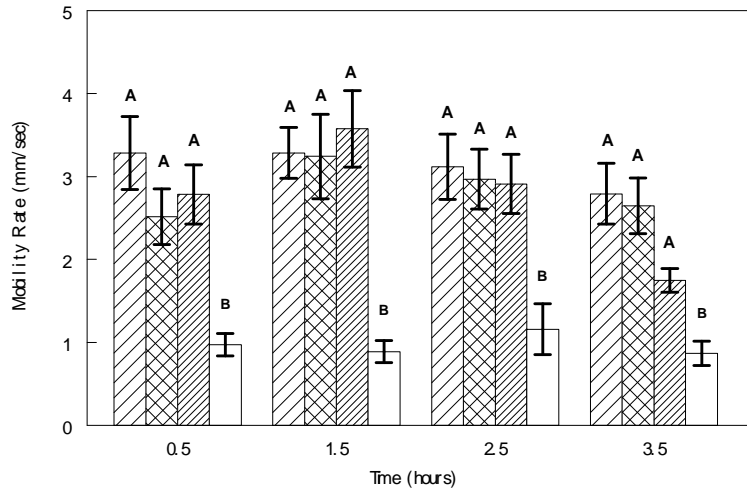
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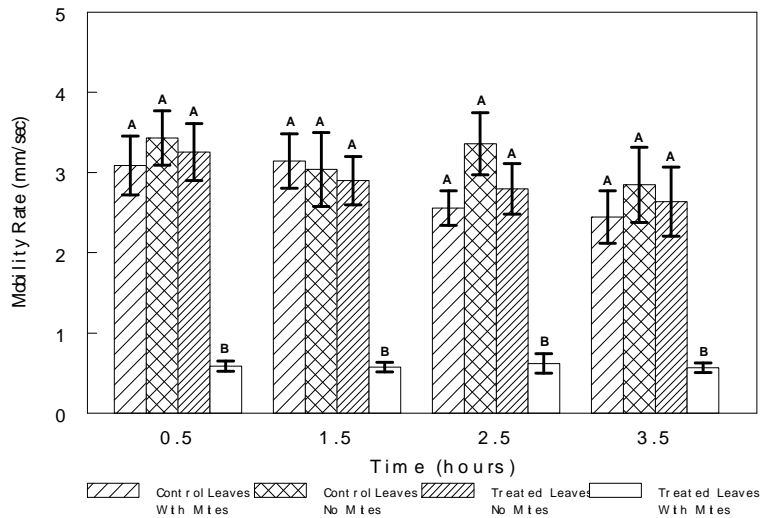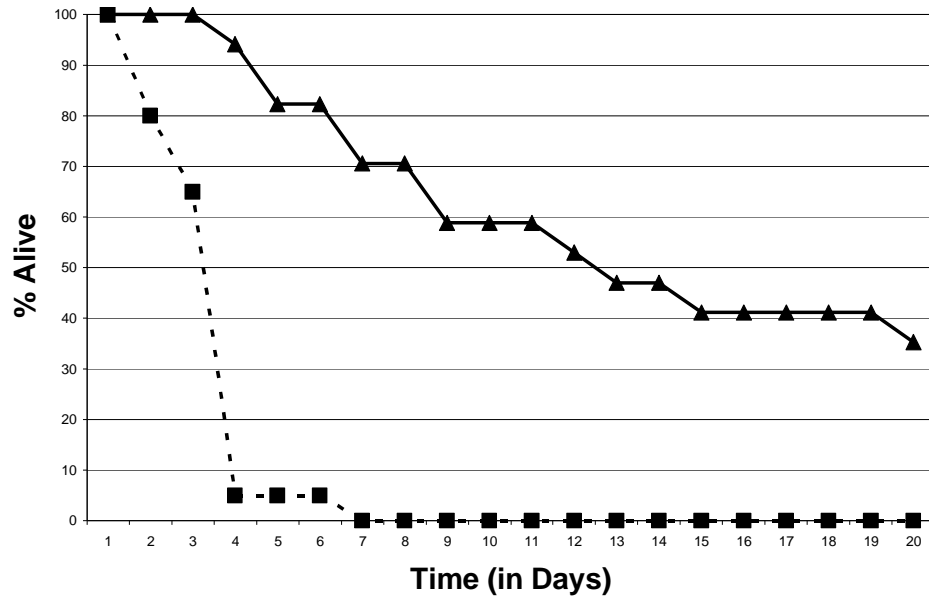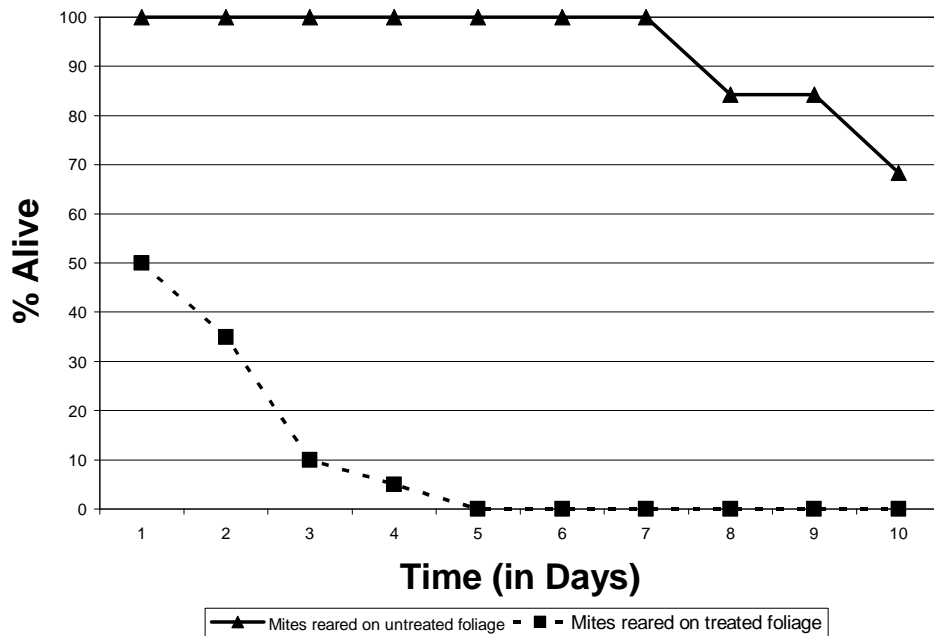Figure 5. Percentage of A) *Chrysoperla rufilabris* larvae and (B) *Stethorus punctillum* adults alive at the time observed after being continuously provided with mites (*Tetranychus schoenii*) on elm foliage treated with imidacloprid and mites on untreated foliage.

Table 1. Repeated measures ANOVA for *Chrysoperla rufilabris* and *Stethorus punctillum* prey consumption and mobility bioassays in the boxwood system

		Prey Consumption				
		C. rufilabris			*S. punctillum*	
Effect	**df**	*F*	**P**	**df**	*F*	**P**
Treatment	1, 18	17.61	0.0005	1, 18	34.52	0.0000
Time	3, 18	14.29	0.0000	3,18	91.42	0.0000
Treatment*time	3, 54	1.73	00.1721	3, 54	9.07	0.0001

		Mobility				
		C. rufilabris			*S. punctillum*	
Effect	**df**	*F*	**P**	**df**	*F*	**P**
Treatment	1,36	6.61	0.0144	1,36	27.34	0.0000
Mites	1,36	8.51	0.0060	1,36	15.01	0.0004
Treatment*Mites	1,36	7.32	0.0104	1,36	15.56	0.0004
Time	3,108	4.01	0.0094	3,72	11.84	0.0000
Treatment*Time	3,108	4.26	0.0069	3,72	1.44	0.2429
Mite*Time	3,108	0.34	0.7960	3,72	0.55	0.5776
Treatment*Mite*Time	3,108	1.41	0.2440	3,72	1.31	0.2772

Table 2. Repeated measures ANOVA for *Chrysoperla rufilabris* and *Stethorus punctillum* prey consumption and mobility bioassays in the elm system

		Prey Consumption				
		C. rufilabris			*S. punctillum*	
Effect	*df*	*F*	*p*	*df*	*F*	*p*
Treatment	1, 12	52.29	0.0000	1, 12	37.5	0.0001
Time	3, 12	31.49	0.0000	3, 12	41.01	0.0000
Treatment*time	3, 36	25.64	0.0000	3, 36	34.07	0.0000

		Mobility				
		C. rufilabris			*S. punctillum*	
Effect	**df**	**F**	**p**	**df**	**F**	**p**
Treatment	1,24	19.76	0.0002	1,24	21.50	0.0001
Mites	1,24	9.62	0.0049	1,24	27.84	0.0000
Treatment*Mites	1,24	16.75	0.0004	1,24	13.21	0.0013
Time	3,72	4.00	0.0108	3,72	2.42	0.0733
Treatment*Time	3,72	0.40	0.7500	3,72	0.46	0.7079
Mite*Time	3,72	1.76	0.1634	3,72	0.38	0.7658
Treatment*Mite*Time	3,72	1.57	0.2039	3,72	0.83	0.4835

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