EFFECTS OF IMPERVIOUS SURFACES ON OVERWINTERING SURVIVAL OF EVERGREEN BAGWORM AND ABUNDANCE OF SCALE INSECT PESTS IN THE URBAN ENVIRONMENT

by

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I dedicate this work to my mother Ambika D Dawadi and father Chakrapani Dawadi.

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ABSTRACT

Urban areas are warmer than surrounding rural areas. During the cold of winter, warming increases surrounding host temperature and may improve the overwintering survival of marginally hardy insects like evergreen bagworms. Similarly, during the summer, it has the potential to increase the fecundity and abundance of sap feeding insect pests such as scale insects in ways that change the capacity of their natural enemies to regulate their populations.

Although in parts of Indiana winters can be cold enough to kill bagworm eggs, they thrive in cities. I conducted field experiments to determine the extent to which impervious surface near an infestation could keep temperatures warm enough to affect bagworm survival during cold of winter. My results suggest that the percentage of live eggs inside overwintering pupae decreased as ambient temperature drops. This response was moderated by the presence of impervious surface around an infested plant. Eggs found in bagworms collected from host trees surrounded by more impervious surface had a higher chance of survival than those collected from trees with low levels of hardscape. However, impervious surface has its limit such that egg mortality was not buffered by impervious surfaces at temperatures at or below -21.67°C. Similarly, I also conducted field experiments with sap feeding insects on honeylocust trees, a commonly planted tree in cities. Hot sites had a mean daily temperature more than 1.5 °C warmer than cool sites and scale insects were more abundant and fecund on trees in the hottest part of Indianapolis compared to cooler areas. No differences were observed in rates of parasitism on the scale insect. However, I found strong density dependence relation between parasitoids and scales abundance at scale density at or below the levels present in cool sites. The top-down regulation was prevalent at or below a critical density of scale hosts. Conversely, bottom-up regulation was prevalent above this host density as pests benefit from bottom-up factors. This suggests that urban habitats helped the scales to escape biological control by resident natural enemies above critical density of scale hosts.

My findings can be useful to landscape designers to design landscapes that are less prone to insect pests. My finding adds to a growing body of evidence that suggests that planting urban trees with lesser amount of impervious surface can help reducing the urban warming effect and increase the regulation from natural enemies.

INTRODUCTION: EFFECTS OF IMPERVIOUS SURFACES ON OVERWINTERING SURVIVAL OF EVERGREEN BAGWORM AND ABUNDANCE OF SCALE INSECT PESTS IN THE URBAN ENVIRONMENT

The Overview

The majority of people live in urban areas with more than 50% of world's population and expected to reach two third population by 2050 (UN-WUP 2018, Balk et al. 2018). Urban forest is key component of the urban ecosystem which can affect the health and quality of human life (Nock et al. 2013). Urban trees are the backbone of the green infrastructure, that provides direct aesthetic, economic, and public health value to the communities in which they grow (Nowak and Dwyer 2007). Numerous studies have quantified the extent to which urban trees can moderate temperatures, carbon dioxide sequestration, as well as reduce pollution, water runoff and soil erosion (McPherson et al. 2005, Dale et al. 2016). This dissertation addresses how urban habitats can influence arthropod pests that alter the health of trees in the urban forest.

Urban habitats have the potential to promote herbivorous pests by increasing host canopy temperature, changing pest phenology and inducing drought stress during the growing season. This phenomenon has been shown to be the case for armored and soft scale insects in cities along the eastern seaboard of the United States (Meineke et al. 2013, Dale et al. 2016, Dale and Frank 2017, Just et al. 2018). During the winter, urban warming has the potential to keep marginally hardy insects like the mimosa webworm (*Homadaula anisocentra*) warm enough to survive the winter (Miller 1984, Sperry et al. 2001). Recent development of a systematic pacing technique (pace to plant) has made it possible to measure the urban microhabitat in ways that can predict warming, drought, and the abundance of scale insects in Southern and Eastern US states (Dale et al. 2016, Just et al. 2018). This pacing process estimates the amount of impervious surface within an approximate 20 m radius of the host plant. I used this pacing technique and standard GIS methods to investigate how urban conditions influence the abundance of armored and soft scale insects and a marginally hardy caterpillar in urban Indiana landscapes.

Urban Conditions on Pest Survival

Urbanization has a wide range of effects on climate (Karl et al. 1988, Emilsson and Sang 2017). The 0.6° C increase in global temperature over the past century (Easterling et al. 1997, IPCC 2013) has greatly affected the phenology and distribution of native and exotic species (Broadmeadow 2002, Prather et al. 2013). For example, changes in climatic conditions have had notable effects on pest abundance and its distribution, population dynamics, intensity, and nature of herbivorous insect damage (Classen et al. 2005, Clissold et al. 2013, Rebaudo et al. 2016). Similarly, periods of extreme drought, heat, and cold occur more frequently and contribute to observed changes in insect survival and distribution (Hance et al. 2007, Rehman and Kumar 2018).

Impervious surface cover around a host tree can reduce water availability and induce heat stress. Heat stress (Cregg and Dix 2001, Jenerette et al. 2009) increases demand for transpiration (Whitlow and Bassuk 1988) in ways that can reduce root growth (Savi et al. 2015, Dale et al. 2016). The resulting conditions are positively associated with increased insect herbivory and loss of urban forest cover (White 1984, Meineke et al. 2013, Dale et al. 2016, Dale and Frank 2017).

Consequences of Pest Survival and Outbreak

High survival and abundance of insects on urban trees are associated with obvious and conspicuous damage. For example, the nature of damage can be seen due to the defoliation of the tree and bark damage. The most pronounced effects from defoliators on trees are direct growth loss, and reduced defenses, that can increase tree mortality due to elevated susceptibility to herbivory (Kulman 1971). Sap feeders, unlike defoliators, feed on sap assimilates from host tissues (Zvereva et al. 2010). The 52-research projects synthesized by Zvereva et al. (2010) quantified damage from sap feeders on trees. A 17% decrease in reproductive success, 29% decrease in growth, and 27% decrease in photosynthesis have been reported. Damage to host tissues and disruption of the photosynthesis apparatus, including xylem and phloem tissues, negatively impacts tree survival and longevity (Meineke et al. 2013, 2014). Loss of the urban forest can reduce ecosystem services that promote both human and environmental health (Sanesi et al. 2011, Donovan et al. 2013, Escobedo and Nowak 2009).

Drought and increased temperature are a major player in pest outbreaks (Meineke et al. 2013, 2014). In addition, physiological stress caused by urban environment increase host susceptibility to insect pests (Foggo et al. 1994, Koricheva et al. 1998, Cregg and Dix 2001, Dale

and Frank 2014, 2017). Because urban conditions weaken tree defense and increase chances of pest survival, many insect pests are capable of increasing their host and geographical ranges in cities (Denman and Webber 2009, Tubby and Weber 2010). However, urban conditions may not have an equal impact on arthropod natural enemies.

Natural enemies are often considered less abundant and more diverse in urban areas (Schultz 1990, Hodges and Braman 2004, Camacho et al. 2018). Natural enemy populations are associated with urban vegetation complexity and are capable of altering pest dynamics (Martinson and Raupp 2013). The enemies hypothesis predicts that natural enemies are more effective at pest regulation in diverse and complex habitat compared to simple habitat (Root 1973). For example, polyculture has higher natural enemy populations with lower pest population compared to monoculture as described in Andow (1991), Letourneau et al. (2011), and O'Rourke and Petersen (2017). However, urban areas are often associated with habitat loss and large areas of impervious surface (Peng et al. 2020, Cooper et al. 2021) that can hinder the dispersal capacity of natural enemy populations (Corcos et al. 2019, Peng et al. 2020). In contrast, urban areas with lesser impervious cover can have greater biodiversity (Lepczyk et al. 2017).

Modification in Urban Habitats

Many strategies are already in place to reduce the effect of urban warming to make human life comfortable. For example, use of air conditioning to control indoor air temperature (Feyisa et al. 2014) and green space establishment to regulate outdoor air temperature have long been in place. However, heat release from indoor air conditioning has enhanced the heat island effect (Tremeac et al. 2012). Similarly, structures and buildings emit carbon to outdoor environment (McKibben 2007). Thus, increased amount of impervious surface in outdoor urban environments has resulted in severe warming effects. Therefore, the minimization of impervious surface area and increase in green space in urban areas can reduce the effect of urban warming. Studies conducted to mitigate heat island effects have shown promising results where urban green space such as parks were relatively efficient in cooling surrounding environments (Oliveira et al. 2011, Susca et al. 2011). Pervious surfaces such as urban greenways not only mitigate thermal problems but provide many environmental and ecological services (Cornelis and Hermy 2004, Escobedo et al. 2010, Jana et al. 2010, Yin et al. 2011).

Despite its contribution to urban warming, impervious surface remains an integral part of development in cities. As such, it is difficult to completely remove impervious surface and introduce green space. Therefore, studies on the interaction between pervious and impervious surface and are needed in different regional and geographical areas to determine ways to balance both. One such study was done by Dale et al. (2016) where they developed a pace to plant technique to measure impervious surface area around 20 m radius of host maple trees. They determined that 33% or more impervious surface area is detrimental to red maple trees and can increase the chances of pest infestations. This pace to plant technique can be utilized by professionals, landscape designers and tree planters (Dale et el. 2016). This technique has been implemented for red maple tree plantings from Delaware to Florida, but has never been evaluated in the Midwestern US.

Two Major Sections of Thesis

I conducted two sets of experiment to identify the effect of urban conditions such as imperious surface area and temperature on overwintering survival of evergreen bagworm and summer outbreaks of scale insect pests. I investigated the effect of impervious surface on host tree canopy temperatures during winter and evaluated its capacity to protect insects when the ambient temperature dropped below levels known to kill them. (Chapter 1). I then conducted field experiments with sap feeding insects on honeylocust trees, a commonly plant tree in cities. I identified the hot and cool sites in Indianapolis using summer air temperature with LANDSAT thermal images and evaluated the performance of two scale insect species (Chapter 2). In addition, I investigated the influence of hot sites and impervious surface on the rate of parasitism on these scale insects (Chapter 2). Then, the capacity of natural enemy (parasitoids) was evaluated based on density dependence mechanism in hot and cool sites (Chapter 2).

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CHAPTER 1. URBAN MICROCLIMATE WARMING AS AN INDICATOR OF OVERWINTERING SURVIVAL OF EVERGREEN BAGWORMS

1.1 Abstract

In the northernmost latitude of its distribution in North America, the evergreen bagworm, *Thyriodopteryx ephemeraeformis* (Haworth), and its distribution is limited by overwintering temperatures. Impervious surfaces in urban landscapes can warm microclimates and create ecological temperature gradients that have the potential to increase winter survival of bagworms and other insects. To test this hypothesis, I evaluated survival of bagworms over gradients of microclimatic conditions. A total of 2255 bagworm bags were collected from 119 sites in Indiana and Illinois during the spring of 2018 and 2019. Up to 25 bagworms were removed from each host plant and assessed to determine the percentage of overwintering egg survival and bags producing viable larvae. I found the percentage of live eggs inside overwintering bags decreased as ambient temperatures fell below the killing threshold. Temperatures observed within the canopy of bagworm-infested plants increased within a 20 m radius of impervious surface cover. Eggs found in bagworm bags collected from host trees surrounded by more impervious surface area had a higher probability of survival than those collected from trees with low levels of impervious surface. Using the probit method, I determined that 50% bagworm eggs survived at maximum daily temperatures of -19.4°C, -20°C and -20.6°C when plants were surrounded by 25.7 %, 48.39% and 50.75% of impervious surface, respectively. Egg mortality was not buffered by impervious surfaces at temperatures at or below -21.67°C. My findings provide insights about how impervious surface can provide refugia for marginally hardy insects and improve their chances of surviving the cold of winter. Thus, given growing evidence that this landscape characteristic can also increase pest problems during the growing season, reducing impervious surface may be an important tool for designing pest resistant urban landscapes.

Keywords: microclimate warming, overwintering survival, impervious surface, evergreen bagworm, impervious surface threshold

1.2 Introduction

Urbanization can create ecological gradients by changing physical environments (Grimm et al. 2008). As such, urban and peri-urban landscapes have been used to test hypotheses about how these gradients impact arthropod communities (eg. Tooker and Hanks 2000, Raupp et al. 2010). Urban warming has the potential to promote herbivorous pests by inducing drought stress in plants during the growing season and changing phenology and fecundity (Meineke et al. 2013, Dale et al. 2016, Dale and Frank 2017). Urban microclimate warming is defined as a localized measure of the climate in the immediate vicinity of a plant and animal (Rosenberg et al. 1983); and is distinct from macroclimate which describes a larger area that extends beyond localized conditions.

The capacity of urban structures and impervious surfaces to increase temperature was first noticed in the 1830s (Howard 1833) and has long been considered to be a contributing factor to urban warming (Biswas and John 1900, Oke 1972, Oke 1981, Grimmond 2007, Lee and Kim 2008, Dale et al. 2016, Cui et al. 2016, Yan et al. 2016, Ren 2017). Recent development of a systematic pacing technique has made it possible to measure the urban microhabitat in ways that predict warming, drought, and the abundance of insect pests in southern and eastern USA (Dale et al. 2016, Just et al. 2018). However, such studies have only focused on sap feeders and their reproductive capacity. The effect of microclimate warming on overwintering survival of insect pests has yet to be fully described.

Microclimate warming has been associated with the abundance of insects at the northern edge of their distribution where it can buffer cold temperatures that cause substantial winter mortality. Mimosa webworm (*Homadaula anisocentra* Meyrick) provides a notable example of this on honeylocust trees (*Gleditsia triacanthos* L.) in Iowa and Indiana (Miller 1984, Sperry et al. 2001). Sperry et al. (2001) related the abundance of mimosa webworm and its natural enemies to the amount of impervious surface within 20 m of its host plants. Since then, little work has been conducted to explore the effect of urban warming on other lepidopterans (Battisti et al. 2005, Robinet et al. 2012). The evergreen bagworm (Lepidoptera: Psychidae, *Thyridopterix ephemeraeformis* Haworth) provides an opportunity to study effects of microclimate warming in cities because this insect is widespread and its geographic distribution is limited by season length and extremes of low temperatures (Lynch et al. 2014).

The evergreen bagworm is a common defoliating pest of ornamental trees and shrubs pests in eastern United States (Ellis et al. 2005, Rhainds et al. 2013). It has a broad host range (Johnson and Lyon 1991) but commonly feeds on *Juniperus* and *Thuja* species (Ghent 1999, Moore and Hanks 2004, Ellis et al. 2005) as well as trees and shrubs like *Acer, Quercus, Ulmus*, and *Gleditizia*. They are called bagworms because their worm-like larvae are concealed by spindle-shaped bags they construct from silken threads and host foliage (Davis 1964, Moore and Hanks 2004). During molting and pupation, they attach bags to the host branch with a band of silk. In late summer winged males mate with wingless females, after which a female lays eggs within its pupal case (Rhainds et al. 2013). Eggs winter within the female pupal case and remain covered by silken bags until they hatch in late spring of the following year (Ellis et al. 2005). Bagworms have only one generation a year (Morden and Waldbauer 1971) and disperse to other hosts or locations by ballooning (Moore and Hanks 2004).

In North America, the bagworm can be found as far north as Michigan and into Ontario, Canada (Rhainds et al. 2013). The winter survival along the northern edge of its range has been explained by season length and winter temperatures. Specifically, exposing bagworms to temperatures of -14 °C and -18 °C for 24 hours killed 50% of the eggs in masses that weighed 0.1 and 0.4 g respectively (Rhainds et al. 2013). Though broad climatic factors have played a significant role in northward expansion of the evergreen bagworm, microclimatic effects on temperature can be crucial to overwintering survival. The potential impacts of urban microclimate warming on overwintering egg survival have not been studied.

In this study, I explore the extent to which urban microclimate warming affects winter survival of bagworms. I hypothesize that impervious surface influence microclimates that affect bagworm overwintering survival in the Midwest. In addition, I also predict that the pace to plant technique for measuring impervious surfaces is sensitive enough to predict the overwintering survival of evergreen bagworms.

1.3   Materials and Methods

1.3.1   Site Selection

Sites for bagworm collection were selected based on the availability of infested plants and the distribution of extreme cold temperatures during the winters of 2017-18 and 2018-19 in Indiana

and Illinois prior to March 1 of each year. Based on data available from the Weather Channel website (weather.com), January 1 was the coldest day in 2018 and February 2 was the coldest day in 2019. I chose the Weather Channel as my data source because it is commonly used and easily obtained by the general public. The maximum temperature extreme cold days ranged from -20°C (-4°F) to -17.22°C (1°F) at 72 sites in 2018 and from -22.22°C (-8°F) to -17.22°C (1°F) in at 47 sites in 2019 (Appendix D). Those sites were selected randomly from bagworm-infested sites within each temperature range.

1.3.2   Infested Host Selection and Bagworm Collection

Bagworm-infested trees and shrubs were identified, collected, and GPS coordinates were recorded with an iPhone 6 during March 12-17, 2018 and during March 11-16, 2019. Briefly, I asked the iPhone voice assistant the question "Where am I?" while standing next to a bagworm-infested host and saved the results as a photograph of the screen image. Each of these screen images contained the GPS coordinates that I used to characterize the location of each tree. A total of 1303 bagworms were collected from 72 sites in Indiana and Illinois with latitude ranging from 39.93° to 40.50° in 2018. Similarly, a total of 952 bagworms were collected from 47 sites with latitude ranging from 39.93° to 41.47° in 2019. However, only 58 sites from 2018 were included in my study because bagworms in the remaining 14 sites had no intact pupal case inside overwintering bags. Depending on the existing infestation level, up to 25 female bagworms (at least 6 in 2018 and at least 8 in 2019) were removed from each host plant and placed into locking plastic bags. Bags were brought into the laboratory and kept at room temperature (25˚C) in lab for 1 week before assessing overwintering survival of eggs, and the capacity of each clutch of bagworm eggs to produce live larvae.

1.3.3   Impervious Surface Area Estimation

I used a pacing technique (pace to plant) (Dale et al. 2016) to estimate impervious surface area within an approximate 20 m radius of the host plant. 'Pace to plant' is a tool developed to measure amount of the impervious surface area that surrounds the tree or specific site. Briefly, this was accomplished by identifying the nearest impervious surface to the host tree and having the same observer use the step technique. The first 25 step transect was walked along a 45° angle from

the tree base location towards nearest impervious surface edge and counting the number of steps that touched impervious surface. Then three more transects were taken at 90° angles to complete the circle. The percentage of impervious surface area around each host plant was calculated as the total number of steps that touched an impervious surface. The average distance (19.81±0.15 m) covered by the single observer who counted these steps was estimated by measuring the distance covered at 15 random landscape locations in West Lafayette, IN.

1.3.4 Overwintering Survival of Eggs

Pupae were extracted from each bagworm and weighed to the nearest 0.001 gm. The body length of each pupa was also measured to the nearest 0.001 mm. Two pupae of near average weight were sampled from each site to estimate the percentage of overwintering survival of eggs. Each of the two pupae were dissected so that the first 50 eggs could be examined to estimate percentage of viable eggs (Rhainds et al. 2013). Live and dead eggs were classified based on their color following Rhainds and Sadof (2009) where white eggs were counted as live and black eggs as dead.

1.3.5 Bags Producing Viable Larvae

Remaining bags were kept in the cups in the lab at room temperature for 12 weeks and monitored until larvae hatched (Rhainds et al. 2013). Overwintering bags that produced at least one live larva were counted as viable bags. All unhatched female bags were dissected to make sure whether they have larvae inside.

1.3.6 Relationship Between Temperature and Impervious Surface

The temperature of the bagworm host trees and shrubs were monitored in 20 sites from West Lafayette, and Lafayette, IN were selected from December 15, 2019 through April 15 2020. A total of 20 iButton® Thermochron® DS1921G (Embedded Data Systems, LLC, Lawrenceburg, KY, U.S) temperature loggers were individually kept inside an inverted 60 ml translucent plastic cup (Dart container cooperation, Mason, Michigan, USA) to protect it from precipitation. Cups were affixed to trees and shrubs at breast height (1.37m from soil line) using plastic zip ties. The temperature loggers were set to record at 2-hour intervals. To determine the buffering effect of

impervious surface during cold snaps I focused my analysis on days when the maximum T was at or below the freezing temperature of water, $\leq 0°C$. On these days at each time point, I assessed the differences between the average measured air temperature at the two sites with no impervious surface and the air temperature at each site with known impervious surface. The ability of impervious surface to predict this temperature difference was determined via simple linear regression.

1.3.7 Statistical Analysis

Each year, sites were grouped by the maximum temperature during the coldest day to the nearest °F. The first analysis was performed to determine if impervious surface, weight, or lengths of overwintering pupae affected one of two estimates of bagworm survival; overwintering eggs and viable bags. This analysis was accomplished using forward stepwise regression (PROC REG, SAS 9.4) for all sites that were in the same temperature grouping. The model starts with a null model and adds or removes predictor variables based on the capacity to improve the p value of each model by 0.05. The proportion of overwintering egg survival (out of 50 eggs) and viable bags were transformed using Arcsine square root transformation for analysis. Original values were presented in graphs. In regression analyses, vif (variance inflation factor) and tol (tolerance) diagnostic options were added to the model to check for collinearities between the predictors. In addition, other criteria such as R^2 (coefficient of determination), adjusted R^2, Mallow's Cp, and Akaike's Information Criterion (AIC) were used in the model to support the results.

A separate probit analysis (PROC PROBIT, SAS 9.4) was conducted to determine the amount of impervious surface required for 50% overwintering egg survival (IS_{50}) for each group of sites with the same maximum daily temperature. In this procedure, the predictor variable, impervious surface cover, was log transformed and the response variable, percentage mortality was transformed to its probit value prior to calculating the regression equation.

1.4   Results

1.4.1   Overwintering Survival of Eggs

Collection sites had different temperature extremes during coldest day of both years. The maximum temperature on extreme cold recorded were -20°C (-4°F) to -17.2°C (1°F) in 2018 and -22.2°C (-8°F) to -17.2°C (1°F) in 2019 respectively. The stepwise model using three different variables to explain overwintering survival of eggs retained impervious surface in the model. Impervious surface was significant when temperature was at or below -18.9°C in 2018 (at -20°C: $F_{(1,4)}$ =7.17, P= 0.05; at -19.4°C: $F_{(1,18)}$ =11.16, P= 0.004; at -18.9°C: $F_{(1,4)}$ =17.68, P= 0.025) (Figure 1.1A) and when the temperature was at or below -20°C in 2019 (at -22.2°C: $F_{(1,8)}$ =15.50, P= 0.004; at -21.7°C: $F_{(1,6)}$ =18.12, P= 0.005; at -20.6°C: $F_{(1,4)}$ =11.73, P= 0.019) (Figure 1.1B). However, the effect of impervious surface was not significant (P< 0.05) when temperature was -18.9°C in 2019. Neither variable, weight or length contributed enough to the model (criteria for entry or exit was p=0.05) to be included in the analysis. As a general rule of thumb, vif higher than 10 and tol lower than 0.1 gives possible sign of the collinearities between predictors (Marcaulides and Raykov 2019). My results were within these criteria and as such that there were no collinearities between predictors. In addition, impervious surface explained much of the variation in overwintering egg survival both years.

The probit analysis estimated the amount of impervious surface required for 50% of bagworm eggs to survive at sites with different maximum daily temperatures (Figure 1.2). Fifty percent of bagworm eggs survived at maximum daily temperatures of -19.4°C, -20°C and -20.6°C when plants were surrounded by 25.7 %, 48.39% and 50.75% of impervious surface, respectively (Appendix A). Egg mortality was not buffered by impervious surfaces at temperatures at or below -21.67°C.

Figure 1.1. Effect of impervious surface on overwintering survival of evergreen bagworm eggs in 2018 (A) and 2019 (B). Impervious surface was measured by the pace to plant technique within a 20 m radius of infested host. Number of eggs live out of first 50 eggs were counted and changed to percentage of egg survival.

Figure 1.2. Estimated impervious surface area (IS$_{50}$) required for 50% survival of evergreen bagworm eggs at sites grouped by maximum daily temperature on the coldest day of winter (Tmax) around 20 m radius of bagworm-infested host tree. Imaginary probit impervious surface area beyond 100 estimated from the regression procedure are displayed to reveal lower confidence limits within the realistic probit range. When a Tmax groupings had an IS$_{50}$ >100, impervious surface was unable to increase probit survival to 50%. The solid lines represent that IS50, lower and upper 95% CI fall within 100% estimated impervious surface threshold. However, dotted lines represent IS50 and upper 95% CI fall above 100% estimated impervious surface threshold.

1.4.2 Capacity of Bagworms to Produce Viable Larvae

Of the three different variables (Impervious surface, weight and length of pupae) examined in the stepwise regression procedure, only impervious surface met the criteria for inclusion in the model that predicted overwintering survival of larvae in 2018 and in 2019. Impervious surface was significant when the temperature was at or below -18.9°C in 2018 (at -20°C: $F_{(1,4)}$ =12.41, $P=$ 0.024; at -19.4°C: $F_{(1,18)}$ =12.16, $P=$ 0.002; at -18.9°C: $F_{(1,4)}$ =12.59, $P=$ 0.023) (Figure 1.3A) and when the temperature was below -18.9°C in 2019 (at -22.2°C: $F_{(1,8)}$ =6.93, $P=$ 0.03; at -21.7°C: $F_{(1,6)}$ =9.55, $P=$ 0.021; at -20.6°C: $F_{(1,4)}$ =7.70, $P=$ 0.039) (Figure 1.3B). At temperatures above 18.9°C there was no effect of impervious surface. There were no collinearities between predictor variables.

Figure 1.3. Effect of impervious surface on production of viable bags (evergreen bagworm bags producing live larvae) in 2018 (A) and 2019 (B) respectively. Impervious surface was measured by pace to plant technique within a 20 m radius of the infested host. Number of bags producing larvae out of total for each temperature location was converted to percentage viable bags.

1.4.3 Determining the relationship between temperature and impervious surface

There were only five days in winter 2019-2020 when maximum daily temperature was ≤ 0 °C (Figure 1.4). Therefore, only five days were analyzed to explore the effect of impervious surface on increase in host canopy temperature. Impervious surface around each infested host had a small, but significant effect on host canopy temperature ($F_{(1,478)} = 5.02$, $P = 0.025$) (Appendix

B). The site with 97% impervious surface was 1.3°C warmer than the site with no impervious surface cover.

Figure 1.4. The daily host canopy temperatures from 01/06/2020 to 02/29/2020 of evergreen bagworm-infested plant in 97% impervious surface cover and no impervious surface cover around 20 m of plant. Temperature loggers were used to monitor canopy temperature within 2-hour intervals. Total 5 days of extreme low temperature (maximum temperature of the day $\leq$ 0 °C) days were analyzed and marked in the graph (see Appendix C). Solid line represents the temperature recorded from infested host having no impervious surface whereas dotted line represents from infested host having 97% impervious surface. The five rectangles and arrow with date in main graphs represent the coldest recorded days during winter of 2019-20.

1.5 Discussion

The survival of evergreen bagworm during two Indiana winters was increased by the microclimate warming caused by impervious surfaces within 20 m of the host plant. The other two predictor variables, bagworm pupal weight and length did not significantly predict bagworm

survival. In addition, the pace to plant method to quantify impervious surface area was sensitive enough to predict increases in bagworm survival. Although I found the impervious surface increased the temperature of the microclimate, its capacity to insulate bagworms from the lethal effects of cold had its limits. When temperatures fell to -21.7° C (-7° F) and below, impervious surface had no effect on survival.

It was impossible for us to monitor the effects of impervious surfaces on temperatures at each of my study sites because they needed to be selected at the end of winter after the cold temperatures occurred. As such, my measurements of winter temperatures in 2020 were used to determine the extent to which impervious surfaces affected tree canopy temperatures in my study region. found that trees completely surrounded by impervious surface (97%) experienced temperatures that were about 1.3 °C warmer compared to hosts not surrounded by impervious surface (0%) during coldest day of 2020. The magnitude of the increase in temperature caused by impervious surfaces is similar to that reported by other investigators. For example, Dale and Frank (2014) found the 2°C increase in summer temperature due to impervious surface in urban areas was enough to allow scale insects to survive, reproduce and damage maple trees. My findings are also consistent with more general studies that demonstrate that impervious surface is capable of augmenting temperatures year-round (Myint et al. 2013).

My results and observations indicate that overwintering survival of evergreen bagworm can be ascertained from the percentage of viable overwintering eggs and overwintering bags producing live larvae. However, the percentage of surviving eggs was more sensitive to temperature and impervious surface for two reasons. First, calculating the mortality from a set number of eggs held for each overwintering female provided a consistent sample from every tree. In contrast, the number of bags collected at each site ranged between 4 and 25 which mathematically altered the possible proportions of live bagworms at each site. Furthermore, the live larval method does not distinguish between bags with one or many survivors. Overwintering survival of eggs has been used by other researchers to examine effects of latitude, and weight of overwintering survival of bagworm (Rhainds et al. 2013). For these reasons my discussion of how microclimate affects bagworm will focus on egg survival.

I observed bagworms surviving at lower maximum daily air temperatures than reported by others. Rhainds et al. (2013) found 50% of bagworm eggs to survive when temperature fell to -18 °C and only about 1% survival when temperature reached about -19.5 °C. This freeze threshold

is mostly calculated from rural environments which lacked the impervious surfaces that I studied. Rhainds et al. (2013) also assessed egg survival across five degrees of latitude (36.5-41.5 °N). Weights of egg clutches varied enough within this range to alter egg survival. In my study I reduced the variation in survival caused by differences in the weight of egg clutches by collecting bags within a smaller latitudinal range of 1.44° (39.93 to 41.47° N). This leaves the effects of impervious surfaces on air temperature as the most likely factor to explain variation in the observed level of egg survival.

Landscape designers seeking to identify sites where plants are more likely to survive, can benefit by being able to recognize threshold values of landscape characteristics associated with increased pest abundance and decreased plant health. Logistic regression and probit analysis have the potential for identifying specific thresholds for plants and pests. Using logistic regression, researchers were able to predict that more than 50% of red maples planted across the Southeastern USA were more likely to be considered in good condition, when surround by 36% or less impervious cover (Just et al. 2018). Similarly, I used a probit analysis to identify impervious surface thresholds associated with 50% mortality of bagworm eggs in years the coldest day had a maximum temperature of -5 to -2° F. While my findings support my hypothesis that impervious surfaces alter the overwintering survival of bagworms, microclimate warming is insufficient to protect bagworms when the temperature dips down below a critical limit. Thus, the capacity to use impervious surface to predict the overwintering mortality of bagworms can only be applied within specific temperature ranges.

My study contributes to a growing body of literature on how cold temperatures affect the survival of lepidoptera. It also demonstrates how the capacity of impervious surface to alter temperature can affect insect survival. The urban landscape provides a natural laboratory to test the effect of urban settings on biological entities. Few studies have examined the effect on overwintering survival of lepidopteran pests in urban conditions. For example, mimosa webworm pupae overwintering in an urban environment which was 2.5-5°C warmer than nearby rural areas had increased levels of survival compared to rural areas (Miller and Hart 1987). Similarly, Sperry et al. (2001) related the abundance of mimosa webworm with the amount of impervious surface within 20 m radius of its host plants and found increased population and damage on plants with increasing amount of impervious surface. Other investigations into the effect of cold temperatures on overwintering caterpillars have mainly focused on cold hardiness and effect on body size and

weight (Pullin and Bale 1989), spring emergence (Mironidis et al. 2010) and survival of overwintering host plants (Liu et al. 2007). My study provides insight into how urban conditions can create refugia from lethal temperatures during the extreme cold of winter.

The relationship I have found between overwintering survival of bagworm and impervious surfaces complements research on how these surfaces affect the abundance of arthropods feeding on plants during the growing season (Coulson et al. 1993, Bale et al. 2002, Meineke et al. 2013, Just et al. 2018). In particular, sap feeding arthropods are widely studied in urban conditions (Koricheva and Larsson 1998, Herms 2002, Huberty and Denno 2004, Dale and Frank 2017). Studies on gloomy scale (*Melanaspis tenebricosa*) (Dale and Frank 2014, Just et al. 2018) and the oak lecanium scale (*Parthenolecanium quercifex*) (Meineke et al. 2013, 2014) in the southern and eastern US showed the higher abundance of overwintering female population in hotter part of the cities compared to cooler areas. The abundance of horsechestnut scale (*Pulvinaria regalis*) (Speight et al. 1998), gall midges and spider mites (Frankie et al. 1987), and lace bugs (Cregg and Dix 2001) abundances have also been reported to be elevated in highly disturbed urban areas.

1.5.1 Conclusion and Future Recommendations

Small changes in temperature associated impervious surfaces in urban areas can profoundly affect pest abundance. In this way, increased pest abundance caused by the elevated temperatures of impervious surfaces may be indicative how climate change could affect pest problems. Increasing urban greenspace is one of many strategies to reduce effects of urban microclimate warming. However, impervious surface responsible for warming and increased pest abundance remains an integral component of city infrastructure. Studies like my s, that elucidate the relationship between impervious surface and survival of overwintering pests complement other studies that focus on changes in the pest populations during the growing season. Armed with a simple method to quantify impervious surface, pest managers can predict which pest problems are likely to be more abundant due to overwintering survival or enhanced growth of pest populations. Conversely, landscape designers can use this tool to design landscapes that are resistant to insect pests. Ultimately, this work is part of a growing number of studies that shows how reducing impervious surfaces can create more sustainable urban landscapes.

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1.6 Acknowledgements

1.7 References

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CHAPTER 2.   RESPONSE OF TWO SCALE INSECTS AND THEIR NATURAL ENEMIES TO URBAN CONDITIONS ON HONEYOCUST

2.1   Abstract

Urban habitats can alter tree physiology in ways that make trees more susceptible to insect pests. Although this phenomenon has been documented extensively along the eastern seaboard of the US on oak and red maple trees, it has not been examined on other tree species and regions. In this chapter I examined how urban conditions (e.g., impervious surface and warming) affected two species of scale insects and the health of honeylocust trees (*Gleditsia tricanthos* L. var. inermis) in Indianapolis, Indiana, USA. The two species included a, soft scale, (Hemiptera Coccidae) *Parthenolecanium corni* Bouché (the European fruit lecanium) and an armored scale (Hemiptera: Diaspididae), *Chionaspis gleditsiae* Sanders (honeylocust scurfy scale). Hot and cool sites were selected for study based on surface air temperature obtained from thermal LANDSAT-8 images overlaid against map of honeylocust tree locations. Based on temperature probe data, study trees located in hot sites had a mean average daily temperature that was up to 1.56 °C and 1.79 °C warmer than cool sites in 2019 and 2020. I hypothesized that abundance and fecundity of scale insects would be higher on trees in hot sites and those surrounded by more impervious surface within a 20 m radius. Although my data supported these hypotheses, impervious surface predicted more of the variation in scale abundance of both scales and the fecundity of *P. corni* in hot sites compared to cool sites. Rates of parasitism of each scale species was not affected by impervious surface area or whether the tree was located in a hot or cool site. My examination of density dependence and parasitism of *P. corni* found a strong density dependence in both hot and cool sites when the scale density was at or below the levels present in cool sites. This suggests that the natural enemies I studied could reduce *P. corni* populations only at or below a critical density of scale hosts through top-down regulation. Above this host density there was no relationship as pests benefit from bottom-up factors. My findings add to a growing body of evidence that suggests that increased global temperatures can increase pest abundance in ways that cannot be mitigated by local populations of natural enemies.

Keywords: Urban habitats, impervious surface, European fruit lecanium scale, honeylocust scurfy scale, abundance and fecundity, top-down regulation, density-dependent

2.2   Introduction

Urbanization is a global phenomenon that describes changes associated with localized increases in the concentration of human populations. This phenomenon often results in covering more areas with impervious surfaces (Bierwagen et al. 2010) to support roads, parking lots, airports, buildings, and walkways (Bauer et al. 2017). Paved infrastructure has been associated with the creation of urban heat islands (UHI) that contribute to the warming of cities (Oke 1972, 1973, 1981, Grimmond 2007, Lee and Kim 2008, Cui et al. 2016, Ren 2017).

Urban areas have been reported to be up to 10 °C warmer than surrounding countryside or rural areas (Oke 1973, Kim 1992, Cregg and Dix 2001). Impervious surface around a tree can reduce water availability and induce drought conditions (Elmes et al. 2017). Heat stress can increase the vapor pressure deficit of plants (Cregg and Dix 2001, Jenerette et al. 2009) and lead to a greater rate of transpiration (Whitlow and Bassuk 1988). During drought, trees can become stressed, and reduce their root growth (Savi et al. 2014, Dale et al. 2016). This can result in conditions that favor damaging outbreaks of herbivorous arthropods (White 1984, Meineke et al. 2013) by changing both pest and natural enemy population dynamics in the urban forest (Ayres and Schneider 2009). Loss of the urban forest can reduce ecosystem services that promote both human and environmental health (Sanesi et al. 2011, Donovan et al. 2012, Escobedo and Nowak 2009). Therefore, cities stand to benefit from a healthy urban forest.

Sap feeding arthropods are particularly positioned to benefit from the changed urban conditions such as impervious surface and increased temperature (Koricheva et al. 1998, Speight et al. 1998, Herms 2002, Huberty and Denno 2004, Dale and Frank 2017). This has been shown to be the case for the gloomy scale (Heteroptera: Diaspidae *Melanaspis tenebricosa*) and the oak lecanium scale (Heteroptera: Coccidae *Parthenolecanium quercifex*) insects feeding on maples and oaks in North Carolina and along the Eastern Seaboard of the United States (Meineke et al. 2013, 2014, Dale et al. 2016, Dale and Frank 2014a, 2017, Just et al. 2018). Populations of these herbivorous insects benefit from improved nutrition on water stressed trees and a reduced and asynchronous natural enemy population (Meineke et al. 2014). A 2°C increase in the temperature of urban areas raised the fecundity of *M. tenebricosa* on *Acer rubrum* trees (Dale and Frank 2014a). Similarly, the scale insect *P. quercifex* was 13 times more abundant on willow oak trees in the hottest parts of Raleigh, NC compared to cooler areas.

As heat and water stresses on urban trees can make them more prone to insect attack, it is imperative for landscape designers and planners to identify the sites least likely to promote these stresses. This has been accomplished by quantifying the impervious surface area around trees that stores soil moisture. An approach called 'Pace-to-Plant' has been used in cities along the Atlantic coast from Georgia to New York to measure the abundance of impervious surfaces within 20 m of a host tree and predict the abundance of scale insects on red maple and oak trees (Meineke et al. 2013, Dale et al. 2016, Just et al. 2018). Impervious surface along with urban warming is associated with both insect pest abundance and tree health decline (Dale and Frank 2014b). In addition, this approach has been used to develop impervious surface thresholds along the southeastern USA for to select suitable sites for planting free of scale insect problems (Just et al. 2018). Because of its success with scale insects on maples and oaks over a large geographic area, I hypothesize that it could also predict summer performance of other scale insects on other trees infested with scale insects in different parts of the USA. As such it could help urban foresters and planners identify sites where certain trees could be prone to problems with sucking insects on other commonly planted trees.

In this chapter I explore the relationships between the abundance of scale insects on honeylocust trees (*Gleditsia tricanthos* L. var. inermis) and the impervious surface around each tree. Honeylocust trees are native to North America and popular in cities because of their compatibility with turf and their capacity to tolerate adverse environmental conditions (Bassuck et al. 2009). Common insect pests of honeylocust include mimosa webworm (*Homaduala anisocentra*), honeylocust plant bug (*Diaphnocoris chlotionis*), and honeylocust spider mite (*Platytetranychus multidigituli*) (Sperry et al. 2001). Several soft scale species (Hemiptera: Coccidae) including calico scale (*Eulecanium cerasorum*) and the European fruit lecanium *Parthenolecanium corni* (Bouché) have been reported to commonly infest honeylocust trees (Willams and Kostarab 1972, Quesada et al. 2018, Quesada and Sadof 2019). Honeylocust scurfy scale (*Chionaspis gleditsiae* Sanders) (Hemiptera: Diaspididae) is a specialist armored scale that attacks mainly *Gleditsia triacanthos* (Kosztarab 1996).

The first objective of the chapter is to identify the relationship between urban conditions (impervious surface cover and temperature) and the abundance and fecundity of scale insects. Here I test the hypothesis that the impervious surface around the host plant will positively affect these scale fitness parameters. The second objective is to identify the impact of scale populations and

the health of street trees. This will be accomplished by testing the hypothesis that scale abundance is related to an objective measure of tree health. Finally, the third objective is to test the hypothesis that populations of scale are regulated by natural enemies or top-down regulation. This will be accomplished by looking density dependence (Solomon 1949, Hassel 1966, May et al. 1981, Morgan et al. 2017) in the relationship between parasitized scale insects and the total abundance of *P. corni* hosts on urban trees.

2.3   Materials and Methods

2.3.1   Urban Forest and Study Location Characteristics

The study was conducted in city of Indianapolis, IN (39.7684° N, 86.1581° W), located in the Midwestern region of the United States. Elevation in this city ranges from 198 to 274 m above sea level. Indianapolis is in a humid climatic zone where summers are usually hot, humid and wet and winters are cold with moderate snowfall. Maximum temperature reaches 32°C during summer and minimum temperature dip down to -18°C during winter. Rainfall usually occurs during spring and summer with higher average of 5.1 to 12.8 cm of precipitation in the month of May (NowData-NOAA 2021).

According to a street tree inventory conducted in 2014 by the Indianapolis Parks Department there were 228,497 trees from 30 genera in its urban forest. Of these, a total of 2816 thornless honeylocust trees (*Gleditsia triacanthos* inermis) were present in the street tree database. Locations of honeylocust trees mapped in the survey were used to assist in selecting study sites.

2.3.2   Thermal Map

A shape-file map of Indianapolis was provided by Purdue University Libraries (Figure 1). I formed the base layer of a thermal map in ArcMap (ArcGIS Desktop 10.5, Redlands, CA) using summer temperature LANDSAT-8 band following methods described by Meineke et al. (2013). The LANDSAT-8 band with WRS path 21, row 32 images were acquired on 16 August, 2017. Thermal band images were converted to at-satellite brightness temperature (°C). The converted images were representative of air temperature where high temperature was represented by red color and blue represents the cool temperature (Figure 2.1). The same color representation was used to select hot and cool sites.

Figure 2.1. Map of Indianapolis, IN overlaid with thermal imaging and 2×2 km square grids with 48 sampled honeylocust trees represented as black dots. These trees were sampled from one hot site (red thermal area) and one a cool site (blue thermal area) in 24 grids selected via a stratified random sampling. The thermal map of Indianapolis, IN was created from Landsat-8 image acquired in 16 August, 2017 as described in the text.

2.3.3   Sites and Tree Selection

The thermal map laid over the Indianapolis map was divided into four quadrants. A fishnet tool in ArcMap was used to create 2×2 km square grids within each quadrant (Figure 1C). Six 2×2 km grids were randomly selected from each quadrant. From each selected grid, two honeylocust trees were randomly picked from each grid so that one was selected from a hot site and the other was from a cool site. All together 48 trees were selected from 24 grids (Figure 2.1). The diameter of the trees at 1.37 m above the ground ranged from 4.46 cm to 60.51 cm during the spring of 2018. Each tree site was at least 345 m away from the other tree site in the grid.

2.3.4   Impervious Surface Area Estimation

I used the pace to plant technique (Dale et al. 2016) to estimate impervious surface area within an approximate 20 m circumference of each host plant. Briefly, this was accomplished by identifying the nearest impervious surface to the host tree and taking four 25 step transects and recording the number of steps taken on an impervious surface. The transect length made by the single observer who counted steps was estimated to be 19.81 ± 0.15 m after measuring the distance covered by 15 transects at random landscape locations in West Lafayette, IN. The percentage of impervious surface area around each host plant was calculated as the total number of steps that touched an impervious surface. The first 25 step transect was walked along a 45° angle from the tree base location towards the nearest impervious surface edge. A second 25 step transect was made at from the tree base at a, 90° angle to the first transect. The process was repeated until four transects were completed.

2.3.5   Canopy Temperature Measurement

A total of 48 iButton® Thermochron® DS1921G (Embedded Data Systems, LLC, Lawrenceburg, KY, U.S) temperature loggers were kept inside 60 ml translucent portion containers (Dart container cooperation, Mason, Michigan, USA) and hung inverted. Cups were tied with zip tie bands at 2.5 meters height on the branch (Appendix E.A). The temperature loggers were set to record temperature every two hours from 10 April to 10 September in 2019 and 22 May to 10 September in 2020. Travel restriction due to COVID in 2020 delayed the installation of data loggers.

2.3.6   *Chionaspis gleditsiae* Abundance

The abundance of overwintering mated female *C. gleditsiae* on each tree was estimated by collecting four 30.5 cm twigs containing one-year old wood were collected from each tree in October of 2018 and 2019. Preliminary studies conducted in the spring of 2018 revealed that crawlers hatching from this univoltine species settled on the twigs before shoots elongated. Therefore, one-year old twigs only contained a single generational cohort of scales. A pole pruner was used to cut branches from each cardinal direction approximately 2.5 m above the soil line. Sampled branches were kept in a black plastic trash bag and labeled with a tree identification

number. Branches were kept inside a refrigerator at 2°C until they were assessed for the abundance of scale insects. Total number of females were counted from each branch under a dissecting microscope. Scale insects were flipped and if body was juicy and expulsed fluid, they were considered alive. Dead scales were dry, dehydrated and flaked off easily.

2.3.7 *Parthenolecanium corni* **Abundance**

Immature stages of *P. corni* scales move from leaves to branches in the fall. Adults mate when accumulated base 10°C degree days is around 150-175 in mid-May (Williams and Kosztarab 1972). After mating females fill with eggs and move the site where they will feed and grow. Females lay eggs that hatch within 2-3 weeks. Therefore, to obtain samples of adult females from the same generational cohort, four twigs (30.5 cm long) were sampled from new growth during first week of June in 2019 and 2020 when accumulated growing degree day was 370 at 10°C base temperature. A pole pruner was used to cut branches from each cardinal direction approximately 2.5 m above the soil line. The lowest branches were collected if they were greater than 2.5 m above the soil. All excised branches were kept in a black plastic trash bag and labeled with a tree identification number. Branches were kept inside walk-in refrigerator at 2°C until they were assessed for female scale insect abundance. Total number of females were counted from each branch under a dissecting microscope. The live and dead scales were identified as described in previously for the scurfy scale.

2.3.8   **Parasitism rate of *Parthenolecanium corni***

Total number of females *P. corni* collected during 2019 and 2020 were counted and dissected under a dissecting microscope to identify those that were parasitized. First, parasitized females were separated based on the presence of exit holes made by parasitoids and counted (Japoshvili et al. 2008). Second, remaining females were dissected to determine if there was any presence of parasitoids inside them, or if they were unparasitized. Rate of parasitism was calculated from the total female scales present on sampled twigs.

2.3.9 Fecundity of *Parthenolecanium corni*

A maximum of 15 non-parasitized *P. corni* scales were kept singly in 75% ethanol. Each scale was examined with a dissecting microscope to determine the fecundity of each female using the following procedure. Each vial containing a single female was poured into a 100 mm × 15 mm petri dish and dissected under microscope to release the all the eggs into the alcohol mixture. After dissection, enough alcohol was added to the petri dish so the liquid was 5mm deep. The petri dish with eggs was placed on the top of a printed counting disk having 12 equal sectors (Appendix E.E). Eggs were distributed equally on the disc by shaking and kept still after settling. All eggs in each petri dish were counted when a female had <200 eggs. When more eggs were present only four sectors from counting disk were used to count number of eggs and multiplied by 3 to get total number collected from each female.

2.3.10 Parasitoid Identification

To identify the parasitoids of *P. corni* scale, four 30.5 cm branches were collected on 4 June 2019. Branches were kept in cardboard tubes (10.5 cm diameter and 18 cm depth) at room temperature at 22 ˚C (Quesada et al. 2018) (Appendix E.B). Parasitoids that emerged from cardboard tubes were separated into morphospecies. Voucher specimens of each morphospecies were sent to Matt Bertone (North Carolina State University) for identification (Appendix E.F).

2.3.11 Tree Health

The health of each tree in this study was assessed during summer 2021. The overall health of trees was estimated following the criteria developed by Dale and Frank (2014a) and Just et al. (2018) and scored as excellent, good, fair, poor or dead. Excellent trees had no dead branches and fully-grown canopies with healthy leaves. Good trees had minimal dead branches with fully-grown canopies (<10% canopy thinning). Fair trees had some dead branches with some root exposure and small canopy dieback (<30% canopy thinning). Poor trees had high root exposure, severe dieback (>30% canopy thinning) and injuries. Dead trees had no or minimal canopy left (>80% canopy thinning) with injuries and severe root exposure.

2.3.12 Statistical Analysis

Differences in the abundance of adult female scale insects, fecundity, and parasitism in hot and cool sites were analyzed, with a repeated measure ANOVA using SAS (PROC GLM with a repeated statement, SAS 9.4). The simple regressions were carried out to assess the effect of impervious surface on abundance, fecundity and rate of parasitism (PROC REG, SAS 9.4). The rate of parasitism was transformed using the arcsine square root transformation for analysis purpose but original percentage value is presented in the graphs. The slopes of regression lines for hot and cool sites for abundance, fecundity and parasitism were compared using a Student's t-test. The effects of year were analyzed comparing the regression slopes for each year using t-test. Delayed density dependence of scale abundance on parasitism rate was examined by comparing abundance of live *P. corni* females in 2020 to rate of parasitism in 2019. The tree health data were analyzed using ordinal logistic regression with the effect of site (hot and cool), impervious surface and abundance of *P. corni* and *C. gleditsiae* (PROC LOGISTIC, SAS 9.4). For this, tree health data were ranked 1-5 (1- Excellent, 2-Good, 3-Fair, 4-Poor and 5- Dead) and site coded '1' for Hot and '0' for Cool. The daily mean temperature was calculated for entire period of temperature recording. The PROC GLM (SAS 9.4) was used to compare average daily mean temperature between hot and cool sites in 2019 and 2020 using entire period in a time series. Average daily temperatures for each tree location data were analyzed separately for each year because differences in the temperature recording periods. A separate regression analysis was carried out between average temperature (of entire period from 22 May – 10 September) for 28 tree locations of scale infested trees and surrounding impervious surface.

2.4 Results

2.4.1 *Chionaspis gleditsiae* Abundance

The mean numbers of females per tree were about 3.5 times higher in hot sites compared to cool sites ($F_{(1,41)} = 10.67$, $P=0.002$; Figure 2.2). There was an overall increase in scale insect population in 2019 compared to 2018 ($F_{(1,41)} = 4.25$, $P=0.046$) but no interactions were there between year and spot ($F_{(1,41)} = 1.81$, $P=0.186$). The regression procedure indicated a significant increase in female abundance due to impervious surface ($F_{(1,45)} = 5.52$, $P=0.023$; Figure 2.3), with R^2 of 0.109. The R^2 values between total scale abundance and impervious surface were 0.05 and

0.12 for cool sites and hot sites respectively. The slope of the lines for hot sites was nearly double that for cool sites ($t_{44}=2.03$, $P=0.048$; $\beta_{Hot}=0.109\pm0.062$, $\beta_{Cool} = -0.027\pm0.025$).

Figure 2.2. *Chionaspis gleditsiae* abundance (±SEM (standard error of the mean)) across Indianapolis, IN in Spring 2018 and 2019 from four 30.5 cm sections of previous year's growth. Scale insect densities were significant higher in hot sites compared to cool sites (*P<0.05*).

Figure 2.3. Effect of impervious surface around 20 m tree host canopy on the abundance of *Chionaspis gleditsiae* from four 30.5 cm twigs collected from honeylocust trees during 2018 and 2019. Effect of impervious surface on female scale abundance was significant at *P < 0.05*.

2.4.2 *Parthenolecanium corni*

Abundance

The mean numbers of total female scales per tree were 3.3 times higher in hot sites compared to cool sites ($F_{(1,40)} = 7.2$, *P*=0.011; Figure 2.4). There was neither a significant effect of year ($F_{(1,40)} = 0.59$, *P*=0.447) nor any interaction between year and designation as a hot or cool site ($F_{(1,40)} = 0.64$, *P*=0.427). The regression analysis showed a significant increase in female abundance with impervious surface ($F_{(1,40)} = 6.28$, *P*=0.016; Figure 2.5), with an R^2 of 0.12. The R^2 values between total scales and impervious surface were 0.02 and 0.16 for cool sites and hot sites respectively. The slope of the lines for hot sites was about 12.5-fold greater than cold sites (t_{44}=2.18, *P*=0.035; β_{Hot}=0.365±0.176, β_{Cool} = -0.029±0.044).

Figure 2.4. Mean number of female *Parthenolecanium corni* (±SEM) across Indianapolis, IN in summer of 2019 and 2020 from four 30.5 cm terminal branches of each tree. Scale insects were significant higher in hot sites compared to cool sites (*P*=0.05).

Figure 2.5. Effect of impervious surface around 20 m tree on the abundance of *Parthenolecanium corni* on four 30.5 cm twigs collected from honeylocust trees during 2019 and 2020. Effect of impervious surface on female abundance is significant at *P < 0.05*.

Fecundity of *P. corni*

      Female scales were about 2 times more fecund in hot sites compared to cool sites ($F_{(1,12)}$ = 72.10, *P*<0.001; Figure 2.6). There was neither a significant effect of year ($F_{(1,12)}$ = 0.43, *P*=0.255) nor any interaction between year and spot ($F_{(1,12)}$ = 1.25, *P*=0.285). Female fecundity increased with increase in impervious surface ($F_{(1,12)}$ = 14.28, *P*=0.003) with an R^2 of 0.45. The R^2 value between total eggs and impervious surface were 0.06 and 0.23 for cool sites and hot sites respectively. There was no significant difference in slope of the lines for hot and cool sites (t_{28}=0.98, *P*=0.337; β_{Hot}=18.015±9.596, β_{Cool} =6.568±6.743).
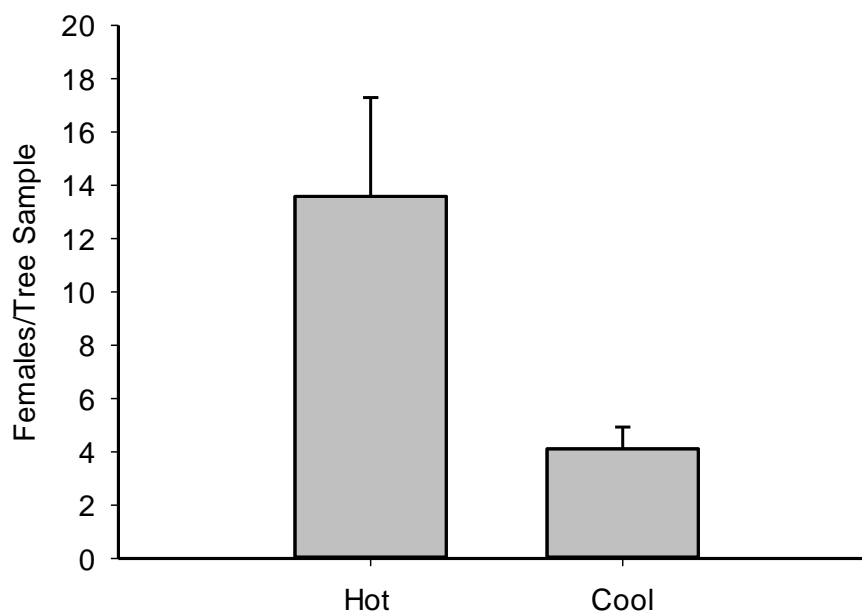

Figure 2.6. *Parthenolecanium corni* fecundity (±SEM) across Indianapolis, IN in Summer 2019 and 2020 from four 30.5 cm terminal branches of each tree. Scale insect were more fecund in hot sites compared to cool sites (*P<0.05*).
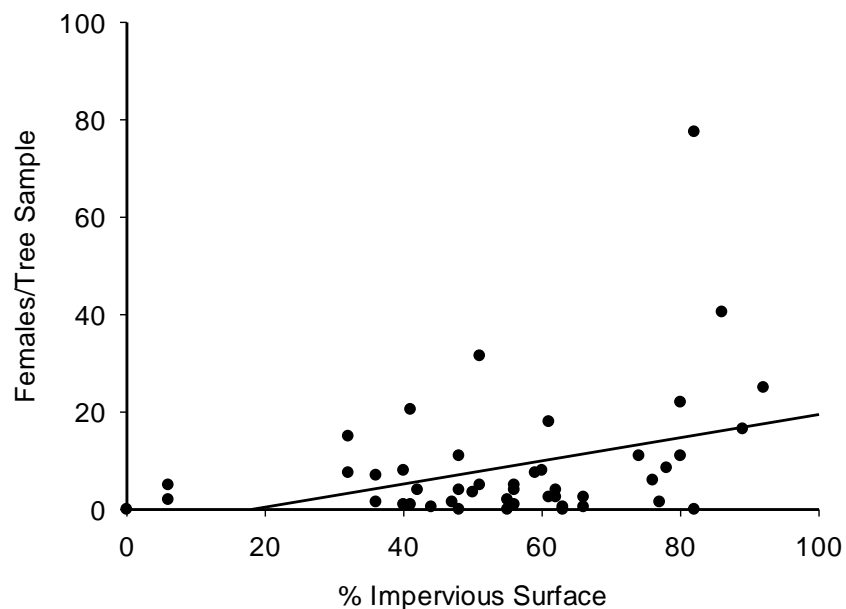
Figure 2.7. Effect of impervious surface on female *Parthenolecanium corni* scale fecundity during 2019 and 2020. Mean number of eggs counted per females are presented. Effect of impervious surface on female scale fecundity is significant at *P < 0.05*.

Parasitism Rate of *P. corni*

The rates of parasitism of *P. corni* were similar in hot sites and cool sites ($F_{(1,31)} = 0.15$, $P=0.705$; Figure 2.8). There was no effect of year ($F_{(1,31)} = 1.77$, $P=0.193$) nor any interaction between year and site designation as either hot or cold ($F_{(1,31)} = 0.08$, $P=0.783$). There was no increase in rate of parasitism with increase in amount of impervious surface ($F_{(1,31)} = 0.18$, $P=0.671$). No evidence for delayed density dependence of scales on parasitism rate was found. The rate parasitism in a given year (n) was not significantly related to the abundance of female scales in the following year (n+1) ($F_{(1,34)} = 2.44$, $P=0.127$).
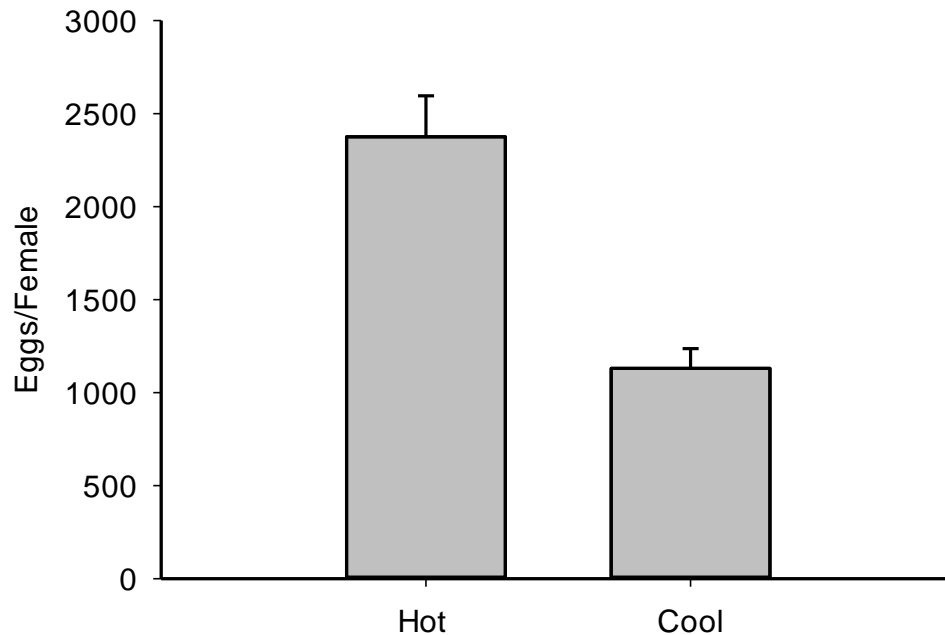
Figure 2.8. The rate of parasitism (±SEM) on *Parthenolecanium corni* across Indianapolis, IN in Summer 2019 and 2020 from all 30.5 cm terminal branches of each tree. No difference in parasitism rate between hot and cool sites (*P*>0.05).

Density Dependence of Scale Parasitism

The average density of total *P. corni* female scale insects was 9.93±2.19 females per four-twigs sampled from each tree. The highest number of females recorded on a tree sample in a cool site were 15 in 2019 and 17 per tree sample in 2020. However, hot sites had up to 90 and 65 scale insects per tree sample in 2019 and 2020 respectively. Overall, there was a significant increase in parasitized scales as the total density of scales increased ($F_{(1,39)}$ = 60.19, *P*<0.001; Figure 2.9), with an R^2 of 0.61. The R^2 value between total scales and parasitized scales were 0.83 and 0.56 in cool and hot sites respectively. Within the overlapping densities of scales in hot and cool sites the respective R^2 values were similarly high (Hot R^2=0.84, Cool R^2=0.83). There was also no difference in slope of the lines (t_{29}=1.46, *P*=0.15; β_{Hot}=0.756±0.094, β_{Cool}=1±0.064). Population densities higher than the 17 per sample found in cool sites had no effect on number of parasitized females ($F_{(1,6)}$ = 0.66, *P*=0.447; Figure 2.9), with an R^2 of 0.09. The parasitoids reared on *P. corni* came from seven different families of Hymentoptera: Aphelinidae, Encyrtidae, Eulophidae, Petromalidae, Platygastridae, Ceraphronidae and Cynipidae (Table 2.1).

Figure 2.9. Relationship between total *Parthenolecanium corni* density and parasitized scale in overall population (black regression line, $R^2=0.61$), in the hot sites (red regression line, $R^2=0.56$) and cool sites (blue regression line, $R^2=0.83$) (B) across Indianapolis, IN in honeylocust trees (n = 40) from four 30.5 cm terminal branches of each tree in 2019 and 2020. Blue dots represent scale insect populations in cool sites whereas red dots represent scale insect population in hot sites.

Table 2.1. List of scale insect parasitoids collected from four 30.5 cm branches of honeylocust tree infested with *Parthenolecanium corni* kept in cardboard tubes (10.5 cm diameter and 18 cm depth) at room temperature at 22°C.

Order	Family	Subfamily	Genus	Comments
Hymenoptera	Aphelinidae	Coccophaginae	*Encarsia*	
Hymenoptera			*Coccophagus lycimnia*	Male/Female
Hymenoptera	Eulophidae	Tetrastichinae	*Aprostocetus*	
Hymenoptera		Eulophinae	*Pnigalio*	Male/Female
Hymenoptera	Encyrtidae	Encyrtinae	*Metaphycus*	
Hymenoptera			*Encyrtus*	
Hymenoptera	Petromalidae	Petromalinae	*Pachyneuron*	
Hymenoptera	Platygastridae	Platygastrinae	*Synopeas*	
Hymenoptera	Ceraphronidae		*Aphanogmus*	Male
Hymenoptera	Cynipidea		*Ceroptresini*	

2.4.3 Tree Health

Out of 37 trees evaluated for tree health during 2021, 34 trees were at least in fair condition (1 excellent, 17 good and 16 fair). Only three trees were found in poor condition and no trees were dead. Three trees found in poor condition are from hot sites. Total 17 trees were in good condition and only 7 of those in hot sites. One tree found in excellent condition was from cool sites. There was no effect of site (hot and cool) (χ^2 (1) = 0.77, $P=0.55$), impervious surface (χ^2 (1) = 0.2, $P=0.65$) and abundance of scale insect (scurfy: χ^2 (1) = 0.15, $P=0.7$; lecanium: χ^2 (1) = 0.44, $P=0.51$) on tree health (Appendix F).

2.4.4 Tree Canopy Temperature

There was significant difference in average daily mean temperature between hot and cool sites in 2019 (F $_{(1,306)}$ = 4.06, $P=0.045$; Figure 2.10A). The hot sites were up to 1.56°C higher in mean daily temperature compared to cool sites in 2019. Similarly, hot sites were up to 1.79°C higher in mean daily temperature compared to cool sites in 2020 (F $_{(1,222)}$ = 16.03, $P=0.001$; Figure 2.10B). The average temperatures of entire recorded period for every tree location were positively associated with impervious surface in both years (F $_{(1,27)}$ = 36.28, $P=0.001$; Figure 2.11). However, the degree of variation explained was higher in hot sites ($R^2=0.59$) compared to cool sites ($R^2=0.46$).

Figure 2.10. Daily average temperatures (±SEM) recorded between hot and cool sites in Indianapolis, IN during 10 April – 10 September, 2019 (A) and 22 May- 10 September, 2020 (B). Red and orange dots represent the mean daily average temperature on hot and cool sites respectively. Temperature probe iButtons were installed on trees to record temperature in 2-hr intervals for entire period.

Figure 2.11. Effect of impervious surface on average temperature recorded during 22 May – 10 September during 2019 and 2020 for 28 tree locations with scale insects. Effect of impervious surface on average temperature is significant at *P<0.05*.

2.5   Discussion

This study provides evidence to support the hypothesis that impervious surface and elevated temperatures are associated with increased abundance and fecundity of scale insects on honeylocust trees in Indianapolis, IN. However, honeylocust tree health was not severely affected. In addition, neither impervious surface nor hot site had any effect on the average rate of parasitism of scale insects. This was likely caused by a lack of density dependent parasitism above the densities of scales found in cool sites. In contrast there was strong density dependent parasitism in both hot and cool sites at or below densities recorded in cool sites. These findings suggest top-down regulation from natural enemy is prevalent at low host densities. However, as host density increases above a critical limit due to increased host suitability in hot sites, bottom up regulation prevails and trees are likely to suffer from heavy infestations of scale insects. This elevated rate of growth in urban pest populations suggest that continued urban development and global warming could increase the stress on the health of urban forests.

Survival and fecundity of arthropods are profoundly influenced by temperature (Brier et al. 1999). The direct measure of canopy temperatures supported the predictions of my LANDSAT-8 thermal map. The average daily temperatures of hot sites were warmer than cool sites. Differences between the average daily temperatures were more than 1.5°C in both years in Indianapolis, IN. Similar results have been reported in other studies where mean average temperature in hot sites were up to 2.4°C higher compared to cool sites in Raleigh, NC (Meineke et al. 2013). Elevated temperature can boost insect metabolic activity (Ray 1960), quicken their developmental rate and increase egg production (Yasuda 1983, Dale and Frank 2014). My study supports these general findings. Both *C. gleditsiae*, and *P. corni*, were more abundant in hot sites compared to cool sites. Although I did not measure the fecundity of scurfy scale, the two-fold increase I found in the fecundity of *P. corni* suggests that warmer habitat benefits scale insects. These findings are consistent with other investigations of scale insects. For example, a key pest of red maple, gloomy scale *Melanaspis tenebricosa*, was found to be physically larger in hot sites compared to cool sites and produced eggs earlier in the season (Dale and Frank 2014a). In addition, Mieneke et al. (2013) found oak lecanium *Parthenolecanium quercifex* was more abundant on willow oak trees in hot sites compared to cool sites in Raleigh, NC.

I found a significant positive relationship between the impervious surface within 20 m of the host plant and the abundance and fecundity of scale insects, that was stronger in hot sites compared to cool sites. This suggests that both elevated temperatures and impervious surface could have also increased scale insect abundance and fecundity. Other investigators found that impervious surfaces reduced the water available to trees in ways that reduced their water potential and increased the abundance of scale insects (Dale and Frank 2014a, 2014b, 2017). This was explained by the putative increases in the availability of nutrients in plant sap based on numerous studies relating the abundance and fecundity of the Coccoidea to sap nutritional content (e.g. Sadof and Raupp 1992, Sadof et al. 2003, Dale and Frank 2017).

Urban habitats accompanied by increased impervious surface (or reduced vegetation cover) and elevated temperature (both urban heat island effect and anthropogenic heat source) are prerequisites to induce stress on plants (Oke 1973, Kim 1992, Dale and Frank 2014). For example, soil compaction and reduced water availability to plant roots (Jenrette et al. 2009), greater vapor pressure deficits with water loss through transpiration (Whitlow and Bassuk 1988), and disruption in the mechanism of stomatal conductance (McDowell et al. 2008) can have detrimental effect on

tree health and growth. In addition, trees in poor health are prone to attack by many herbivorous pests (Meineke et al. 2013, Just et al. 2018). Studies on gloomy scale infestations on red maple (Dale and Frank 2014, Just et al. 2018), and oak lecanium on willow oak trees (Meineke et al. 2013, 2014) also demonstrate the influence of impervious surface on scale abundance and tree health.

Although my findings support other research on the impacts of impervious surfaces, it differs in a number of key respects. Unlike the work of Just et al. (2018), the densities of scales I encountered had little impact on host tree health. In addition, I saw no increase in the populations of *P. corni* during my study. Nevertheless, on average, populations of scale insects were threefold higher in hot spots compared to cool spots. This contrasts with the 200-fold increase in populations of *M. tenebricosa* in red maple trees in hot sites compared to cool sites in Raleigh, NC (Dale and Frank 2014b). Furthermore, *M. tenebricosa* population increases are thought to be part of a long-term trend associated with the warming of cites the southeastern USA (Youngsteadt et al. 2015). Nevertheless, overall, the health of honeylocust trees were poorer in hot sites than cool sites. This suggests that as temperatures warm, the health of trees in hot sites could continue to decline and that increases in the reproductive rate of scale insects could further contribute the deterioration of their health in the future.

Although the fecundity and abundance of *P. corni* increased in hot sites, there was no corresponding increase in the average rate of parasitism in overall population. Meineke et al. (2013) also found similar rates of parasitism in hot and cool sites in Raleigh, NC. That means that the rate of biological control did not respond to population differences in hot and cool sites. Despite the lack of difference, my regression analysis shows the overall relationship between total scale insects and parasitized scale insects is significant ($R^2=0.61$). This suggests the kind of positive density dependence with successful biological control of scale insects (eg. Tooker and Hanks 2000). However, separate analyses revealed that scale density explained more of the variation in parasitism in cool sites ($R^2=0.83$) than in hot sites ($R^2=0.56$). Because the average scale density in hot sites is greater than in cool sites, this effect could be the result of both differences in scale density and temperature. When I examined rates of parasitism in hot and cool sites on trees where the densities of scales were all less than or equal to the maximum density in cool sites, I found no differences in variation explained by scale density (Cool $R^2=0.84$, Hot $R^2=0.83$). In contrast, there was a weak relationship ($R^2=0.09$) between density of scales and parasitism in hot sites at

infestation densities greater than those found in cool sites. Taken together, at lower scale host density there was density dependent response of natural enemy.

The relationship between natural enemy and prey host as a numerical and functional response is first described by Solomon (1949). Hassell (1966) described these terms more precisely by classifying as a natural enemy response to prey density relating them to the three types of functional response given by Holling (1959). The relationship I observed between parasitism and density of scale insects is similar to a type I functional (Hassel 1966) where number of parasitized *P. corni* increased with density of *P. corni* population until it reaches to maximum density at cool sites. Density dependent parasitism has been shown to be a critical to the regulation of scale insects by their parasitoids, most notably in the example of how the California red scale, *Aonidiella aurantia* is regulated by hymenopterous parasitoid *Aphytis melinus* in citrus orchards (Murdoch et al. 2006). Yet despite my observation of density dependence, there was no evidence of delayed density dependence of *P. corni* on the previous year's rate of parasitism. This may be due the relative stability of the population of *P. corni* as evidence by a lack of change in scale populations during the two years of my study.

Although density dependence is popularly used to justify the case of biological control (Crawley 1992, VanDriesche et al. 1996), host-parasitoids interaction can persist in systems with top-down and bottom up regulation (Hassell 2000). The top-down regulation or consumer-based hypothesis is common regulation in natural systems such that host populations are often determined by impact from higher trophic level (Leroux and Schmitz 2015, Chidawanyika et al. 2019). On the other hand, the bottom-up or resource-based hypothesis states that the resources such as light, nutrients and primary producers at lower trophic level are key determinants of success at higher trophic level (Albittar et al. 2019, Chidawanyika et al. 2019). My study provides evidence of enhancement in populations of higher trophic level (parasitoids) especially at or below host densities recorded in cool sites were capable of regulating the lower trophic level (scale insects). This top-down regulation is common in agricultural systems (Balmer et al. 2013, Tan et al. 2019), forest ecosystem (Cuevas-Reyes et al. 2007, Staab and Schuldt 2020) and urban landscape (Rebek et al. 2006, Raupp et al. 2010). On the other hand, trees in hot sites in urban habitats are prone to water stress which increases the sugar and nitrogen concentrations in plants (White 1984, Koricheva et al. 1998). In addition, hot sites are warmer which also allows pest to develop and reproduce faster. Therefore, resources such as warmer temperature, sugar and nitrogen

are key elements in the success of pest herbivores in hot sites. This is indicative that bottom-up regulation is more influential at higher host densities in hot sites than in cool sites where top-down regulation prevails.

I found parasitoids from ten genera in seven families. The study conducted by Camacho et al. (2018) in the Southeastern United States with *Parthenolecanium* spp. and their parasitoids found parasitoids from 13 genera of four families. It is not surprising that the study like my s in one city the size of Indianapolis would have lower richness of parasitoid species compared to a study conducted in 4 states (Camacho et al. 2018). In addition to similar parasitoids reported in Camacho et al. (2018), I also found few morphospecies in genera that are not previously mentioned as parasitoids of Coccidae, *Pnigalio* spp. (Eulophidae) *Ceroptresini* spp. (Cynipoidea) and *Synopeas* spp. (Platygastridae). As these parasitoids were reared from twigs and not isolated scale hosts, it is difficult to be sure if they are parasitoids of scales, hyperparasitoids or inquiline species.

Natural enemies are often considered more diverse (Schultz 1990, Hodges and Braman 2004, Camacho et al. 2018) and less abundant (Meineke et al. 2013) in urban areas. Change in natural enemy populations alter the arthropod abundance and diversity (Dreistadt et al. 1990, Frankie and Ehler 1978, Rickman and Connor 2003). In addition, vegetation complexity also plays role to provide food source for natural enemies to alter the pest dynamics in urban areas (Martinson and Raupp 2013). The natural enemies (the enemies hypothesis) hypothesis states that regulation in pest populations can be achieved by increasing habitat complexity which support natural enemies (Root 1973). Numerous studies have examined the effect of plant diversity on the abundance of herbivores arthropod pests and natural enemies. These studies found host plant diversity (polyculture) increases the natural enemies while decreases the pest populations compared to monoculture (Andow 1991, Letourneau et al. 2011, O'Rourke and Petersen 2017). Loss of biodiversity and species loss is well documented in urban areas (Peng et al. 2020, Cooper et al. 2021). In addition, anthropogenic structures in cities can limit the dispersal of natural enemies and hence negatively impact natural enemy abundance and diversity (Corcos et al. 2019, Peng et al. 2020). However, the habitat complexity differs spatially where cool sites in cities (e.g. green space or proximity to water) can facilitate biodiversity conservation (Lepczyk et al. 2017). This variability results in increase in pest fitness and decrease in control from natural enemies in hottest part of cities as found in my study. Other studies also related urban areas are prone to pest outbreak (Hanks and Denno 1993, Raupp et al. 2010) or loss of biological control (Meineke et al. 2014).

Overall, the abundance of scales on Midwest honeylocust trees is not as severely threating to tree health as gloomy scale is to red maple trees in the Eastern United States. However, the scale insects are still benefiting from impervious surface and elevated temperature as bottom-up regulation identified especially in hot sites. Despite the difference in scale insect populations, biological control was not responding similarly. Though hottest part in cites lack biological control, density dependent response of parasitoids was seen and identified as a top-down regulation at low scale host density especially in cool sites.

2.5.1   Conclusion and Recommendations

As human populations grow and urbanization increases, pest problems are likely to worsen when the capacity of pests to reproduce is enhanced beyond the regulatory capabilities of local populations of parasitoids and predators. Therefore, it is important to manage urban forest with sustainable practices that can mitigate pest threats stemming from site conditions. My finding suggests that planting urban trees with lesser amount of impervious surface can help reduce the urban warming effect and increase the regulation from natural enemies. Other sustainable practices may include planting trees tolerant to urban conditions, less susceptible to pests and increasing planting diversity or vegetation complexity (Dale and Frank 2014b). Even though urban areas with high impervious surface are harmful to urban forests, this relationship can be used to identify high risk areas where susceptible trees need to be more intensively monitored and managed, or simply replaced with tree species less prone to pest outbreaks. Future studies should focus on long term evaluation of pest abundance, biological control and tree health with changing urban environment.

2.6   Acknowledgements

2.7 References

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APPENDIX A. IMPERVIOUS SURFACE THRESHOLD FOR 50% EGG SURVIVAL

Table A1. The regression equations for each maximum daily temperature group that was $\leq$ -19.4°C (-3°F) during winter 2017/2018 and 2018/2019. The impervious surface area threshold (IS_{50}) is calculated based on regression equation for 50% overwintering egg survival of evergreen bagworm. Locations were sampled from Indiana and Illinois. Evergreen bagworms were sampled form evergreen trees.

Maximum daily Temperature	Regression Equation	Impervious Surface Threshold* (IS_{50})	95% CI of Probit-Mortality at IS_{50}	
			Lower	Upper
-19.4°C (-3°F)	Y=0.6307x+4.0817	25.7%	12.26	53.86
-20°C (-4°F)	Y=4.6204x-2.9219	48.39%	37.06	63.18
-20.6°C (-5°F)	Y=2.9382x-0.0268	50.75*	43.31	59.46
-21.7°C (-7°F)	Y=0.9132x+3.0906	100%**	56.27	251.02
-22.2°C (-8°F)	Y=1.4751x+1.9008	100%**	79.82	187.08

*The percentage of impervious surface within 20 m of the host plant required to survive 50% of overwintering eggs (IS_{50}).

**No amount of impervious surface increased survival of overwintering eggs to 50%. Imaginary Probit estimated impervious surface threshold beyond 100 are displayed as 100% impervious surface. However, the estimated impervious surface requirement at -7 and -8°F were higher than 100%.

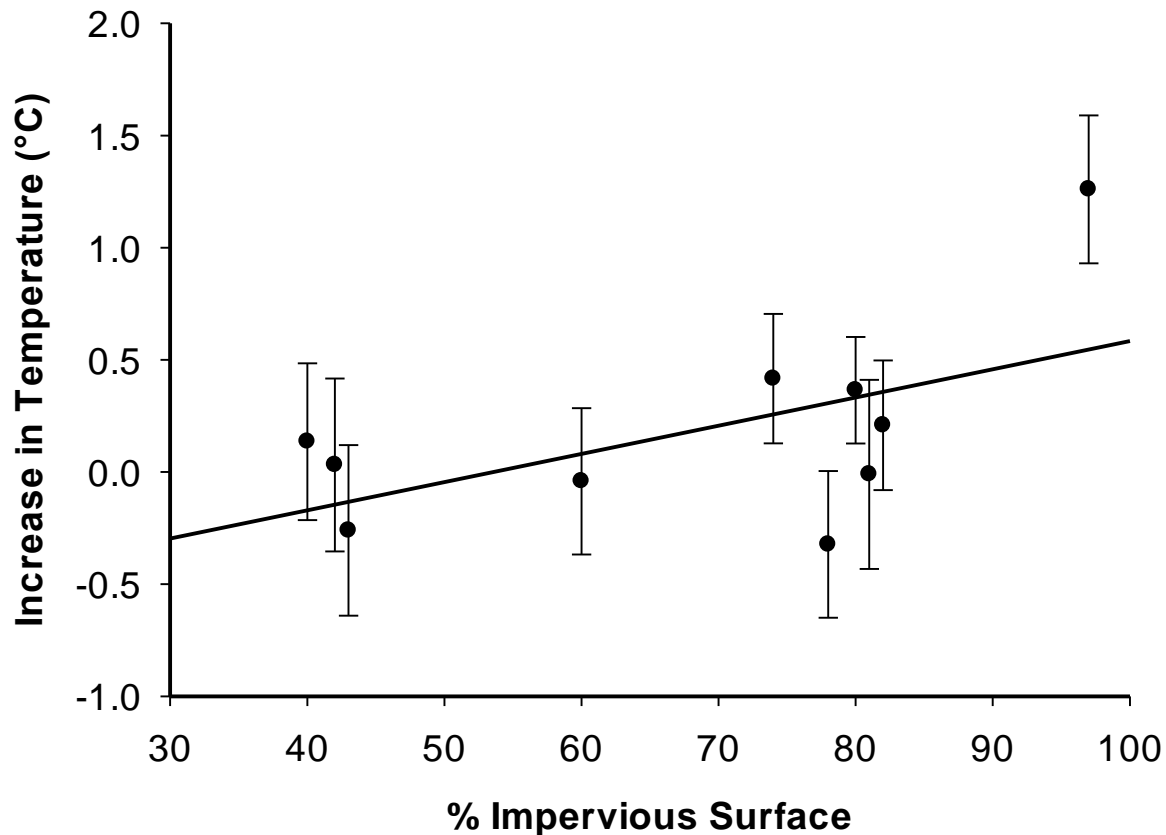# APPENDIX B. IMPERVIOUS SURFACE EFFECT ON TREE CANOPY TEMPERATURE

Figure B1. Effect of impervious surface on increase in evergreen tree host canopy temperature ($F_{(1,478)} = 5.02$, $P=0.025$). Temperature loggers were used to monitor canopy temperature within 2-hour intervals in winter 2020/2021. Total 5 days of extreme low temperature (maximum temperature of the day ≤ 0 °C) days were analyzed. Increase in temperature is difference between host canopy temperature when host is surrounded by gradients of impervious surface and no impervious surface. Impervious surface was measured by pace to plant technique around 20 m radius of infested host. The study trees were from Lafayette and West Lafayette, Indiana.
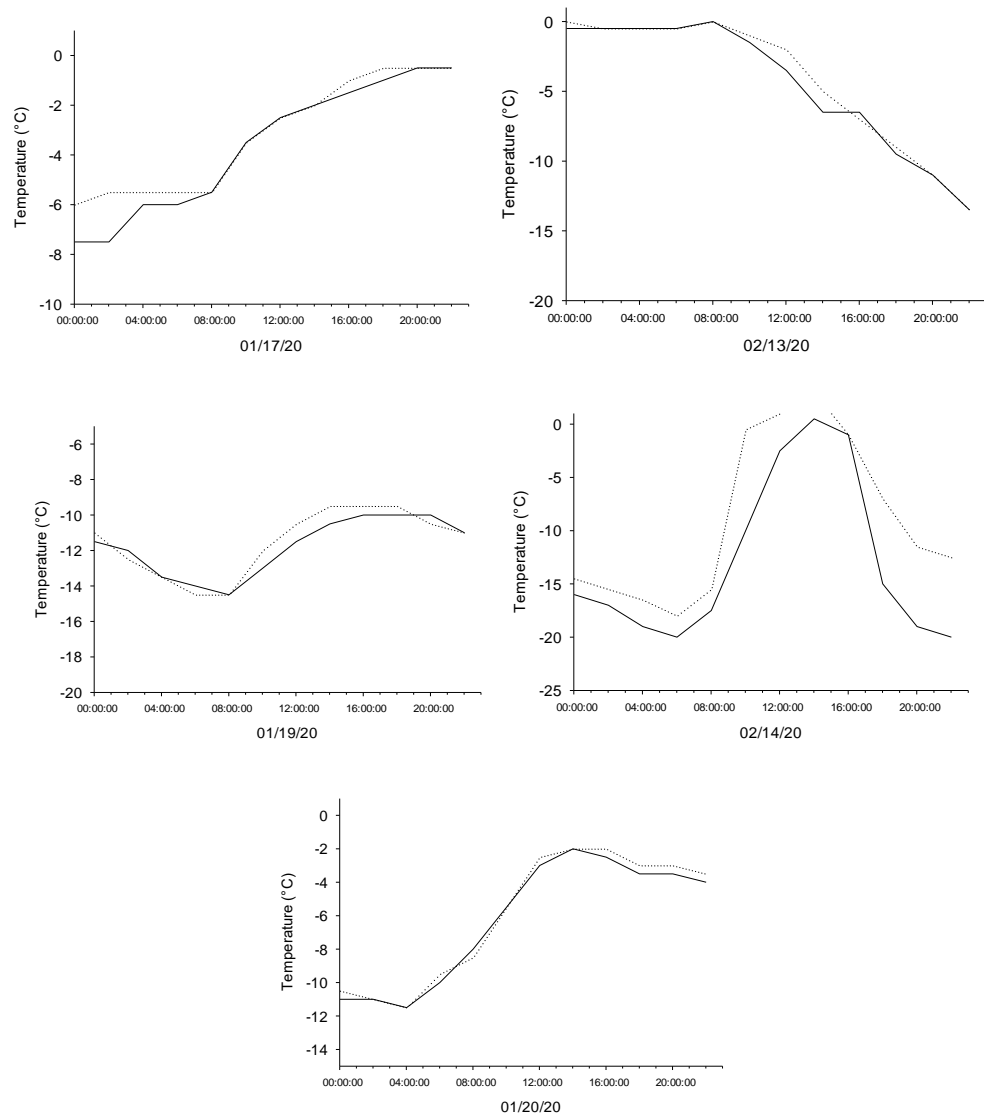
Figure C1. Total 5 days of extreme low temperature (≤ 0 °C) days were analyzed and presented in this figure from my main figure (see Figure 1.4 from Chapter 1). Solid line represents the temperature recorded from infested host having no impervious surface whereas dotted line represents from infested host having 97% impervious surface. Five individual graphs presented here shows the effect of impervious surface on evergreen tree host canopy temperature when daily maximum temperature is ≤0 °C. Impervious surface was measured by pace to plant technique around 20 m radius of infested host. The study trees were from Lafayette and West Lafayette, Indiana.

APPENDIX D. BAGWORM COLLECTION SITES

Table D1. Bagworm collection sites during 2018. Maximum of 25 bagworms were sampled from each evergreen tree.

Site Selection	Max Temp. (°C) on 01/01/018	No. of Sites
Danville, IL	-20°C (-4°F)	9
Lafayette/WL, IN	-19.44°C (-3°F)	22
Frankfort, IN	-18.89°C (-2°F)	7
Ogden, IL	-18.89°C (-2°F)	1
Kokomo, IN	-18.83°C (-1°F)	13
Russiaville, IN	-18.83°C (-1°F)	1
Lebanon, IN	-17.78°C (0°F)	11
Carmel, IN	-17.22°C (1°F)	4

Table D2. Bagworm collection sites during 2019. Maximum of 25 bagworms were sampled from each evergreen tree.

Site Selection	Max Temp. (°C) on 02/02/019	No. of Sites
Merrillville, IN	-22.22°C (-8°F)	10
Valparaiso, IN	-21.67°C (-7°F)	8
Plymouth, IN	-20.56°C (-5°F)	7
Kokomo, IN	-18.89°C (-2°F)	8
Lafayette/WL, IN	-18.33°C (-1°F)	8
Lebanon, IN	-17.22°C (1°F)	6

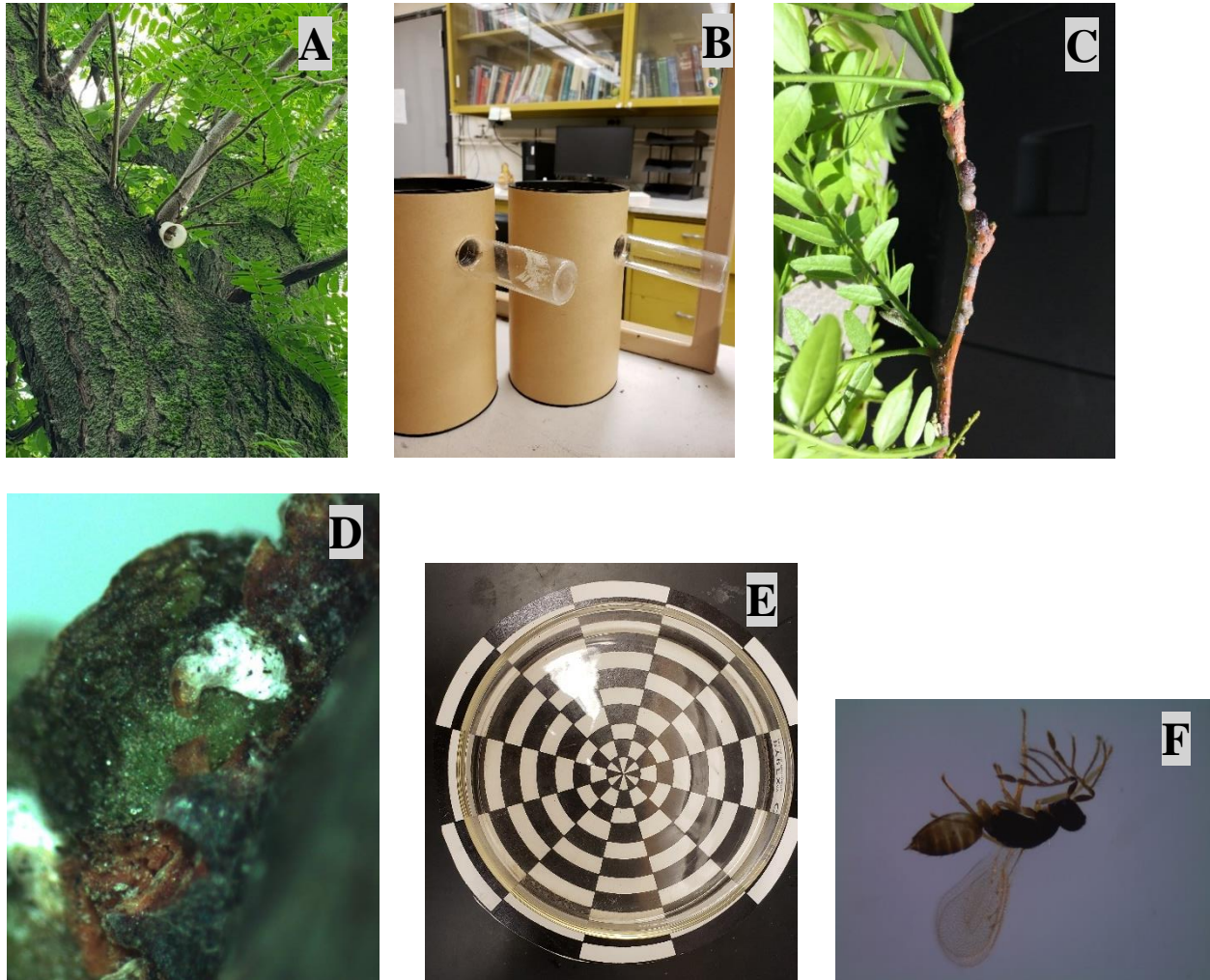# APPENDIX E. SCALE INSECT COLLECTION

Figure E1. An iButton installed on honeylocust tree to measure tree canopy temperature during 2019 and 2020 at 2.5 m height from soil line on trees sampled from Indianapolis, Indiana (A). Cardboard tubes used to rear parasitoids of lecanium scale insect (B). Scale insect infested twigs were kept inside cardboard tubes and parasitoids were collected after adult emerges. European fruit lecanium scale insect (C) and Honeylocust scurfy scale insect (D). A mite counting disc used to count eggs after dissection of female lecanium scale insect (E). An example of parasitoid (*Pnigalio* spp) emerged from infested twigs (F).

APPENDIX F. SCALE INSECT ABUNDANCE IN TREES WITH DIFFERENT HEALTH CONDITIONS
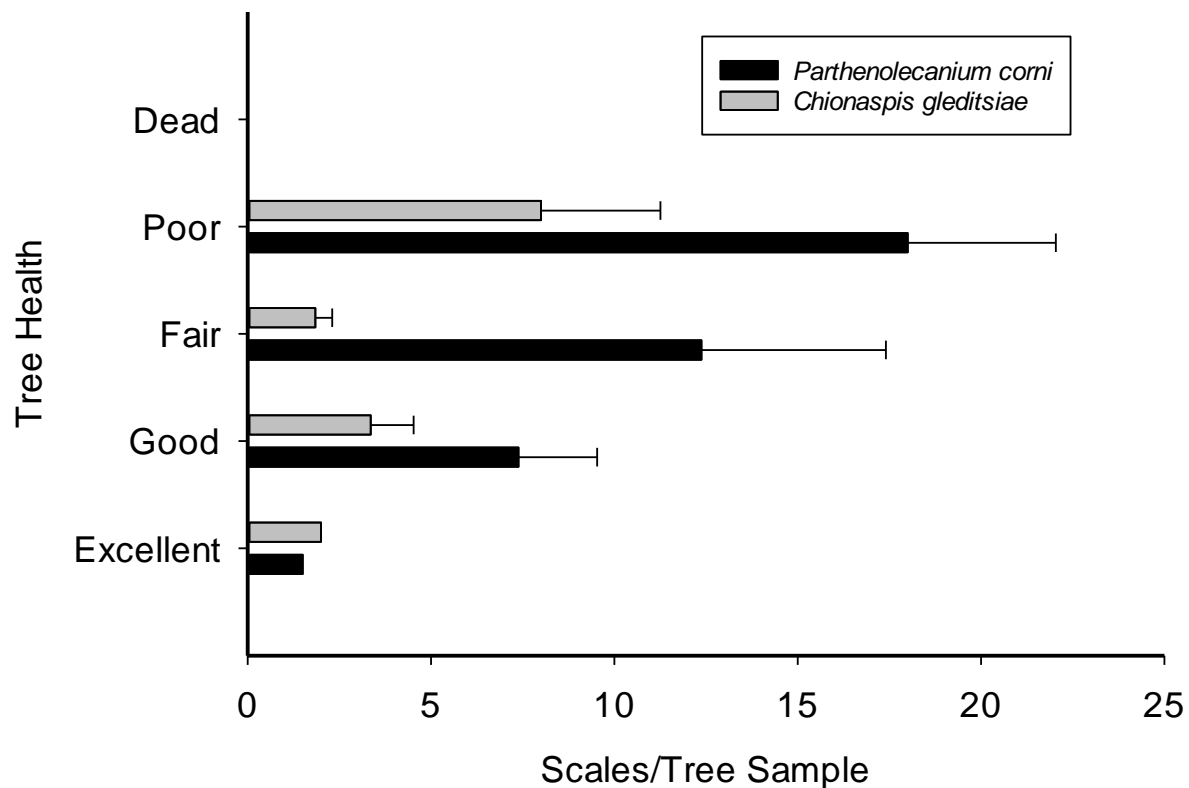

Figure F1. Mean number of female *Parthenolecanium corni* (±SEM) and *Chionaspis gleditsiae* abundance (±SEM (standard error of the mean)) across the Indianapolis, IN in Spring 2018 and 2019 (*C. gleditsiae*) and summer 2019 and 2020 (*P. corni*) from four 30.5 cm twigs. Tree health was examined as described in text in 2020. Of the 37 trees I examined, 1 was Excellent, 17 were Good, 16 were Fair and 3 were Poor.