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# Effects Of Selected Pesticides On Calico Scale And Its Natural Enemies

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# EFFECTS OF SELECTED PESTICIDES ON CALICO SCALE AND ITS NATURAL ENEMIES

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# ABSTRACT

Quesada, Carlos R. M.S., Purdue University, December 2013. Effects of Selected Pesticides on Calico Scale and its Natural Enemies. Major Professor: Clifford S. Sadof, College of Agriculture

Calico scale (*Eulecanium cerasorum*) is an exotic pest of shade and ornamental trees. It feeds on phloem sap, reduces tree vigor and can ultimately kill trees. We observed effects of four foliar applied (bifenthrin, pyriproxyfen, chlorantraniliprole and cyantraniliprole) and two soil applied pesticides (dinotefuran, imidacloprid) on calico scales and their natural enemies on infested thornless honeylocust trees (*Gleditsia triacanthos inermis*) over three years. Bifenthrin, dinotefuran and cyantraniliprole provided the largest reductions in scale populations when they were applied to egg laying females on branches early in the season. In contrast, bifenthrin and pyriproxyfen provided the most consistent reductions in scales when they were applied to settled scales on leaves later in the season. None of the insecticides reduced natural enemy abundance when they were applied to egg laying females. In contrast, bifenthrin and pyriproxyfen reduced natural enemy abundance when they were applied to settled scales on leaves. Laboratory assays indicated that foliar applications of bifenthrin were highly toxic to larval *Chrysoperla rufilabris* (Chrysopidae: Neuroptera), 4 DAT but not 50 DAT. In contrast, toxicity of pyriproxyfen and chlorantraniliprole were moderately toxic to *C. rufilabris* at 4 DAT and 50 DAT. This suggests physiological selectivity of a pesticide

and the timing of its application can influence its activity against scales and their natural enemies.

# CHAPTER 1. INTRODUCTION

## 1.1 Introduction

Selective and broad spectrum insecticides are commonly used to manage insects. However, the use of broad-spectrum products can create problems with secondary pests by killing their natural enemies. Selective pesticides tend to have fewer problems with secondary pest outbreaks. This research will determine the capacity of labeled pesticides to increase mortality of calico scale *Eulecanium cerasorum* (Cockerell) (Homoptera: Coccideae) on honeylocust trees, and their effects on calico scale natural enemies. Calico scale is one of the most destructive invasive pests of shade and ornamental trees. It was introduced to California from China, Japan and Korea in the 1920s (Miller et al. 2002) and has since moved into the eastern and north central US, where it is causing substantial damage to urban trees (Gill 1988).

Calico scales feed on the phloem sap and produce copious amounts of honeydew and black sooty mold on heavily infested trees. This reduces photosynthesis, the rate of carbohydrate assimilation, and can reduce tree vigor and kill branches (Hubbard and Potter 2005; Rebek and Sadof 2003). Calico scale is difficult to manage with insecticides. Hubbard and Potter (2005) could only achieve a 66% reduction when spraying pyrethroids on the whole canopy of the trees. Applications of the systemic insecticide imidacloprid to the soil also failed to provide control.

In the proposed research, I will measure the sources of mortality of calico scale to determine how each insecticide reduces scale abundance. When a female scale dies after it lays all its eggs, its death will be considered a result of natural causes. Other sources of mortality such as death from pesticide application or from natural enemies will be recorded separately. Mortality caused by parasitoids (*Prospaltella* sp. and *Physcus* sp.) and predators (*Chysoperla* sp.) will also be recorded separately. My goal is to identify products that can be used in management strategies that kill calico scale while conserving their natural enemies.

## 1.2 Biology of Calico Scale and its Relatives

There are more than 7,300 species of scale insects and their relatives in the order Hemiptera. They are divided among mealybugs (Pseudococcidae), soft scales (Coccidae), pit scales (Asterolecaniidae), giant scales (Margarodidae), felt scales (Eriococcidae), kermes scales (Kermesidae) and armored scales (Diaspididae) (Miller and Davidson 2005).

Soft and kermes scales feed on the phloem sap of the plant and produce honeydew while armored scales and pit scales feed on the contents of plant cells. They do not produce honeydew (Krischik and Davidson 2004; Rebek and Sadof 2003). In the Midwest the most common and important scale pests are soft and armored scales. The most problematic armored scales in the Midwest are *Unaspis euonymi* (euonymus scale), *Lepidosaphes ulmi* (Linnaeus) (oystershell scale), *Chionaspis pinifoliae* (pine needle scale), and *Quadraspidiotus perniciosus* (San Jose scale). The most common soft scale pests in the Midwest are *Eulecanium cerasorum* (calico scale), *Pulvinaria innumerabilis* (cottony maple scale), European fruit lecanium, *Parthenolecanium corni* (pine tortoise scale), *Magnolia grandiflora* (magnolia scale) and *Toumeyella liriodendri* (tulip tree scale) (Krischik and Davidson 2004).

Armored and soft scales generally have only one to two generations per year and three different instars that are called crawler, settler and adult respectively (Krischik and Davidson 2004; Sadof and Sclar 2000). After eggs hatch, they crawl to a place where they will settle. They often use the same host plant as their parents. Some disperse using wind or can move phoretically on insects such as *Musca domestica*, *Cryptolaemus montrouzieri* and *Linepithema humile* or on birds (Magsig-Castillo *et al*. 2010). In temperate areas, armored scales crawl after they hatch to the trunk or twig, settle, become flat and grow a clear wax shell. Females do not move again. In contrast, soft scales crawl to the leaves after they hatch from overwintering females on twigs. They then settle on the leaves for most of the summer and then crawl back to the trunk and twigs before leaves fall in autumn. Once they are on twigs, females are sessile (Hubbard and Potter 2005). Both armored and soft scale females release sex pheromones to attract the searching males (Kosztarab and Kozar 1988). Both soft and armored scales have adult winged males, who lack mouthparts and live just long enough to find female mates (hours to days) (Kosztarab and Kozar 1988; Miller and Davidson 2005).

Calico scale (*Eulecanium cerasorum*) was accidentally introduced on nursery stock from China, Japan and Korea into California in the 1920s, and has become a hugely destructive pest on shade and ornamental trees (Miller et al 2002). Females can be identified by black and white markings on their bodies. Female adults are 6-8 mm in diameter. Each female produces between 3,700 to 4,700 eggs, depending on the host plant (Hubbard and Potter 2005). Eggs hatch in the middle of May. First instars crawl to the leaves where they settle and feed on leaf tissue. In the middle of July, calico scales molt to the second instar and remain on the leaves until the middle of September, when they move to the trunk and twigs to overwinter. Females do not move again. From March to the middle of April they develop into adults, and males fly to mate with wingless females. Calico scale has been found on honeylocust, maples, buckeye, hackberry, dogwood, walnuts, sweetgum, flowering crabapple, pears, elm and all stone fruit and their ornamental cultivars (Hubbard and Potter 2005).

### 1.3 Managing Scale Insects

Biological control is critical to the management of most scale insects. In rural forests, the biodiversity of predators and parasitoids is supported by the availability of alternative prey and other food resources, resulting in more predators and parasitoids exerting more effective biological control of scale (Hanks and Denno 1993). In contrast, in landscaped or urban forests, natural enemies are affected by dust, extreme temperatures, and pesticide residues from treatment to control human problems such as mosquitoes and other phytophagous insects (Hanks and Sadof 1990, Luck and Dahlsten 1975, Meineke et al. 2013, Raupp et al. 2001, 2010). Even though calico scale is an exotic pest, Hubbard and Potter (2005) reported fourteen species of parasitoids representing 11 genera reared from calico scale adults and nymphs (Table 1.1). This includes *Encarsia* sp., reared only from calico scale nymphs; *Coccophagus* sp, *Blastothrix americana, Metaphicus* sp reared from calico scale nymphs and adults; and *Cheiloneurus albicornis* reared from calico scale adults, where the parasitoids were present for a longer time. The rate of parasitism was 38% on settled second instars.

Additionally, predators attack calico scale (Table 1.1). Vanek and Potter (2010) observed that lacewings (*Chrysopa rufilabris*), are the most significant predator. Lady beetles (*Harmonia axyridis*) prey on calico scale as well. However, lady beetles only consume calico scales moving in the crawler stage, which lasts approximately 19 days (Hubbard and Potter 2005), so they do not contribute significantly to mortality. When ants were excluded, Vanek and Potter (2010) found predation rates of calico scale to be reduced by 68%, whereas excluding ants from the native magnolia scale reduced populations by 82%. This finding is consistent with other studies of scale and ant mutualism (Hanks and Sadof 1990, Kondo and Gullan 2004).

### 1.4 Insecticides Used to Manage Armored and Soft Scales

Scale outbreaks occur when trees are under the stresses of drought, pollution, or nutritional imbalance. When this happens, scales can reduce tree vigor and kill branches, but rarely kill trees. Generally, the management of trees heavily infested with scales is accomplished by the application of oils, soaps, or synthetic organic insecticides.

*Oils* are used to reduce population levels of branch-infesting scales, mites, plant bugs, psyllids, and certain moths in dormant season. Oil is used during the winter to prevent injury to leaves; however, dormant oils do not work on scales that overwinter in the egg stage because it only kills the upper layers of eggs. Nevertheless, it is important to know the life cycle of the scale when using this product. Oil kills scale insects by penetrating their waxy cover and smothering them (Sadof and Sclar 2000). Baxendale and Johnson (1990) reported that the oil product Sunspray 6E Plus was highly effective against cottony maple scale (*Pulvinaria innumerabilis*), golden oak scale (*Asterolecanium variolosum*), and calico scale. Also, Sadof and Sclar (2000) obtained positive results using oil to control euonymus scale (*Unaspi euonymi*) on Japanese pachysandra (*Pachysandra terminalis*). In the April census, they found 65.6% fewer live scales than the untreated control on plots treated in March with dormant oil. In the July census, there were 99.5% fewer scales than the untreated control when the second generation scale crawler was treated with summer oil. However, Hubbard and Potter (2006) only suppressed calico scale on hackberry by 33% when they used whole-tree oil sprays on 6 and 12 March.

*Insecticidal soaps* are long-chain fatty acids. They are usually sprayed on the leaves and bark of infested plants to control the crawler stage. Insecticidal soaps kill scales only when they are wet, which allows predators and parasites to re-colonize the sprayed part of plants after the soap dries. Soaps kill insect by obstructing spiracles, causing asphyxia (Tremblay et al 2008, Cating et al 2010). It is important to know the host of the pest or insect targeted because some plants can be injured by the application of soap (Miller and Davidson 2005)

*Synthetic organic insecticides* can be classified in different ways. One of them is by the mode of action. Depending on the mode of action synthetic organic insecticides may inhibit, antagonize, mimic, disrupt, block, or receive some substances such as antigens, hormones or neurotransmitters affecting the nervous system (primarily) and digestive system (Silcox and Vittum 2008). Another way to classify insecticides is by chemical classes. Insecticide classes are organochlorines, organophosphates, carbamates, pyrethoids, neonicotinoids, ryanoidinoids, insect growth regulators, biological, and anti-feedants. Also they can be classified by systemic or contact insecticide. Systemic insecticide kills insects when they feed on plants where the toxin has been incorporated into the tissue. Contact insecticide kills insects on direct contact.

Hubbard and Potter (2006) concluded that acephate, bifenthrin, carbaryl, cyfluthrin and pyriproxyfen kill young, settled calico scale crawlers on leaves of individually sprayed branches of hackberry trees. Also, in separate trials they compared trunk-injection of dicrotophos and imidacloprid with whole canopy sprays with bifentrhin on sugar maple, sweetgum, and Zelkova on different dates. They achieved significant mortality with bifenthrin and dicrotophos compared with the control; however, their percent mortality was only 65.6 and 55.8 percent. Dicrotophos applied on sugar maple (28 June) and sweetgum (2 July) only produced 42 and 39% mortality, respectively. Imidacloprid applied on sweetgum (23 May and 31 July) and on Zelkova (on 20 May) only produced 22, 9.4, and 17% mortality respectively.

### 1.5 Interactions Among Insecticides and Natural Enemies

When insecticides are applied to manage calico scale, predators and parasitoids may be affected in different ways. Direct effects result when the insecticide comes in contact with the natural enemy and/or when natural enemies consume the insecticide. Indirect effects can occur when a predator preys on a victim who has consumed the insecticide or when the host is killed by the insecticide before parasitoids complete their development. Pesticides can kill predators by eliminating their food supply (Cloyd 2011). Rebek and Sadof (2003) suggested that the effect of insecticides on natural enemies should be assessed as part of integrated pest management programs for armored scales.

They showed that several insecticides reduced parasitism up to 100% when they were applied to manage euonymus scales.

## 1.6 Objectives

1. To determine how to manage calico scale with a minimal effect on natural enemies.

    a. Determine the percentage of induced mortality caused by insecticide, predation, and parasitism on female adults, crawler and settled stages.

    b. Determine the effect of the insecticide on calico scale's natural enemies.

2. Assess the impact of application timing to target ovipositing females (May) and settled first instar scales on leaves (June) to manage calico scale.

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Table 1.1 Predators and parasitoids associated with calico scale (after Hubbard and Potter 2005)

| **Order** | **Family** | **Genera** | **Species** | **Stage attack** | **Mortality (%)** |
|---|---|---|---|---|---|
| Hymenoptera | Aphelinidae | *Encarsia* | sp | Nymphs | Unknown |
| | Aphelinidae | *Coccophagus* | sp | Nymphs and adults | Unknown |
| | Encyrtidae | *Blastothrix* | *americana* | Nymphs and adults | Unknown |
| | Encyrtidae | *Metaphicus* | sp | Nymphs and adults | Unknown |
| | Encyrtidae | *Cheiloneurus* | *albicornis* | Adults | Unknown |
| Coleoptera | Coccinellidae | *Harmonia* | *axyridis* | Active crawler | 40 |
| | | | | Settled scale | 0 |
| Neuroptera | Chrysopidae | *Chrysoperla* | *rufilabris* | Settled scale | 70 |
| Araneae | | | | | 1 |
| Coleoptera | Coccinellidae | *Chilocorus* | *stigma* | Settled scale | 0 |

# CHAPTER 2. EFFECTS OF SELECTED PESTICIDES ON CALICO SCALE AND ITS NATURAL ENEMIES

## 2.1 Abstract

Calico scale (*Eulecanium cerasorum*) is an exotic pest of shade and ornamental trees. It feeds on phloem sap, reduces tree vigor and can ultimately kill trees. We observed effects of four foliar applied (bifenthrin, pyriproxyfen, chlorantraniliprole and cyantraniliprole) and two soil applied pesticides (dinotefuran, imidacloprid) on calico scales and their natural enemies on infested thornless honeylocust trees (*Gleditsia triacanthos inermis*) over three years. Bifenthrin, dinotefuran and cyantraniliprole provided the largest reductions in scale populations when they were applied to egg laying females on branches early in the season. In contrast, bifenthrin and pyriproxyfen provided the most consistent reductions in scales when they were applied to settled scales on leaves later in the season. None of the insecticides reduced natural enemy abundance when they were applied to egg laying females. In contrast, bifenthrin and pyriproxyfen reduced natural enemy abundance when they were applied to settled scales on leaves. Laboratory assays indicated that foliar applications of bifenthrin were highly toxic to larval *Chrysoperla rufilabris* (Chrysopidae: Neuroptera), 4 DAT but not 50 DAT. In contrast, toxicity of pyriproxyfen and chlorantraniliprole were moderately toxic to *C. rufilabris* at 4 DAT and 50 DAT. This suggests physiological selectivity of a pesticide

and the timing of its application can influence its activity against scales and their natural enemies.

## 2.2 Introduction

Calico scale (*Eulecanium cerasorum*) is a destructive exotic pest of shade and ornamental trees. It was introduced to California from China, Japan and Korea in the 1920s (Miller et al. 2002) and has since moved into the eastern and north central U.S. where it causes substantial damage to urban trees (Gill 1988). Calico scales feed on phloem sap and produce copious amounts of honeydew, which leads to the growth of black sooty mold on heavily infested trees. This reduces tree vigor and can ultimately kill branches by reducing rates of photosynthesis and carbohydrate assimilation (Hubbard and Potter 2005).

Female calico scales can be identified by characteristic black and white markings on their bodies. Body sizes of adult females are 6-8 mm in diameter (Hubbard and Potter 2005). Each adult female produces between 3,700 to 4,700 eggs, depending on the host plant (Hubbard and Potter 2005). Eggs normally hatch in the middle of May in Indiana. First instars crawl to the leaves where they settle and feed on leaf tissue. In the middle of July, calico scales molt to the second instar and remain on the leaves until the middle of September, when they move to the trunk and branches to overwinter. From March to the middle of April, they develop into adults, and males fly to mate with wingless females.

Parasitoids and predators of calico scale have been reported (Hubbard and Potter 2005). However, the efficacy of natural enemies against these and other soft scale insects can be affected by the presence of ants, pesticide residues, dust, temperature, and pollution (Luck and Dahlsten 1975, Hanks and Sadof 1990, Meineke et al. 2013, Raupp et al. 2001, 2010, Vanek and Potter 2010). For these reasons pesticides may be needed to supplement population managment.

Work by Hubbard and Potter (2006) identified a number of insecticides that could be useful for managing calico scale when applied at specific stages of the scale's life cycle. Although they have described natural enemies associated with this pest in the urban landscape (Hubbard and Potter 2005), they did not directly report how use of these products could affect these beneficial insects. Several new products have been developed since conducted their work. Insectides with potential activity against soft scales include the neonicotinoid dinotefuran (Elbert at al 2008, Johnson 2010), and the ryanidine inhibitors, chlorantraniliprole, with purportedly low impacts on natural enemies and pollinators (Brugger et al. 2010, Gradish et al. 2010, Larson et al. 2012). For these reasons, we conducted a study to compare the efficacy of selected pesticides against calico scale and their effects on natural enemies.

## 2.3 Materials and Methods

*Field Studies.* Four experiments were conducted on thornless honeylocust trees (*Gleditsia triacanthos* var. *inermis*) that were naturally infested with calico scale (*Eulecanium cerasorum*) over three years at four sites in the Indianapolis area. Three sites were for a one-year study, while another site was used for two years. In a fifth experiment, effects of insecticides were assessed on artificially infested trees located at the Agronomy Center for Research and Education (ACRE), in West Lafayette, IN.

*2011 Urban infestations.* Two urban plantings of honeylocust heavily infested with calico scale were used tor study the effectiveness of foliar and systemic insecticides in Indianapolis, IN (Table 2.1). Experiments in both sites followed a randomized complete block design with treatments randomly assigned to trees blocked by location. At the first site, seven foliar insecticide treatments were applied to seven replicate blocks of trees ranging in size from 8.1 to 29.1 cm DBH (Diameter Breast Height) in downtown Indianapolis near Market Street (39.768263 N, -86.152452 W). These insecticides were applied on 3 May, (152 $DD_{10\,°C}$) (Degree days, base 10 °C) to target ovipositing females. Each insecticide was applied to the tree canopy until run off with an FMC John Bean (Philadelphia, PA) high pressure sprayer at ($2.9 \times 10^{-6}$ kg/cm$^2$).

Systemic insecticides were evaluated at a second site located along the Indianapolis Canal (39.777448 N, -86.164640 W). There, 54 trees ranging in size from 8.6 to 24.6 cm DBH were divided into, six replicate blocks. Nine treatments of trunk and soil applied insecticides (Table 2.1) were randomly assigned to each block. Trunk insecticides were applied with a 5.68 liter hand sprayer at low pressure (0.56 Kg/cm$^2$). Soil insecticides were applied at 1.41 Kg/cm$^2$ with a subsoil injector whose probe penetrated 20 cm below the soil surface at 4 points within 0.3 m of the trunk. Imidacloprid was applied on 10 April (73 $DD_{10\,°C}$) and dinotefuran on 3 May (152 $DD_{10\,°C}$) to target ovipositing females.

Mortality of overwintered egg laying females at each site was evaluated after treatment by collecting them on four infested twigs, sampled randomly from each tree canopy at the four cardinal direction points. Twigs were taken to the laboratory and examined visually to determine if overwintered egg laying females were alive or dead. When a scale was shriveled and the color was faded brown, it was counted as dead. If the scale was black and smooth with white tufts of wax it was counted as alive. At both study sites, twenty individual scales were used to assess mortality on each tree on 18 May, 15 DAT for all compounds except for dinotefuran (38 DAT). Simple mortality was estimated as the proportion of dead females collected.

Efficacy of products against scales on leaves was assessed by collecting four to six leaves from treated trees at both study sites on 14 June and 15 July in 2011, 55 and 84 DAT for all compounds except for dinotefuran (79 and 108 DAT). Scale mortality on leaves was assessed by examining a total of 100 settled scales from two randomly chosen leaflets of each leaf. Each scale was visually examined to determine if it was dead or alive. Dead scales were brown to orange in color and live scales were yellow. Mortality was expressed as a proportion of dead to living scales. The abundance of live scales on each leaf was estimated using a ranking process. Leaflets with no live scales were given a rank of zero. Leaflets with 1 to 10 were assigned a rank of 1. Leaflets with 11 to 20 scales were ranked at level of 2. Leaflets with more than 20 scales were assigned a ranking of 3. To estimate the number of live scales per leaf, ranks were multiplied by the midpoint value of each range 0, 5, 15 and 25, respectively. Density of scales was estimated by dividing counts by the total leaf area examined. Leaf area of each sample was measured with a LiCor (Lincoln, NE) leaf area meter.

*2011 Artificial infestation.* Relative capacity of selected foliar and soil applied systemic insecticides to kill scale crawlers was compared on artificially infested trees located at ACRE in Purdue University (40.4708676 N, -86.9905682 W). A total of 35 trees were selected to be infested with calico scales that were collected on twigs from the Indianapolis Canal. Two branches were infested on each tree by tying two twigs containing 20 ovipositing females on 2 June. Leaves from infested branches were inspected six days after infestation to determine the number of live calico scales crawlers that survived and settled on leaves after treatments were applied (Table 2.1). Seven trees were allocated to each of five treatments in a completely randomized design. Soil insecticides were applied at the base of trees, while foliar insecticides were applied to the branches with a Solo 3 gallon sprayer. Insecticides were applied on 8 July, six days after the artificial infestation. Mortality on leaves was assessed on 22 June from 100 scales as described previously.

*2012-2013 Urban infestations*. Egg-laying overwintered females were the target of our first experimental site located along River Road Ave. in Carmel, IN (39.979093 N, -86.051424W). A total of ninety five trees ranging in size from 11.7 to 28.7 cm DBH were selected from a group of 300 infested honeylocust trees. Trees were selected to allow untreated trees to serve as buffers and prevent cross contamination with insecticides during treatment applications. Eight treatments were randomly assigned to two trees within each of five insecticide blocks, excluding treatments containing pyriproxyfen, bifenthrin, and water control which were assigned to three trees per block (Table 2.1). Insecticides were applied on 20 March (88 $DD_{10\,°C}$). Mortality of overwintered egg laying females was determined by examining at least one hundred female scales collected from 4 branches at each cardinal direction on and 3 May in 2012 (44 DAT) as well as 23 May in 2013 (22 DAT). Simple mortality was estimated as the proportion of dead females collected as described for the 2011 site.

A second experiment located at 96th street in Fishers, IN (39.928119 N, -86.0336224 W) was designed to target first instar calico scales that had settled on leaves in 2012 followed by applications that targeted overwintering females in the spring of

2013. Five treatments (Table 2.1) were allocated to each of five trees in a completely randomized design. Trees selected ranged in size from 17.7 to 40.6 cm DBH. Foliar and soil applications were applied on 6 June (558 $DD_{10\,°C}$) 2012. In 2013, these treatments were applied on 2 May (120 $DD_{10\,°C}$) and 3 May (130 $DD_{10\,°C}$) to target ovipositing females. In both experiments, insecticides were applied either to tree canopy until run off, or to soil with a subsoil injector, as described previously. Mortality and abundance of settled scales on leaves at both sites were estimated in a different manner than in 2011. Five leaves were collected from different locations on the trees for each treatment near sites where there was evidence of egg laying females. Six leaflets from the middle of each leaf were used to obtain a total of thirty leaflets per sample. Settled scales were determined to be alive or dead as described previously. Leaf area of each sample was measured using a Licor® leaf area machine and the density of scale was determined as the total number per square cm of leaf. Mortality was calculated as the proportion of the examined scales that were dead. In 2012, leaf samples were collected on 31 May (72 DAT) at the Carmel site that targeted egg laying females. At the second site, where initial insecticide applications targeted crawlers, leaves were collected on 20 June (14 DAT), 16 July (40 DAT) and 30 July (58 DAT). In 2013, overwintering scale populations on these trees were examined by randomly choosing four branches of each treatment and counting the number of overwintering scales present as dead or alive in the terminal 30 cm on 16 May. After overwintering females were treated with insecticides described previously, leaf samples were collected from the same trees 17 June (46 DAT), 17 Jul (76 DAT), and 29 July (88 DAT).

*Assessing impacts of natural enemies.* In 2012 and 2013, parasitoid populations of overwintering females were evaluated by collecting at least 100 overwintering females on two to three twigs from each treated tree on 18 April and 3 May in 2012 (29 and 44 DAT) and 16 and 26 May in 2013 (13 and 23 DAT). For each sampling date, 100 overwintering calico scale females were left intact on the twigs collected from each tree. Females on the twigs were placed in cardboard tubes (10.5 cm diameter and 18 cm depth) at room temperature with an 18:6 L:D photoperiod. Parasitoids were collected from tubes everyday over three weeks, separated out to morphospecies, counted and preserved in 75% ethanol. Representative samples were sent to Andrew Ernst (North Carolina State University) for identification.

Populations of parasitoid of settled scales on leaves were evaluated in two ways. First, parasitism rates were estimated during mortality and population census. Parasitized scales were rounded and black or had visible exit holes while dead scales were flat and brown to orange. Live scales were rounded and yellow. All dissected black scales contained parasitoids. Second, parasitoid and predator populations on branches were examined by using an inverted leaf blower vacuum machine (Rebek et al. 2005) to collect all insects from four, 0.5 m lengths of branch in each cardinal direction while females were laying eggs, as well as during the first and second stage of settled scale on leaves. During 2012, samples were collected in the field on 25 April, 7, 14, 28 June, 16, 30 July, 6, 15, and 23 August, taken to the laboratory and preserved in 75% ethanol. In 2013, vacuum samples were collected on 16, 24 May, 3, 17 June, 1, 17, 29 July, 12, and 26 August. Numbers of natural enemies were tallied during laboratory inspections. Also, yellow sticky cards were placed on the trunk approximately at two meters from the soil surface for one week periods throughout the second stage of settled scale. In 2012, sticky cards were placed on 14, 21 August, 12, and 19 September whereas in 2013, sticky cards were placed on 2, 16, 30 August and 7 September.

*Laboratory studies on scale crawlers*. In 2012, an assay was conducted to determine effects of insecticide application timing on calico scales. In this study, we used scale-free leaves collected from honeylocust trees located in a nursery in Westfield, IN (40.020767 N, -86.188962 W) that had been treated with soil and foliar insecticides (Table 2.2) on 8 June, two weeks prior to the assay as well as on the day of the assay. Soil insecticides were applied with a subsoil injector, described previously. Foliar applications were made with a Solo 4 gallon sprayer to run off. Leaves treated the day of the assay were collected from untreated trees in the same place and sprayed to run-off with foliar insecticide (Table 2.2) using a 32 oz. bottle sprayer on 22 June and allowed to dry.

Six leaflets receiving each treatment were placed in Petri dishes atop of cotton wool dampened with water at a constant temperature of 25°C and 16 hours light L:D where humidity was maintained. There were six replicates for each of the nine treatments in a completely randomized design. On the day of the assay, 22 June, ovipositing female calico scales (collected from Indianapolis) were placed on each leaflet. After 12 hours, the ovipositing females were removed, leaving only crawlers on the leaflets. Mortality of calico scale crawlers was estimated seven days later on 29 June. Crawlers that died before and after settling on the leaflet were used to calculate mortality (those that crawled off the leaf into the cotton substrate were not counted).

*Laboratory studies on predator larvae Chrysoperla rufilabris* was used as a proxy to determine impacts of insecticides on natural enemies. Leaves infested with calico scale were collected on 17 June 2013 from the Fishers, IN site (39.928119 N, -86.0336224 W) which were sprayed with pesticides on 2 and 3 May (Table 2.2) to represent an early season application. Extra leaves were collected from the water control and treated with foliar insecticides (pyriproxyfen, bifenthin, and chlorantraniliprole) with 32 oz. bottle sprayer on 18 June to represent late application (Table 2.2). There were 30 replicates for each of the eight treatments. *Chrysoperla rufilabris* was obtained from Hummert International (1835 North Glenstone Ave. Springfield, Missouri 65803). On 18 June, two leaflets with at least 100 calico scales and one larva of *C. rufilabris* were placed in 1 oz. plastic soufflés. Leaflets with calico scale were replaced every 24 hours. Mortality was assessed at 4 days by probing larvae with a camel's hair brush and counting unresponsive individuals. Individuals that responded by moving a leg or mandible were considered alive. A Chi square test was used to determine whether the mortality of *Chrysoperla rufilabris* observed on leaves treated with insecticide were different from the water control.

*Statistical Analysis.* Data from field studies were analyzed using Analysis of Variance with a Randomized Complete Block or a Completely Randomized design as described above using Statistica Software 7.0 (Statsoft Inc., Tulsa, OK, USA). Means of significant treatment effects were compared with Fishers Least Protected Means. Comparisons of mortality were conducted on arcsin square root transformed proportions of dead individuals to adjust for non-normality. Actual percentages of mortality are reported in results tables. A generalized linear model was used to conduct an analysis of covariance to determine whether populations of calico scale natural enemies collected in vacuum samples were affected by insecticides or the abundance of calico scale hosts. A Shapiro-Wilk's test was conducted to determine if data were normally distributed. If data were not normal a log 10 transformation was made and the assumption retested. If data were still not normal after log 10 transformation, we conducted a repeated measures analysis using PROC GLIMMIX for Generalized Linear Mixed Models (SAS® 9.3 Institute Inc., Cary, NC). LSMEANS were separated using Tukey's HSD Test at an α-level of 0.05.

## 2.4 Results

*Mortality and population density of overwintered females on branches*. Mortality of the overwintering generation of scales increased significantly ($F = 10.580$; df = 6, 36; $P < 0.001$) in 2011 after applying insecticides to target egg-laying females (Table 2.3). Among the foliar insecticide sprays, the highest mortalities were observed in the bifenthrin treatments with (77%) and without (70%) a surfactant. Cyantraniliprole plus surfactant (46%) treatments also had significantly higher mortality than the control (10%). In contrast, mortality of overwintered females on trees treated with systemic insecticides during egg laying was not significantly different ($F = 1.388$; df = 8, 32; $P = 0.241$) from the control (Table 2.4). In 2012, no significant differences ($F = 1.960$; df = 6, 74; $P = 0.082$) were found among the mortalities of overwintering-female scales on trees treated with foliar or soil applied insecticides during egg laying female stage (Figure 2.1). In 2013, mortality of overwintering-female scales were significantly affected ($F = 24.232$; df = 4, 20; $P < 0.001$) by soil and foliar treatments of insecticides with the highest mortality found in treatments of bifenthrin (55%). Also, mortalities caused by pyriproxyfen (23%) and dinotefuran (23%) were small, but significantly larger than water control (11%). In 2013, densities of overwintered female scales on trees treated with foliar and soil insecticides during the emergence of crawlers in 2012 were significantly ($F$ = 4.491; df = 4, 20; $P$ = 0.009) lowered by applications of bifenthrin (42%) and pyriproxyfen (73%) when compared with the control (Figure 2.2).

*Live crawler density on leaves in urban infestations.* During 2011, live crawler density was significantly ($F$ = 3.895, df = 6, 36; $P$ = 0.004) reduced by foliar applications of insecticides that targeted egg laying females during the crawler emergence (Table 2.3). All treatments except Capsil surfactant had statistically lower survivorship than the control. The highest reduction in live scales was provided by the combination of bifenthrin plus Capsil surfactant and the combination of cyantraniliprole plus Capsil surfactant with reductions of 72% and 74% respectively, compared with the control. Chlorantraniliprole, bifenthrin, and chlorantraniliprole plus Capsil surfactant were also significantly different than the control with reductions of 58%, 56% and 39% respectively. Live crawler density was significantly reduced ($F$ = 3.05, df = 6, 33; $P$ = 0.016) during the second instar. However, only chlorantraniliprole treated trees had a density that was lower (68%) than the control. In addition, systemic insecticides significantly reduced live crawler density ($F$ = 5.320; df = 8, 32; $P$ < 0.001) during the first instar (Table 2.4) when applied during egg laying. Soil applications of dinotefuran 2G, dinotefuran 70WSP, imidacloprid and trunk application of dinutefuran plus surfactant had reduction of 66, 52, 56 and 52% respectively when compared with the control. In contrast, during second instar live crawler density on trees treated with systemic insecticides during egg laying was not significantly different ($F$ =0.969; df = 8, 32; $P$ = 0.466) from the control (Table 2.4).

In 2012, live crawler density was significantly different ($F$ = 2.290; df = 6, 74; $P$ = 0.044) during the first instar on trees treated with foliar or soil applications of insecticides during egg laying in 2012 (Figure 2.1). Population reductions relative to the control caused by dinotefuran (54%), cyantraniliprole (40%), chlorantraniliprole (35%) and bifenthrin (34%) were significant. At the second site where insecticides were applied during the emergence of crawlers, no significant effects were found on first instar crawlers ($F$ = 2.851; df = 4, 20; $P$ = 0.051) (Figure 2.1). However, significant reductions were produced by pyriproxyfen (47%) and bifenthrin (42%) on 30 July on second instar crawlers, but not by the soil applied insecticides. In 2013, live crawler density was not significantly affected ($F$ = 2.251; df = 4, 20; $P$ = 0.099) during first instar by soil and foliar treatments of insecticides during female laying eggs (Figure 2.1). In contrast, live crawler density was significantly reduced ($F$ = 10.647; df = 4, 20; $P$ <0.001) by pyriproxyfen (68%) and bifenthrin (55%) when compared with the control during second instar on 30 July. Also, dinotefuran significantly reduced the number of crawlers (39%) compared to the control.

*Mortality of settled scales on leaves.* Although mortality of 100 scales sampled on leaves was assessed at each sampling interval, we only found significant differences on 14 June, 13 July, 2011 when insecticides targeted overwintered females, 20 June and 16 July when insecticides targeted scale on the leaves (Tables 2.5, 2.6). During 2011, mortality of settled crawlers was significantly increased ($F$ = 3.895; df = 6, 36; $P$ = 0.004) by applying foliar insecticides that targeted egg laying females. Cyantraniliprole plus surfactant (50%) and chlorantraniliprole (42%) had significantly greater mortality than the control (14%). Mortality of scales on leaves was significantly higher ($F = 6.381$; df = 6, 36; $P < 0.001$) during second instar on trees treated with chlorantraniliprole (77%) when compared with the control (39%) (Table 2.5). However, mortality of scales on leaves of trees treated with systemic insecticides during egg laying, was not significantly different during first ($F = 1.777$; df = 8, 35; $P = 0.118$) nor second instar ($F = 1.395$; df = 8, 34; $P = 0.241$) when compared to the control. Also, only the foliar treatment of bifenthrin on artificially infested trees significantly increased ($F = 15.237$; df = 4, 29; $P < 0.001$) mortality of settled crawlers (80%) on trees compared to the untreated control (27%) (Table 2.6).

In 2012, applications of insecticides that targeted egg laying females failed to significantly increase ($F = 1.092$; df = 6, 74; $P = 0.375$) mortality of settled crawlers on leaves (Table 2.5). However, mortality of settled crawlers scale was significantly greater ($F = 11.664$; df = 4, 20; $P < 0.001$) during first instar on trees treated during the emergence of crawlers. Bifenthrin showed 69% mortality. During the second instar (16 July), bifenthrin (51%) was significantly different ($F = 22.299$; df = 4, 20; $P < 0.001$) than the control (12%). During second instar, no significant difference ($F = 1.540$; df = 4, 20; $P = 0.229$) was found on trees treated during the emergence of crawlers. Insecticides toxicity at 7 and 21 DAT significantly ($F = 11.830$; df = 8, 53; $P < 0.001$) increased mortality of crawler when compared to the control (Table 2.7). Bifenthrin had a mortality of 78% at 7 and 21 DAT while Chlorantraniliprole had a mortality of 60% and 72% at 7 and 21 DAT, respectively. In 2013, mortality of settled scale crawlers was not significantly different during first (*F* = 2.563; df = 4, 20; *P*= 0.070), or second instar (*F* = 2.817; df = 4, 20; *P*= 0.053).

*Natural enemies of calico scale.* Over the course of this study, morphospecies of parasitoids were collected from five genera in two families: *Coccophagus lycimnia*, *Encarsia* (Aphelinidae: Hymenoptera), *Blastothrix*, *Metaphycus*, and *Encyrtus* sp. (Encyrtidae: Hymenoptera). Whereas, predators came from three families *Chrysoperla* sp (Chrysopidae: Neuroptera), *Orius* sp. (Anthocoridae: Hemiptera) and (Coccinellidae: Coleoptera).

In 2012, a total of 411 parasitoids were collected from overwintering adult female placed in cardboard tubes. *Coccophagus lycimnia* was the most abundant with 62% of the total collected followed by *Blastothrix* sp. (25%) and *Encyrtus* sp. (13%) (Figure 2.3). The abundance of total parasitoids emerging from overwintering adult females treated during early season with bifenthrin and cyantraniliprole was significantly lower (67 and 34%, respectively) (*F* = 4.560; df = 6, 74; *P* = <0.001) than the control (Table 2.8). In 2013, only 37 parasitoids emerged from overwintering females placed in cardboard tubes, *C. lycimnia* (70%) was the most abundant. The abundance of total parasitoids emerging from overwintered egg laying females was not significantly affected (*F* = 2.560; df = 4, 20; *P* = 0.070) by insecticides applied during the previous year in June of 2012 or May 3 2013.

In 2012, a total of 554 natural enemies were collected from trees in vacuum samples, 336 were parasitoids and 218 were predators (Figure 2.4). *Coccophagus lycimnia* was the most predominant parasitoid (78%) while *Chrysoperla* sp. was the most abundant predator (59%) followed by *Orius* sp. (35%). The abundance of natural enemies collected from vacuum samples of overwintering females on branches during egg laying was not significantly affected (*F* = 2.150; df = 6, 70; *P* = 0.058) by insecticide treatment early in the season (Figure 2.5). Similarly, insecticide use failed to affect the natural enemies collected in vacuum samples during first instar crawler stage (*F* = 0.96; df = 4, 20; *P* = 0.450) and settled first instar stage (*F* = 1.522; df = 4, 20; *P* = 0.234). In contrast, when applications of bifenthrin and pyriproxyfen were made during the crawler stage, they reduced (*F* = 4.804; df = 4, 20; *P* = 0.007) natural enemy abundance collected from vacuum samples of second instar scales by 69% and 43% respectively. Analysis of covariance indicated that reductions in natural enemies in these treatments were due to the effects of the pesticides applied (*F*=3.41; df=4,44; *P* = 0.0163), and not due to the effects of scale density (*F*=2.13; df=1,44; *P* = 0.152).

In 2013, 374 natural enemies were collected from vacuum samples of all stages of scale (Figure 2.4). Eighty–five percent of the 237 parasitoids were *C. lycimnia*. In contrast, the 137 predators were evenly distributed across three taxonomic groups *Chrysoperla* sp. (39%), *Orius* sp. (37%) and Coccinellidae (24%). Natural enemy abundance from vacuum samples during overwintering female stage (*F* = 2.59; df = 4, 20; *P* = 0.068), first instar crawler stage (*F* = 1.9; df = 4, 20; *P* = 0.1504), settled first instar scale stage (*F* = 1.045; df = 4, 20; *P* = 0.409) and second instar scale stage (*F* = 0.670; df = 4, 20; *P* = 0.622) was not significantly reduced by insecticide applied during early in the season when compared with the untreated control trees (Figure 2.5).

In 2012, a total of 559 parasitoids were collected from sticky cards (Figure 2.6). *Coccophagus lycimnia* (44%) and *Encarsia* sp. (38%) were the most abundant taxa followed by *Encyrtus* sp. (15%). In 2013, 72% of 627 parasitoids of calico scale present in the sticky cards were *C. lycimnia* follow by 16% of *Metaphycus* sp. Total parasitoids collected on sticky cards placed in trees was not significantly affected by treatment of trees with foliar and systemic insecticides during the emergence of crawlers ($F$ = 2.043; df = 4, 20; $P$ = 0.127) in 2012, or when females were laying eggs in 2013 ($F$ = 0.916; df = 4, 20; $P$ = 0.474) (Table 2.9).

In 2012 and 2013, rates of parasitization of leaf feeding calico scales were less than one percent. Percentage of calico scales parasitized was not significantly different in 2012 ($F$ = 2.502; df = 4, 19; $P$ = 0.077) or in 2013, ($F$ = 0.100; df = 4, 20; $P$ = 0.982) (Table 2.9).

Insecticide toxicity at 4 and 50 DAT significantly increased mortality of *Chrysoperla rufilabris* when compared to the control expected frequency (Table 2.10). Bifenthrin chlorantraniliprole and pyriproxyfen produced mortalities of 100%, 45% and 25% respectively 4 DAT. Pyriproxyfen and chlorantraniliprole produced mortalities of 46% and 42% respectively 50 DAT.

## 2.5 Discussion

Although many insecticides have the capacity to kill scales on trees, effective use of these products requires an understanding of their relative effectiveness against different life stages, their longevity and their impacts on natural enemies (Frank 2012, Szczepaniec and Raupp 2012). Several insecticides were used in this study to determine when use of each product is most effective at reducing calico scale abundance and how these applications affect natural enemy communities on honeylocust trees in an urban setting.

*Effect of insecticides on Calico Scale.* Bifenthrin was the only insecticide we tested that was capable of reducing the population of calico scale when it was applied either on egg laying females with or without surfactant or later in the season on settled scale crawlers. Early season applications of bifenthrin caused 70% mortality of egg laying females. These findings are consistent with anecdotal evidence reported by Hubbard and Potter (2006) and other studies demonstrating efficiency of bifenthrin on others scale species (Clarke et al. 1988, 1992, Frank 2012). This reduction in overwintering female survival reduced densities of first and second instar calico scales on leaves. In the same manner, when bifenthrin was applied to crawlers on leaves late in the season, survivorship was reduced to the point that it lowered densities of surviving egg laying females on twigs during the following season.

Pyriproxyfen failed to control calico scale when applied to egg laying females. This may be because it is an insect growth regulator which cannot kill adult stages. However, when pyriproxyfen was applied to crawler stages, it significantly decreased survival of second instar calico scales that molted from settled first instars. This reduced the number of overwintering females on stems available to lay eggs the following season. Toxicity of this product is consistent with Hubbard and Potter’s work (2006) on calico scale and those working on other species of scale insects (Frank 2012, Grafton-Cardwell et al. 2003, 2006, Rebek et al. 2003, Schneider et al. 2003, Suma et al. 2009).

Soil applications of both imidacloprid and dinotefuran reduced the abundance of first instar calico scale on leaves during at least one of the years they were applied on egg laying females. However, neither was as effective as bifenthrin or pyriproxyfen. Furthermore, imidacloprid was effective during only one of the years when it was applied against egg laying females. These inconsistent results may be partly due to the fact that they both are soil applied systemic insecticides, which need time and sufficient irrigation to be translocated from the roots through the stems where scales were feeding (Tattar et al. 1998). Application of these products at the beginning of egg laying may simply have not allowed these products enough time to intoxicate females before they completed laying eggs. Dinotefuran failed to control calico scale when applied during the crawler stage in 2012. Lack of effectiveness in that case may have been due to the historic drought of 2012 when the study trees received 31% of the average precipitation from May to July (NOAA 2013). We did not test effectiveness of imidacloprid drenches during the crawler stage because Hubbard and Potter (2006) had previously reported its failure to control scales during that time.

Of the trunk applied insecticides, only the dinotefuran with the pentrabark surfactant provided significant reduction in surviving egg laying females or subsequent first instar crawlers. This may be explained by the capacity of pentrabark to promote absorption of insecticide through the trunk into the xylem (Garbelotto and Schmidt 2009). In the absence of this surfactant, dinotefuran failed to provide any significant control. Failure of imidacloprid to reduce scale numbers during the same year that dinotefuran controlled scales may be due to its relatively low solubility when compared to dinotefuran (Cloyd and Bethke 2011).

The two ryanidine inhibitors differed in their capacity to control calico scale when applied to egg laying females. During both years of testing, cyantraniliprole reduced survival of egg laying females and reduced the densities of calico scale on leaves when it was applied early in the season. In contrast, chlorantraniliprole reduced densities of calico scale on leaves during the first year, but not the second year of the study.

*Effect of insecticides on natural enemies of calico scale*. During the course of this study, eight species of natural enemies were found. The parasitoid *C. lycimnia* was used to represent the effect of pesticides on parasitoids because it accounts for 80% of parasitoids collected in this study. Similarly, *Chrysoperla* sp. was used to represent impacts of pesticides on predators because it accounted for 50% of the predators recovered and demonstrated impacts on calico scale (Vannek and Potter 2010).

*Coccophagus lycimnia* is a facultative hyperparasitoid that feeds on scale and opportunistically parasitizes them. In the presence of parasitized hosts, *C. lycimnia* prefer to oviposit eggs in parasitoid larvae over scales (Compere and Smith 1932, Flanders 1937, Timberlake 1913). According to Tower (1914), hyperparasites species can overwinter as an early stage larva inside other parasitoid larvae. *Coccophagus lycimnia* adults live an average of 12 days and need 36 to 72 days to develop from egg to adult (Flanders 1937, Muegge and Lambdin 1989). We found this parasitoid to be active from April through September with peak abundance in late June and July during both years of our study. This pattern of parasitoid abundance is consistent with that reported previously (Hubbard and Potter, Schultz 1984).

The failure of our analysis of covariance to find a significant relationship among densities of live scale insects and natural enemies suggests that pesticides were responsible for differences in natural enemies collected in vacuum samples and not densities of live hosts. Two weeks after application of insecticides to egg laying females there was no effect of insecticide treatment on the few parasitoids collected from the canopy in vacuum samples. In contrast, when insecticides were applied during the crawler stage of calico scale, bifenthrin and pyriproxyfen reduced natural enemy populations because these applications occurred just before the normal peak of parasitoid flight. This would explain why fewer parasitoids were found on trees treated with both of these compounds when compared to the water control during second stage instars.

Patterns of natural enemy abundance found in our vacuum sample observations are also supported by laboratory bioassays of *Chrysoperla rufilabris*. Here we found no significant mortality to be caused by bifenthrin 50 DAT, whereas the same treatment caused 100% mortality 4 DAT. Similarly, the two purportedly biorational products, pyriproxyfen and chlorantraniliprole, had Abbots corrected mortalities of < 50% at 50 and 4 DAT. This finding is consistent with other studies of scale insects that showed that applications of bifenthrin and pyriproxyfen are toxic to scales and parasitoids during this developmental period (Rebek and Sadof 2003, Frank 2012). Yet, even though bifenthrin and pyriproxyfen both reduced natural enemies, pyriproxyfen had twice the number of natural enemies present as trees treated with bifenthrin. This observation is consistent with several other studies that found that pyriproxyfen is a reduced risk insecticide that impacts natural enemies, but poses less risk than other synthetic insecticides (Frank 2012, Grafton-Cardwell et al. 2003, 2006, Mendel et al. 1994, Rebek and Sadof 2003, Sadof and Sclar 2000, Schneider at al. 2003, Suma at al. 2009). It is also consistent with other studies suggesting physiological selectivity of chlorantraniliprole (Brugger et al 2010, Gradish et al. 2010 and Larson et al. 2012). Interestingly, when egg laying females were examined in May of 2013, 11 months after the June 2012 crawler sprays, there was no effect of any compound on the number of parasitoids emerging from 100 egg laying females that wintered on twigs.

Although two neonicotinoid compounds also failed to consistently impact natural enemy abundance, it is difficult to make generalizations about the toxicity of these compounds on natural enemies. This is largely due to the inconsistencies in calico scale control resulting from lack of product uptake into the trees lacking supplemental irrigation during periods of drought (Tattar et al. 1998).

In conclusion, we find that both the timing of application and physiological selectivity can greatly influence the toxicity of insecticides to scales and their natural enemies. In the present study, however, levels of natural enemies observed were too low to provide effective control of calico scale. For this reason, insecticides are likely to be an important management tool for managing scales when natural enemies are lacking. Not all insecticides capable of killing calico scale are appropriate for use when managing this pest on honeylocust trees. Compounds that produced the greatest immediate reduction in calico scale populations were bifenthrin and pyriproxyfen. Although use of bifenthrin once early in the season can reduce scales while sparing natural enemies of scale insects later in the season, these applications have been associated with outbreaks of honeylocust spider mite, *Platytetranychus multidigituli* (Witte 2013). Although pyriproxyfen was only effective against scale crawlers, it was able to substantially reduce calico scale populations with a relatively low impact on resident scale natural enemies.

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Table 2.1 Formulations, rates, application methods, field sites and application dates used to apply insecticides on honeylocust trees infested with calico scale.

| | | | | 2011 | | 2012 | | 2013 |
|---|---|---|---|---|---|---|---|---|
| | | | Insect stage | Egg laying female | Crawler | Egg laying female | Crawler | Egg laying female |
| Active ingredient | Trade name | Rate (A.I.) | Method | Systemic and Foliar[b] | Artificial infestation[c] | Systemic and foliar[d] | Systemic and foliar[e] | Systemic and foliar[e] |
| Bifenthrin + S[a] | Talstar S | 0.118 g/L | Foliar | 3-May | - | - | - | - |
| Chlorantraniliprole + S | Acelepryn | 0.058 g/L | Foliar | 3-May | - | - | - | - |
| Cyantraniliprole + S | HWG-355 | 0.060 g/L | Foliar | 3-May | - | - | - | - |
| Surfactant | Pentra-Bark | 0.599 g/L | Foliar | 3-May | - | - | - | - |
| Cyantraniliprole | HWG-355 | 0.060 g/L | Foliar | | - | 20-Mar | - | - |
| Chlorantraniliprole | Acelepryn | 0.058 g/L | Foliar | 3-May | - | 20-Mar | 6-Jun | 3-May |
| Bifenthrin | Talstar S | 0.118 g/L | Foliar | 3-May | 27-May | 20-Mar | 6-Jun | 3-May |
| Pyriproxyfen | Distance | 0.099 g/L | Foliar | | - | 20-Mar | 6-Jun | 3-May |
| Dinotefuran | Transtect 70WSP | 0.567 g/cm DBH | Soil | 3-May | 27-May | 20-Mar | 6-Jun | 3-May |
| Imidacloprid | Xytect 75WSP | 0.580 g/cm DBH | Soil | 10-Apr | 27-May | - | - | - |
| Dinotefuran | Safari 2G | 0.470 g/cm DBH | Soil | 3-May | 27-May | - | - | - |

Table 2.1 Continued

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Imidacloprid + S | Xytect 75WSP | 0.699 g/cm DBH | Trunk | 10-Apr | - | - | - | - |
| Dinotefuran + S | Safari 2G | 0.470 g/cm DBH | Trunk | 3-May | - | - | - | - |
| Imidacloprid | Xytect 75WSP | 0.699 g/cm DBH | Trunk | 10-Apr | - | - | - | - |
| Dinotefuran | Safari 2G | 0.699 g/cm DBH | Trunk | 3-May | - | - | - | - |
| Water | | | | 3-May | 27-May | 20-Mar | 6-Jun | 3-May |

[a] S refers to Surfactant.

[b]Indianapolis, IN study site.

[c]Agronomy Center for Research and Education, West Lafayette, IN study site.

[d]Carmel, IN study site.

[e]Fishers, IN study site.

Table 2.2 Pesticides evaluated during laboratory assays of crawler and lacewing larvae exposed to honeylocust leaves.

| **Active Ingredient** | **trade name** | **Rate (A.I.)** | **Application Method** |
|---|---|---|---|
| Chlorantraniliprole | Acelepryn | 0.058 g/L | Foliar |
| Bifenthrin | Talstar S | 0.118 g/L | Foliar |
| Pyriproxyfen | Distance | 0.099 g/L | Foliar |
| Dinotefuran | Transtect 70WSP | 0.567 g/cm DBH | Soil |
| Imidacloprid | Xytect 75 WSP | 0.580 g/cm DBH | Soil |
| Water | | | |

Table 2.3 Effects of early season application of foliar insecticides on different life stages of calico scale on honeylocust trees that were treated on 4 May, 2011 in downtown Indianapolis.

| | | Mortality (%) | | Live scales/cm$^2$ | |
|---|---|---|---|---|---|
| **Treatment** | **N** | **Overwintering females 18-May (15 DAT)** | **Abbott's correct** | **First instar crawler 14 June (55 DAT)** | **Second instar 13 July (84 DAT)** |
| Bifenthrin | 7 | 70.00 ± 11.34a[a] | 66.90 | 6.47 ± 1.84abc | 2.41 ± 0.25ab |
| Bifenthrin + S[b] | 7 | 77.10 ± 14.20a | 74.74 | 4.14 ± 0.83ab | 1.68 ± 0.43ab |
| Capsil Surfactant | 7 | 10.71 ± 02.02c | 1.49 | 10.58 ± 3.11cd | 2.89 ± 0.92b |
| Chlorantraniliprole | 7 | 23.93 ± 05.56bc | 16.07 | 6.18 ± 2.40abc | 1.88 ± 0.93ab |
| Chlorantraniliprole + S | 7 | 22.62 ± 03.29bc | 14.63 | 8.96 ± 1.82bc | 1.25 ± 0.33ab |
| Cyantraniliprole + S | 7 | 44.48 ± 06.85b | 38.75 | 3.83 ± 1.37a | 0.95 ± 0.50a |
| Water | 7 | 9.36 ± 01.99c | 0.00 | 14.85 ± 2.10d | 2.99 ± 1.03b |
| F; df=6,36; P value | | 10.580; <0.001 | | 3.895; 0.004 | 3.050; 0.016 |

[a]Means within columns followed by the same letter are not significantly different ($P <$ 0.05; Fishers Protected LSD).

[b] Surfactant.

Table 2.4 Effects of early season application of systemic insecticides on different life stages of calico scale on honeylocust trees along the Indianapolis Canal that were treated with insecticides on May 3, 2011 with dinotefuran or on 10-April for the other treatments.

| | | **Mortality (%)** | **Live scales/cm$^2$** | |
|---|---|---|---|---|
| **Treatment** | **N** | **Overwintering females 18-May (15[c], 38 [d] DAT)** | **First instar crawler 14 June (55[c], 79 [d] DAT)** | **Second Instar Crawler 13 July (84[c], 108[d] DAT)** |
| **Trunk application** | | | | |
| Imidacloprid | 6 | 23.98 ± 05.59 | 13.56 ± 2.14b[a] | 5.81 ± 1.64 |
| Imidacloprid+P[b] | 6 | 20.00 ± 06.71 | 11.81 ± 2.72b | 6.81 ± 2.93 |
| Dinotefuran | 6 | 18.94 ± 02.79 | 12.58 ± 2.06b | 7.70 ± 3.16 |
| Dinotefuran +P | 6 | 42.94 ± 08.87 | 5.59 ± 2.48a | 2.47 ± 1.18 |
| **Soil application** | | | | |
| Dinotefuran 70WSP | 6 | 36.67 ± 14.70 | 5.60 ± 1.13a | 1.92 ± 1.20 |
| Dinotefuran 2G | 6 | 23.45 ± 06.49 | 3.94 ± 1.87a | 2.95 ± 1.72 |
| Imidacloprid 75 WP | 6 | 43.81 ± 16.79 | 5.15 ± 2.11a | 4.50 ± 1.78 |
| Water | 6 | 20.00 ± 07.91 | 11.84 ± 3.06b | 4.71 ± 2.73 |
| F; df; P value | | 1.388; 8, 32; 0.241 | 5.320; 8, 33; <0.001 | 0.969; 8, 32; 0.466 |

[a]Means within columns followed by the same letter are not significantly different (*P*< 0.05; Fishers Protected LSD).

[b] Pentrabark.

[c] Imidacloprid.

[d] Dinotefuran

Table 2.5 Mortality of 100 first and second instar calico scales examined on leaves collected from infested honeylocust tree that were treated with insecticides in the Indianapolis area in 2011 and 2012.

| Treatment | Insecticides targeting egg-laying female (2011) | | | | Insecticides targeting scales on leaves 2012 | | | |
|---|---|---|---|---|---|---|---|---|
| | First Instar crawler 14 June (55 DAT) | Abbott's correct mortality (%) | Second Instar Crawler 13 July (84 DAT) | Abbott's correct mortality (%) | First instar crawler 20 June (14 DAT) | Abbott's correct mortality (%) | Second Instar Crawler 16 July (40 DAT) | Abbott's correct mortality (%) |
| Chlorantraniliprole + S[c] | 25.44 ± 5.79b[a] | 15.56 | 67.51 ± 1.75ab | 45.85 | - | | - | |
| Cyantraniliprole + S | 50.11 ± 8.01a | 43.50 | 62.76 ± 9.59ab | 37.93 | - | | - | |
| Bifenthrin + S | 15.74 ± 2.69b | 4.58 | 27.71 ± 7.11cd | -20.48 | - | | - | |
| Capsil Surfactant | 21.77 ± 5.75b | 11.40 | 46.73 ± 9.41cd | 11.22 | - | | - | |
| Bifenthrin | 16.98 ± 4.99b | 5.98 | 24.83 ± 2.23d | -25.28 | 69.32 ±10.94a | 63.91 | 51.13 ± 6.48a | 44.32 |
| Chlorantraniliprole | 42.14 ± 4.67a | 34.47 | 77.32 ± 5.67a | 62.20 | 10.64 ± 03.33b | -05.13 | 22.88 ± 3.51b | 12.13 |
| Pyriproxyfen | - | | - | | 28.60 ±10.17b | 16.00 | 13.76 ± 2.13bc | 1.74 |
| Dinotefuran 70WSP[b] | - | | - | | 12.01 ± 02.12b | -03.52 | 10.57 ± 1.25c | -01.89 |
| Water | 11.70 ± 2.14b | 0.00 | 39.78 ± 7.02cd | 0.00 | 15.04 ± 01.42b | 0.00 | 12.23 ± 1.63bc | 0.00 |
| F; df; P value | 6.843; 6, 36; <0.001 | | 6.381; 6, 36; <0.001 | | 11.664; 6, 36; <0.001 | | 22.299; 6, 36; <0.001 | |

[a]Means within columns followed by the same letter are not significantly different (*P*< 0.05; Fishers Protected LSD).

[b] Dinotefuran was applied to the soil, all others were applied to leaves.[c] Surfactant

Table 2.6 Effects of insecticides targeting calico scale crawler on artificially infested trees (honeylocust) that were treated on 8 June, 2011 at Agronomy farm Purdue University.

| **Treatment** | **N** | **Crawler Mortality (%) 22 Jun (15 DAT)** | **Abbott's correct mortality (%)** |
|---|---|---|---|
| Bifenthrin | 7 | 80.30 ± 11.54a[a] | 72.91 |
| Imidacloprid | 7 | 22.61 ± 03.33b | -6.40 |
| Dinotefuran | 7 | 27.80 ± 05.45b | 0.73 |
| Water | 7 | 27.27 ± 04.02b | 0.00 |
| F; df = 4, 25; P value | | 15.237; < 0.001 | |

[a]Means within columns followed by the same letter are not significantly different (*P*< 0.05; Fishers Protected LSD).

[b] Dinotefuran was applied to the soil, all others were applied to leaves.

Table 2.7 Survival of first instar crawlers placed on honeylocust leaf on 29 June that were treated in the field on 8 and 22 June and incubated in the laboratory for a week.

| **Treatment** | **N** | **DAT** | **First instar crawlers mortality** | **Abbott's correct mortality (%)** |
|---|---|---|---|---|
| **Insecticide applied 8 June** | | | | |
| Dinotefuran [b] | 6 | 21 | 48.62 ± 9.85c[a] | 28.0 |
| Imidacloprid [b] | 6 | 21 | 54.39 ± 6.57c | 35.65 |
| Chlorantraniliprole | 6 | 21 | 80.31 ± 4.69ab | 72.22 |
| Pyriproxyfen | 6 | 21 | 45.74 ± 8.66c | 23.45 |
| Bifenthrin | 6 | 21 | 83.79 ± 7.36a | 77.13 |
| **Insecticide applied 22 Jun.** | | | | |
| Pyriproxyfen | 6 | 7 | 45.29 ± 8.83c | 22.81 |
| Bifenthrin | 6 | 7 | 84.37 ± 4.92a | 77.95 |
| Chlorantraniliprole | 6 | 7 | 71.77 ± 3.97b | 60.17 |
| Water | 6 | 7 | 29.12 ± 6.58c | 0.00 |
| F; df = 8, 53; P value | | | 11.830, <0.001 | |

[a]Means within columns followed by the same letter are not significantly different ($P <$ 0.05; Fishers Protected LSD).

[b] Dinotefuran and imidacloprid were applied to the soil, all others were applied to leaves.

Table 2.8 Effects of early season application of foliar and systemic insecticides on the natural enemies of calico scale when overwintering females were treated on 20 March in Carmel, IN and effect of late applications directed against first instar stage on leaves on 6 June, 2012 and 3 May 2013 in Fishers, IN.

| | **Parasitoids emerged from overwintering females** [c] | |
|---|---|---|
| **Treatment** | **18 April and 3 May (29 and 44 DAT), 2012** | **16, 26 May (10, 20 DAT), 2013** |
| Imidacloprid [b] | 4.34 ± 0.78ab[a] | - |
| Cyantraniliprole | 3.06 ± 0.63b | - |
| Bifenthrin | 1.51 ± 0.30c | 0.60 ± 0.35 |
| Pyriproxyfen | 3.15 ± 0.52ab | 3.00 ± 0.77 |
| Chlorantraniliprole | 4.34 ± 0.73ab | 1.20 ± 0.49 |
| Dinotefuran [b] | 3.15 ± 0. 64ab | 1.00 ± 0.45 |
| Water | 4.66 ± 0.72a | 1.60 ± 0.57 |
| F; df; P value | 4.56; 6, 74; < 0.001 | 2.56; 4, 20; 0.070 |

[a]Means within columns followed by the same letter are not significantly different (*P*< 0.05; Fishers Protected LSD).

[b] Dinotefuran was applied to the soil, all others were applied to leaves.

[c] Collected from twigs containing100 females placed in a cardboard tube.

Table 2.9 Effects of early and late season application of insecticides on hymenopteran parasitoids collected on sticky card and percentage of second instar calico scale on trees that were treated on 6 June, 2012 and 3 May 2013 respectively in Fishers, IN.

| | | 2012 | | 2013 | |
|---|---|---|---|---|---|
| **Treatment** | **N** | **Average NE[a] (Sticky card)** | **Percent of calico scales parasitized[b]** | **Average NE[a] (Sticky card)** | **Percent of calico scales parasitized[b]** |
| Bifenthrin | 5 | 34.60 ± 9.45 | 0.191 ± 0.06 | 33.00 ± 3.95 | 0.05 ± 0.10 |
| Pyriproxyfen | 5 | 13.80 ± 4.21 | 0.174 ± 0.05 | 28.40 ± 3.20 | 0.10 ± 0.14 |
| Chlorantraniliprole | 5 | 20.60 ± 5.91 | 0.128 ± 0.04 | 36.40 ± 7.15 | 0.03 ± 0.08 |
| Dinotefuran[c] | 5 | 19.20 ± 3.41 | 0.070 ± 0.02 | 30.40 ± 4.13 | 0.01 ± 0.05 |
| Water | 5 | 23.60 ± 4.96 | 0.255 ± 0.10 | 40.80 ± 6.20 | 0.07 ± 0.12 |
| F; df; P value | | 2.043; 4, 20; 0.127 | 2.502; 4, 19; 0.077 | 0.916; 4, 20; 0.474 | 0.100; 4, 20; 0.982 |

[a] Sum of all counts per tree.

[b] Second instar stage on leaves.

[b] Dinotefuran was applied to the soil, all others were applied to leaves.

Table 2.10 Mortality of *Chrysoperla* sp. larvae placed on honeylocust leaves on 18 June that were treated in the field on 3 May and 18 June and incubated in the laboratory for four days.

| Treatment | N | DAT | Mortality (%) | Chi-square [b] | p- Value | Abbott's correct mortality (%) |
|---|---|---|---|---|---|---|
| **Insecticide applied 3 May** | | | | | | |
| Bifenthrin | 30 | 50 | 30.00 | 01.50 | 0.221 | 12.50 |
| Pyriproxyfen | 30 | 50 | 56.67 | 20.17 | <0.001 | 45.83 |
| Chlorantraniliprole | 30 | 50 | 53.33 | 16.67 | <0.001 | 41.66 |
| Dinotefuran[a] | 30 | 50 | 30.00 | 1.50 | 0.221 | 12.50 |
| **Insecticide applied 18 June** | | | | | | |
| Bifenthrin | 30 | 4 | 100 | 96.00 | <0.001 | 100 |
| Pyriproxyfen | 30 | 4 | 40.00 | 6.00 | 0.014 | 25.00 |
| Chlorantraniliprole | 30 | 4 | 56.67 | 20.17 | <0.001 | 45.83 |
| Water | 30 | 4 | 20.00 | 0.00 | 1.000 | 0.00 |

[a] Dinotefuran was applied to the soil.

[b] Test to determine if mortality was significantly different from the control.

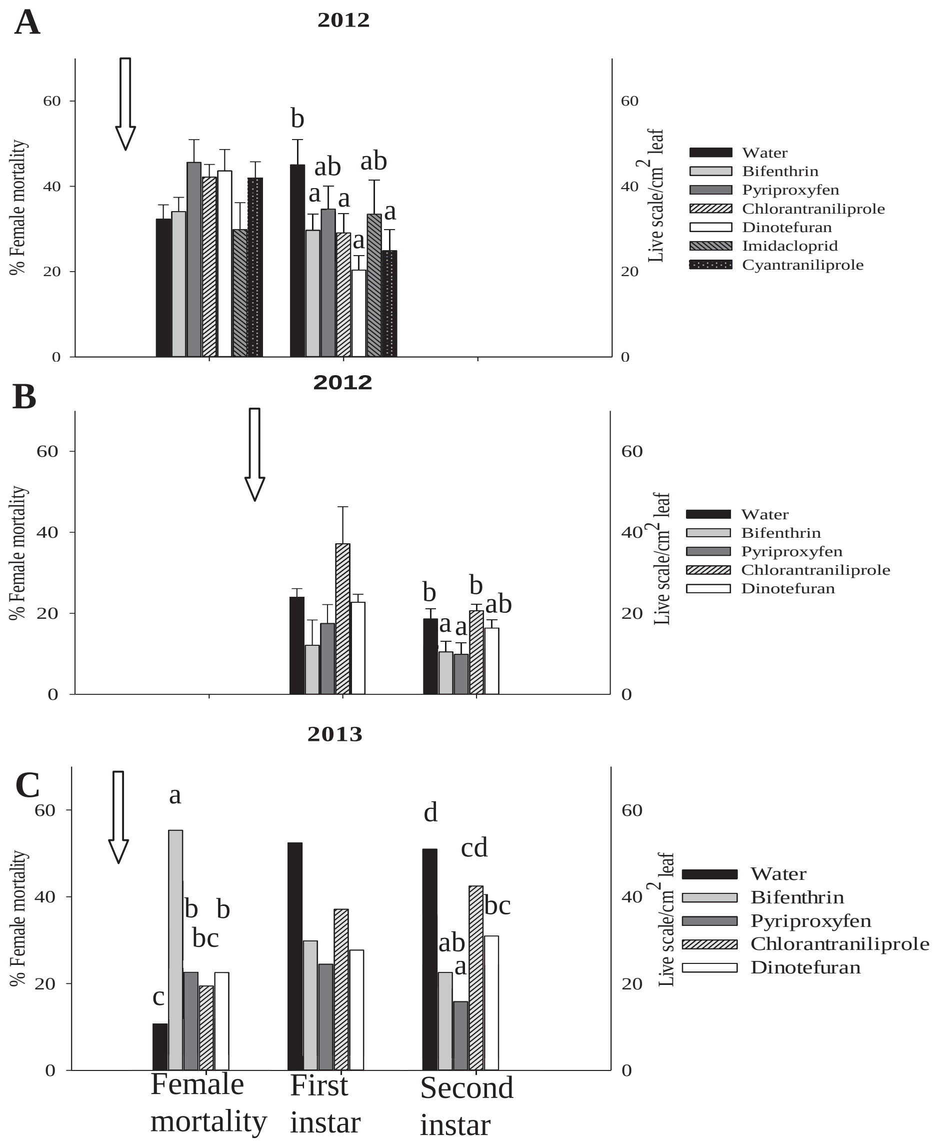


Figure 2.1 Percentage mortality of calico scale females overwintering on branches and calico scale density on leaves during first and second instar on honeylocust. Arrows indicated when pesticides treatments were applied. Graph A contain data from Carmel, IN (application 20 March), B and C Fishers, IN (application 6 June and 3 May respectively).

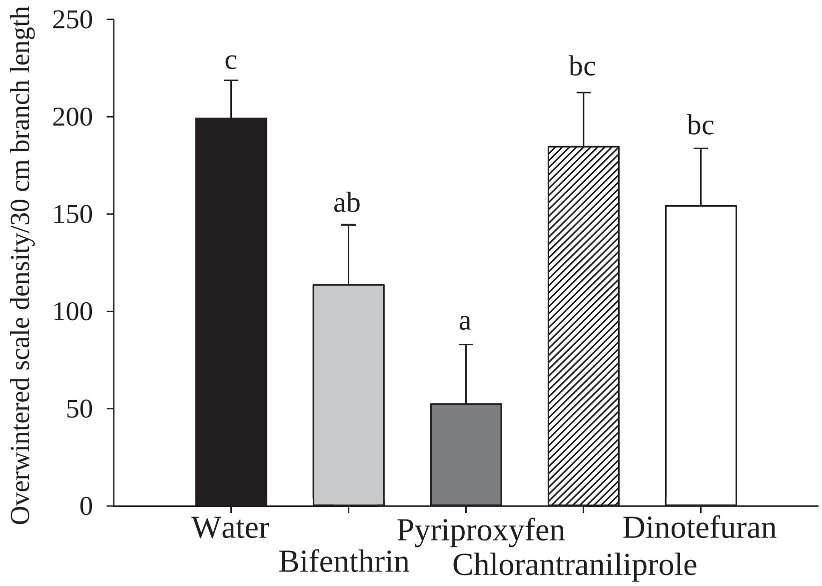


Figure 2.2 Effects of pesticide applications that targeted calico scale crawlers on honeylocust trees during June 2012 on the abundance of egg laying females during the May 2013 in Fishers Indiana.

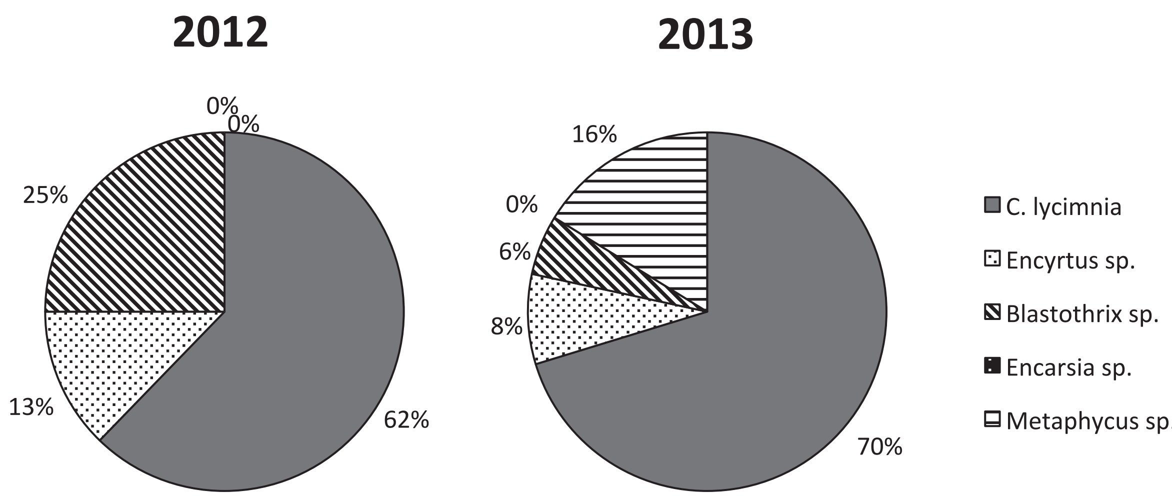


Figure 2.3 Percentage of parasitoids emerged from egg laying female calico scales that were collected on honeylocust branches and reared from cardboard tubes in Carmel and Fishers, IN during 2012 and 2013 respectively.

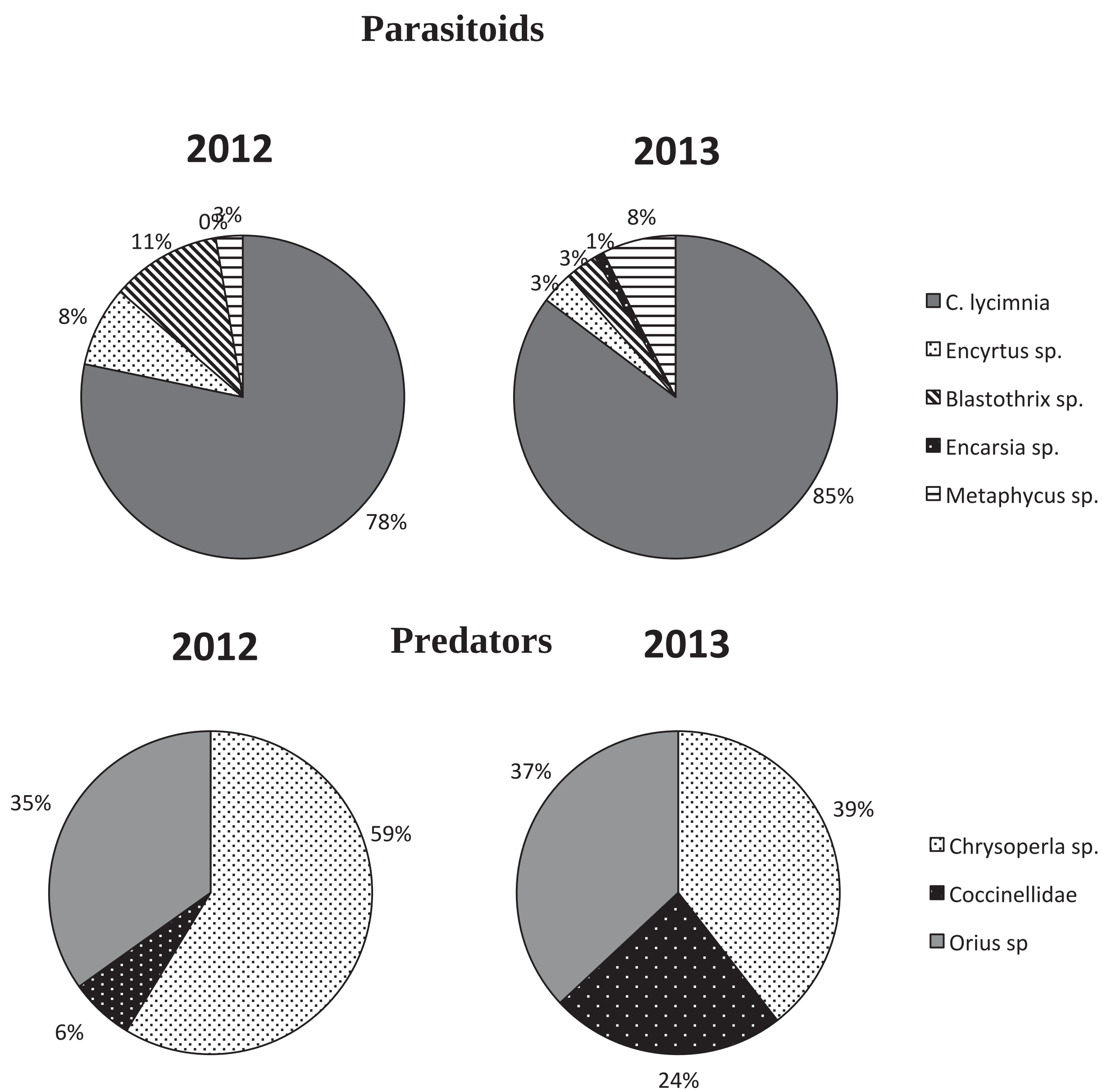


Figure 2.4 Percentage of parasitoids and predators collected from vacuum samples of four 50 cm long branches of honeylocust at different life stages of calico scale in Carmel, IN 2012 and Fishers, IN 2012 and 2013.

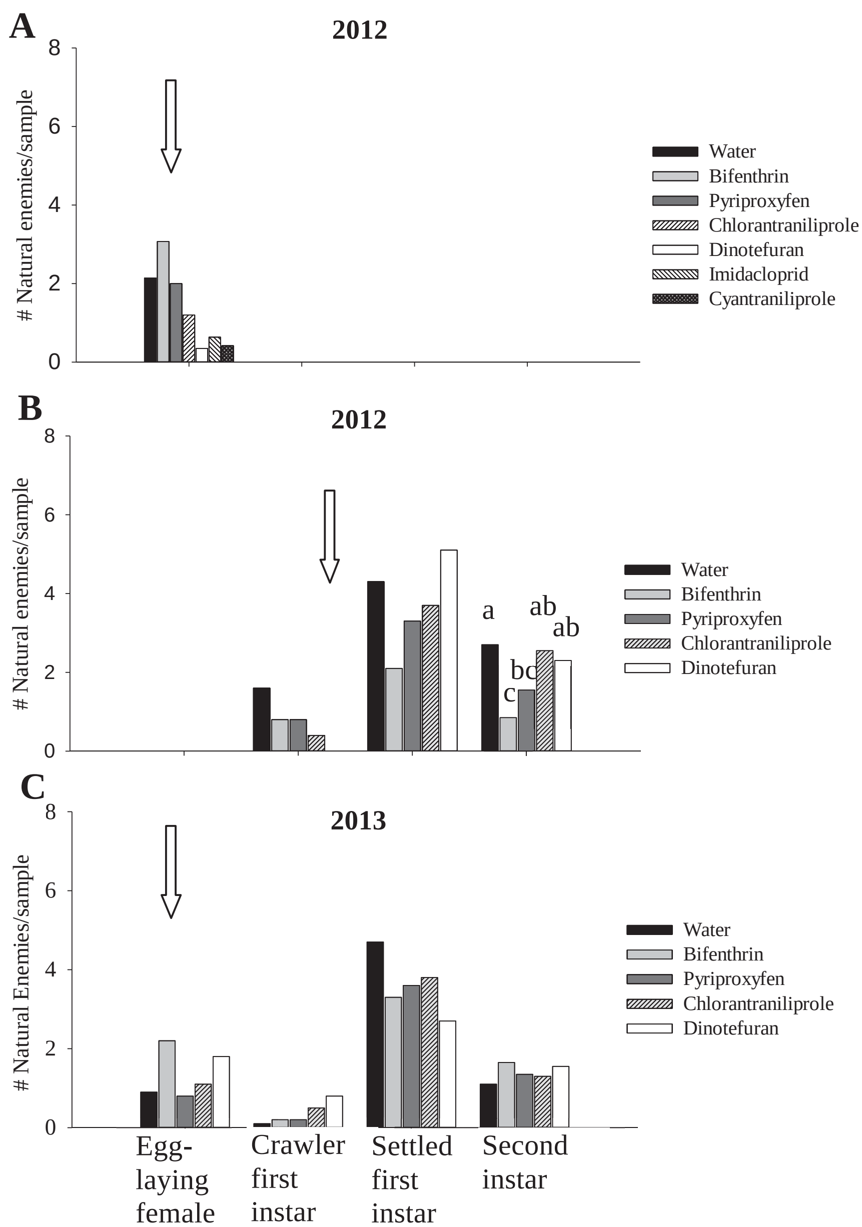


Figure 2.5 Number of natural enemies collected from vacuum samples of four 50 cm long on branches of honeylocust at different life stages of calico scale. Arrows indicate when pesticide applications were applied. Graph A contain data from Carmel, IN (application 20 March), B and C Fishers, IN (application 6 June and 3 May respectively).

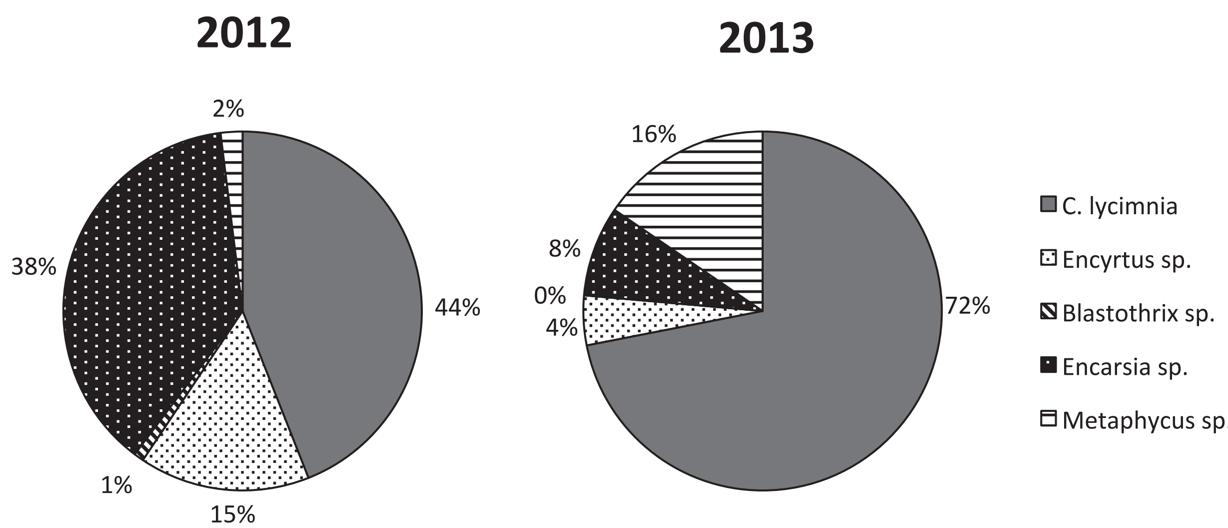


Figure 2.6 Percentage of parasitoids collected on sticky card placed on honeylocust trees during second instar of calico scale in Fishers, IN.

# CHAPTER 3. SUMMARY

Natural enemy efficacy may differ in natural environments than in urban areas. Forests can enhance the survival, fecundity and longevity of natural enemies of scale insects and their capacity to reduce insect populations (Hanks and Denno 1993). In contrast in urban areas the ability of natural enemies to regulate pest is affected by temperature, biodiversity, drought, pesticides, pollution, or nutritional imbalance (Hanks and Sadof 1990, Luck and Dahlsten 1975, Meineke et al. 2013, Raupp et al. 2001, 2010). Thus, effects of the urban ecosystem on natural enemies are generally believed to be an important contributor to pest outbreaks(Raupp et al. 2010). My research was conducted to determine how use of selected insecticides at labeled rates could control calico scale and alter the abundance of natural enemies of calico scale on naturally infested honeylocust trees (*Gleditsia triacanthos* var. *inermis*) in urban plantings.

We observed that both timing and choice of insecticide can influence how their application impacts both calico scale and its natural enemies. Over the course of this study, morphospecies of parasitoids were collected from five genera in two families: *Coccophagus lycimnia*, *Encarsia* (Aphelinidae: Hymenoptera), *Blastothrix*, *Metaphycus*, and *Encyrtus* sp. (Encyrtidae: Hymenoptera). Predators came from three families *Chrysoperla* sp (Chrysopidae: Neuroptera), *Orius* sp. (Anthocoridae: Hemiptera) and Coccinellidae: Coleoptera). Three insecticides were capable of reducing calico scale populations when they were applied to egg-laying females early in the season. Bifenthrin rapidly killed calico scale females causing mortality of up to 77%. This reduced crawler populations on the leaves. However, this application of bifenthrin failed to kill settled scales and reduce natural enemy populations on leaves later in the season. This is important for managing populations of scales because natural enemies were less abundant on both treated and untreated trees when scales were in this stage. By the time natural enemies reached their peak after crawlers had settled in mid June, residues of bifenthrin were no longer able to affect scales or natural enemies. This explanation is consistent with laboratories assays, where I detected that bifenthrin was not toxic to *Chrysoperla rufilabris* 50 DAT. However, there is evidence that application of bifenthrin early in the season might reduce predatory mites on honeylocust trees and cause spider mites (Witte 2013).

Dinotefuran also reduced scale populations when it was applied during the egg-laying female stage. Application of dinotefuran to the soil did not significantly increase mortality of these females. As a systemic insecticide, dinotefuran moves from the soil through the trunk into the leaves. Once in the leaves, it has the capacity to kill scales trying to settle and feed on leaf tissue. Although dinotefuran reduced scale density on leaves it did not reduce the abundance of scale natural enemies collected from branches. This would suggest that these natural enemies are not exposed to this compound when flying in the tree canopy. Nevertheless, there is evidence that some neonicotinoids can harm natural enemies of scale (Rebek and Sadof 2003). Other work suggests that their impacts on the natural enemies of mites and other beneficials can contribute outbreaks of mites (Szczepaniec et al 2011, 2013, Szczepaniec and *Raupp* 2012) and the death of pollinators (Krupke et al. 2012).

Cyantraniliprole was the third insecticide I found to reduce scale populations when applied during egg-laying female early in the season. Cyantraniliprole reduced females and calico scale densities on leaves, during both years of testing. Effect of cyantraniliprole on natural enemy abundance was not assessed.

In contrast to early season applications, only two insecticides were capable of reducing calico scale populations when they were applied to settled first instar scales in early June. Bifenthrin and pyriproxifen reduce both the densities of calico scales densities and their natural enemies. We suggest that even though they were applied during the crawler stage before the peak activity of natural enemies, these insecticides were still toxic to natural enemies when they were most abundant in late June and July. Even though statistically bifenthrin and pyriproxifen had the same effect on natural enemies, trees treated with bifenthrin had half the populations of natural enemies as pyriproxifen. This is consistent with our laboratory assay where bifenthrin reduced populations by 100% whereas pyriproxifen reduced them by 25% at 4 DAT respectively. These findings also support studies that compare the effect of insect growth regulators such as pyriproxifen and synthetic insecticides such as pyrethroids on natural enemies (Frank 2012, Grafton-Cardwell et al. 2003, 2006, Mendel et al. 1994, Rebek and Sadof 2003, Sadof and Sclar 2000, Schneider at al. 2003, Suma at al. 2009).

In conclusion, in situations where natural enemies are not capable of managing calico scale, applications of insecticide may be needed. When densities of calico scales are high and branches are being killed the landscape manager needs to consider the cost of each strategy in terms of how it will affect the tree and the natural enemy community. If trees can survive another spring with a relatively high population of scales, then a later season application of pyriproxifen may be the best strategy since it would avoid spider mite outbreaks and have less of an impact on scale natural enemies. In contrast, if tree health was severely threatened, early and late season applications of bifenthrin may be needed to reduce the population of scales without causing spider mite outbreaks. Although this may greatly reduce the natural enemies of scales in the tree canopy, it is likely to greatly lower the densities of scales and protect the tree. In the following year after the scale densities have been lowered a single application of pyriproxifen later in the season may be sufficient to reduce the populations and allow natural enemies to recover. Monitoring will be critical to evaluation the need for management each year.

## 3.1 Literature Cited

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