Ecology and life history of *Coccophagus gossypariae* (Hymenoptera: Aphelinidae), a parasitoid of *Eriococcus spurius* (Hemiptera: Eriococcidae)

by

Caitlin Elaine Mader

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University of Alberta

Abstract

The American elm (*Ulmus americana*) is a valuable component of urban forests in Alberta. In many Alberta municipalities, the health of these trees is being heavily impacted by the invasive scale insect *Eriococcus spurius* (Hemiptera: Eriococcidae). Biological control of scales using hymenopteran parasitoids can be an effective replacement or addition to control using chemical insecticides. However, no effective biological control agent has been found for *E. spurius.* This project investigates the life history and host interactions of *Coccophagus gossypariae* (Hymenoptera: Aphelinidae), a parasitoid wasp that was discovered parasitizing *E. spurius* in Calgary. Field studies took place at 46 sites in Calgary during 2015, and 44 of the same sites in 2016. At each site, I conducted repeated-measures sampling every two weeks for eight rounds in 2015, and 5 rounds in 2016. At each site and sampling round, I collected three types of samples: Adult *C. gossypariae* that had emerged from their hosts and had been caught in mesh traps over the preceding two weeks; first instar *E. spurius* nymphs that had been caught on sticky traps over the preceding two weeks; and live adult scale insects, which I later dissected in the laboratory for fecundity and parasitism data. I used various combinations of the resulting datasets, along with climate data and urban forest inventory data supplied by the City of Calgary to answer different questions in three investigative chapters. The chapters 2-4 each provide a different area of focus in better understanding this tri-trophic system. In the first chapter, I undertook basic investigations of *C. gossypariae* life history traits. I found that it is well established throughout Calgary, and accounts for 98% of the parasitism of *E. spurius.* Parasitism rates varied greatly between sites, ranging from 6.8% to 81.0%. I recorded a sex ratio of 7% males, and a strong avoidance of superparasitism. In the second chapter, I adopted a much broader focus, conducting spatial analyses on how urban landscape factors influence *E. spurius* populations. I found that the amount of impermeable surfaces within 10 m from a tree, and the number of other elm trees upwind of it are both positively associated with higher *E. spurius* densities. Finally, I examine how *E. spurius* and *C. gossypariae* interact over the course of the season, comparing their relative phenologies, and the effects of parasitism on *E. spurius* reproduction. I found that *E. spurius* have already finished reproduction before the point in the season when the majority of parasitism-induced mortality occurs. Because *E. spurius* adults will not live to reproduce again, most mortality induced by *C. gossypariae* emergence after reproduction will have little impact on its host’s population. However, *E. spurius* that are parasitized by *C. gossypariae* produce significantly fewer eggs than those that are not. I conclude that if *C. gossypariae* has any suppressive effects on its host`s population, it is likely due to fecundity effects, rather than mortality effects. I did not, however, document any influence of *C. gossypariae* parasitism on changes in *E. spurius* population. This study provides some first steps for future work on whether *C. gossypariae* can be used in biological control of *E. spurius*.

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Chapter 1: Thesis Introduction

Urban trees are extremely valuable to municipalities, but are particularly vulnerable to invasive species due to the favourable invasion conditions of the urban ecosystem (Gulick 2014). The value of urban forests is based largely on their contribution to ecosystem services such as storm water management, soil stability, carbon storage, air pollution reduction, energy conservation, and human quality of life improvements (Tyrväinen & Väänänen 1998; Government of Alberta Alberta 2016). In Western Canada, American elms (*Ulmus americana* Linnaeus) are a key component of urban forests (Sandalack and Marce-Santa 2002) and are collectively valued at $634 million CAD in Alberta municipalities alone (Government of Alberta 2016). Although not native in western North America, urban elm populations outside of their native Carolinian forests have become refugia from Dutch elm disease, which devastated the tree throughout most of its native range (Bey 1990).

Despite the current absence of Dutch elm disease, elm populations in Alberta are vulnerable to other pests, particularly European elm scale (*Eriococcus spurius* Modeer) (Hemiptera: Eriococcidae). *E. spurius* (Figure 1.1) are fluid feeders on elm trees (*Ulmus* spp.) (Williams 1985). They are a persistent problem on elms in southern Alberta, particularly in Calgary, as well as many other locations in North America and the world. Heavy infestations of *E. spurius* can cause branch dieback and severely stress the host tree, making it more susceptible to other mortality risks, such as drought stress (Williams 1985), and other viral, fungal, and bacterial pathogens (Bey 1990). If Dutch elm disease should enter a population weakened by *E. spurius* it can have potentially devastating impacts (Dreistadt and Hagen 1994).

The first indication of infection by a parasitoid in *E. spurius* in Calgary was in 2010, when City of Calgary's Integrated Pest Management (IPM) technicians found parasitoid exit holes on dead adult *E. spurius* on two elms, but could not identify the causal species. In 2011, while working with the City of Calgary's IPM group, I recovered some of these parasitoids from two sites (Figure 1.2). I identified them as *Coccophagus gossypariae* Gahan with the help of Jason Mottern of University of California Riverside, and physical descriptions by Viggiani (1998, 1999). This species belongs to the family Aphelinidae, which contains important control agents of insect pests (Tang and Yokomi 1995; Gordh and Beardsley 1999; Babcock et al. 2001; Qiu et al. 2005). Members of the genus *Coccophagus* vary in their effectiveness as biological control agents, but are suppressors of several pests in the suborder Sternorrhyncha (Flanders et al. 1961; Charles 1993; Flaherty and Wilson 1999; van Lenteren 2012).

The discovery of *C. gossypariae* parasitizing *E. spurius* in Calgary leads to the question of whether it can be a useful biological control agent in lowering *E. spurius* populations. Calgary and surrounding municipalities are currently using neonicotinoid insecticides to control *E. spurius* outbreaks, but the long-term availability of neonicotinoids for *E. spurius* control is questioned due to concerns about their non-target effects (Health Canada 2017). Because of this reason, Alberta urban forest managers are seeking alternatives to neonicotinoids to reduce *E. spurius* impacts, including biological control. When effective biological control agents are found, they are capable of replacing or reducing the requirement for chemical insecticides (Van Driesche et al. 2010; Hoddle et al. 2014), and can delay the onset of insecticide resistance in hosts (Tabashnik and Croft 1985; Hill and Foster 2000). While attempts at finding an effective biocontrol agent against *E. spurius* have been made since the 1930's (Flanders 1952), there are none in widespread use.

Biological control, as defined by DeBach (1964), is “the action of parasites, predators, or pathogens in maintaining another organism’s population density at a lower average than would occur in their absence”. There are several possible approaches to biological control if a species is deemed capable of supressing pest populations (van Lenteren 2012). If a biological control agent is not established in an area where its host’s populations are at pest levels, but significantly suppresses its host in areas where it is present, then it is a candidate for classical biological control; the control agent is released in the new area, where it will hopefully have a similarly suppressive effect. Alternatively, if a biological control agent is established in areas where its host is at high populations, but it only suppresses its host to a satisfactory level when its own populations are extremely high, then it is a candidate for augmentative biological control; agents are bred in captivity and released at targeted sites to artificially raise parasitism rates to levels that will suppress host populations. Finally, if a biological control agent is established in a system where its hosts are considered pests, and can cause a useful reduction in pest populations if known environmental conditions are favorable for its survival and reproduction, then it is a candidate for conservation biological control; in which managers attempt to alter the environment in favour of the agent’s proliferation (Naranjo 2001).

In order to use *C. gossypariae* in any of the above approaches, it is necessary to understand its biology, as well as its interactions with its host and the environment. Basic information on parasitoid life history and host discrimination is important to the success of captive breeding, release, and establishment success (Mills and Gutierrez 1996; Mills and Kean 2010), and should be investigated before the use of any organism in biocontrol (Latham and Mills 2010). Information on host-parasitoid interactions, including field behaviour of parasitoids and the direct effects that a parasitoid has on its host are vital, as these can influence the population dynamics of the system (Briggs et al. 1995). It is also important to understand what other environmental factors might influence pest populations, as they may be more influential than natural enemies, or have an interactive effect with natural enemy populations.

The purpose of this study was to provide the first comprehensive investigation of the ecology of *C. gossypariae* in the field, its affects on the host, *E. spurius*, and the environmental aspects important to *E. spurius* populations. This thesis is presented in three data chapters following the introductory chapter: Chapter 2 investigates several basic aspects of *C. gossypariae* life history and ecology in Calgary, including its distribution of establishment, whether it is the main parasitoid of *E. spurius* or part of a complex, its sex ratios, host discrimination, and whether its populations are affected long-term by historical imidacloprid insecticide injections. The third data chapter examines how environmental factors other than *C. gossypariae* influence *E. spurius* populations, with attention to urban forest specific features, such as impermeable surfaces and dense-monocultural tree stands. The fourth chapter examines both species in tandem, and how they interact over the course of the season. This study is guided by the question: "Is *C. gossypariae* capable of significantly suppressing *E. spurius* populations in Calgary?". While I do not seek to answer this question fully, I do aim to provide the first steps towards future work that may.

Tables and Figures

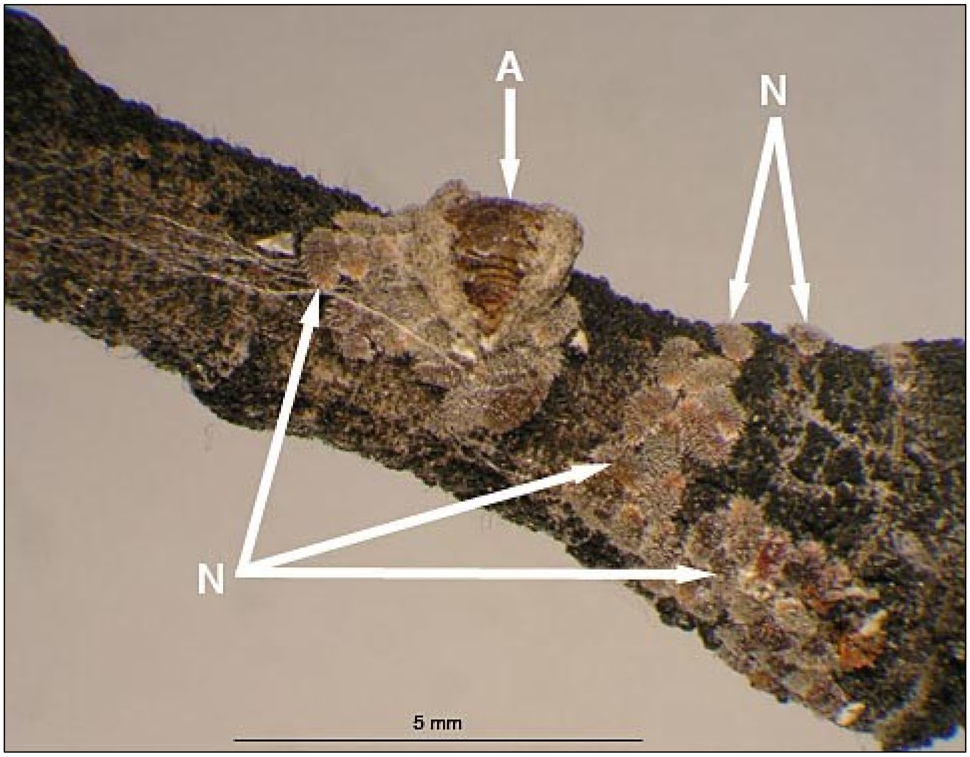


Figure 1.1: Eriococcus spurius collected from an American elm (*Ulmus americana* L.*)* in Calgary, Canada, September 2011. Adult females (A) are sessile, and secrete a waxy coating. Second instar nymphs (N) return to branches from the leaves in the fall and form cocoons to overwinter.

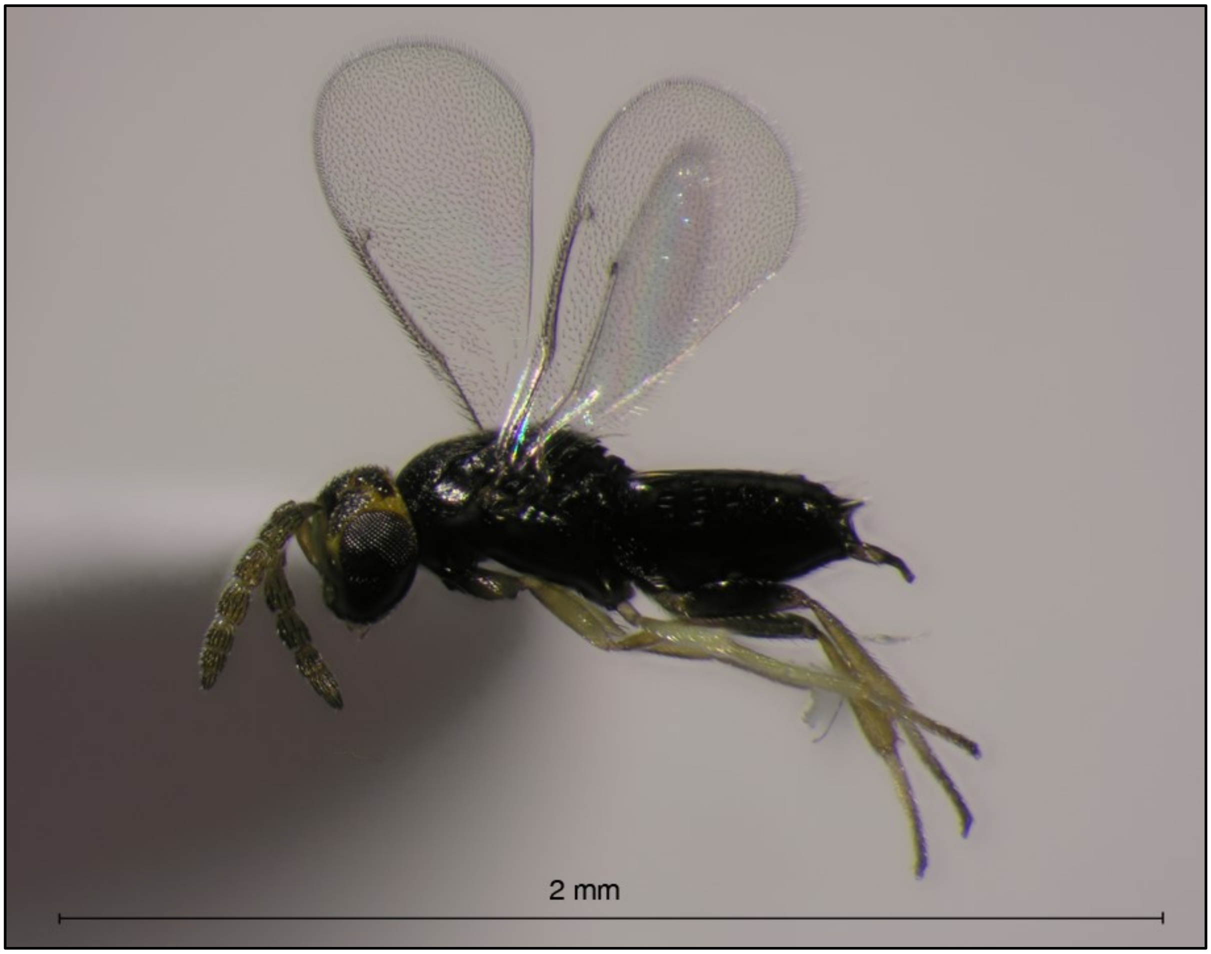


Figure 1.2: Adult female *Coccophagus gossypariae*, emerged in lab from adult female *Eriococcus spurius* in September 2011.

Chapter 2: Life history traits of *Coccophagus gossypariae* (Hymenoptera: Aphelinidae) in field studies

Abstract

European elm scale (*Eriococcus spurius*) is a pest of urban elm trees in western North America, but no effective biocontrol agent has been found to manage it. Basic life history studies of parasitoid species are essential in evaluating a potential biocontrol agent. I documented several life history traits of *Coccophagus gossypariae,* a little studied aphelinid parasitoid of *E. spurius*. This wasp is native to Europe, but is established on *E. spurius* in Calgary, Alberta. I found that *C. gossypariae* comprises 98% of the parasitoids of *E. spurius* in Calgary, and had established at 89% of study sites throughout the city in 2015. They have a female biased sex ratio (93% female), and both males and females parasitize the same host species. I found evidence for a highly effective aversion to superparasitism by ovipositing females. The goal of this study was to provide some insight on key life history traits as a baseline for further studies of the interactions and population effects of *C. gossypariae* and its host *E. spurius*.

Introduction

American elms (*Ulmus americana* Linnaeus) are an important component of urban forests in Alberta, making up a large proportion of public trees in Calgary, Edmonton, Red Deer, and Lethbridge. Elms in these cities are often infested with the invasive scale insect *Eriococcus spurius* Modeer (Hemiptera: Eriococcidae). This study focuses on Calgary, where heavy infestations of *E. spurius* on large proportions of the urban elm forest are causing branch die-back, canopy thinning, and a general decline in tree values throughout the city. The City of Calgary's Urban Conservation department has been mitigating *E. spurius* outbreaks using systemic injections of the neonicotinoid insecticide imidacloprid since 2005. However, concerns about the environmental effects of neonicotinoids (Health Canada 2017) make future availability of imidacloprid uncertain. Because of this, urban forest managers are currently looking for other methods of controlling *E. spurius*, including biological control. However, no current biological control agent is known to be effective in suppressing *E. spurius.*

In 2010, City of Calgary integrated pest management technicians found parasitoid exit holes on dead adult *E. spurius* at two sites in Calgary. No parasitoids were captured at the time, but this discovery prompted interest in the integration of natural enemies into the City's management practices. The parasitoids were captured and identified the following year as *Coccophagus gossypariae* Gahan following descriptions in Viggiani (1999 & 1998). The genus *Coccophagus* contains several economically important biological control agents of scale insect and mealybug pests (Enkegaard and Brødsgaard 2006), but little is known about *C. gossypariae* and its effect on *E. spurius* populations to evaluate its viability as a biological control agent. Adults and preimaginal stages of *C. gossypariae* have been morphologically described by Viggiani (1998, 1999) and Griswold (1927). Griswold (1927) noted that *C. gossypariae* may have had at least two generations per year in New York, during the 1927 growing season; the first emerging from male second instar *E. spurius* nymphs in late May, and the second emerging from adult female *E. spurius* in mid June. She made note at the time of the lack of records of the parasitoid's overwintering behavior. Viggiani (1999) expanded on the knowledge of the biology and behavior of *C. gossypariae*, observing that Females *C. gossypariae* are synovigenic, containing 50-90 mature eggs at emergence, which can be resorbed if no hosts are present. He also noted that unmated females attach their eggs (assumed to be haploid and therefore male) externally to their *E. spurius* hosts, indicating that the males may develop as ectoparasitoids, unlike their endoparasitic female conspecifics, but did not observe whether these eggs developed successfully to adulthood. A variation in the development strategy of males and females of the same species is not unusual for species of the genus *Coccophagus*. Males of this genus may be endoparasitoids, ectoparasitoids, of the same or different species as their female counterparts, and can also be hyperparasitoids of their own species, or other parasitoids (Walter 1983).

These studies provided useful insight into the biology of *C. gossypariae*, but were performed in laboratory settings on adult and larval parasitoids that emerged or were dissected from *E. spurius* on cut elm branches. Little is known about the life history of *C. gossypariae* in a natural setting, or its interactions with its hosts throughout the season. Environmental and density dependent factors affecting *C. gossypariae* populations in the field have also never been studied.

Studies on basic life history and host interactions are required before *C. gossypariae* is considered for biological control of *E. spurius*. Behavioral studies are important to inform captive breeding and release and establishment success (Mills and Kean 2010), and should be investigated before any use of an organism in biocontrol (Latham and Mills 2010). Observations on sex allocation, host discrimination, and sexual ontogenies are important for successfully rearing biocontrol agents for release (Mills and Gutierrez 1996). Information on in-situ host-parasitoid interactions, including field behavior of parasitoids, can determine the population dynamics of the system (Briggs et al. 1995).

Prior to this study, *C. gossypariae* had been recovered from only one site in Calgary, and was suspected to be present at another based on exit holes in adult scale insects. It was not known whether the species was widespread throughout the city, or present only in isolated patches, nor was there any measure of population density in locations where the wasp was present. Natural enemies of pests are best suited for classical biological control if they are not yet established in a location, whereas they are candidates for augmentative or conservation biological control if they are established but not satisfactorily suppressing pests (van Lenteren 2012). Approaches to any future biological control measures using *C. gossypariae* would therefore vary depending on its pre-existing prevalence in Calgary. Furthermore, I could not assume that *C. gossypariae* was the only parasitoid of *E. spurius* in Calgary. An assessment of the parasitoid assemblage in this system is another essential preliminary step to understanding its potential for biological control.

Host discrimination to avoid superparasitism is a potentially important aspect of parasitoid life history that has never been studied in *C. gossypariae*. Superparasitism by parasitoids is the deposition of more eggs into a host than can survive to maturity. It is common in parasitic Hymenoptera (Gordh et al. 1999), although most species of hymenopteran parasitoids possess mechanisms for its avoidance which are generally based on olfactory cues (Rogers 1975; Quicke 1997). Superparasitism can be performed by a single female, or multiple females of the same species (Gordh et al. 1999). Costs to fitness in superparasitizing individuals are inherent, as only one larva will survive to adulthood in solitary parasitoids (Gordh et al. 1999). However, costs of superparasitism are not limited to mortality: there can be fitness costs even to the survivor, such as longer development time, smaller size and shorter life span as an adult (Tunca et al. 2016). Due to the costs of superparasitism, its occurrence was long believed to be maladaptive- a result of mistakes by ovipositing females (Salt 1934). However, host selection in parasitoids is under strong evolutionary selection, and more recent research has recognized that superparasitism is adaptive in many situations, and it is much more likely to occur in situations when its benefits outweigh its costs for ovipositing females (van Alphen and Visser 1990). Generally, superparasitism becomes advantageous when host density is low, competition for hosts is high, and the cost to a female of searching for unparasitized hosts outweighs the risk of her offspring losing the inter-larval competition within a superparasitized host (van Alphen and Visser 1990). In some species, the surviving parasitoid larvae from a superparasitized host do not suffer losses of fecundity and longevity, and such species indeed show a tendency to superparasitize fairly frequently (González et al. 2007). Furthermore, species that suffer a high encapsulation rate seem to use superparasitism as a mechanism to overwhelm host defenses (Blumberg and Luck 1990).

Parasitoids can be rendered poor biological control agents if they limit their reproductive efficiency through conspecific interference at high densities (Eliopoulos et al. 2016). Superparasitism at moderate to high parasitoid-to-host ratios can have this effect (Bai and Mackauer 1990; Quicke 1997). Mass-rearing operations often suffer decreases in quality and efficiency, as they are prone to creating parasitoid-to-host ratios that are artificially high, resulting in high rates of superparasitism even in species with high fitness consequences associated with the behavior (Hamelin et al. 2007; Ho and Ueno 2008; Tunca et al. 2016). Thus, superparasitism is often disadvantageous when mass-reared biocontrol agents due to its potential decrease in rearing efficiency (Tunca et al. 2016) and lower quality of parasitoids produced (Smith 1996). Successful gregarious parasitism has never been observed in previous studies of *C. gossypariae* (Griswold 1927; Viggiani 1998; Viggiani 1999), and it is therefore likely to be a solitary parasitoid. In this project, I investigate the frequency of superparasitism of *C. gossypariae* on *E. spurius* in a field setting, to better understand the effects host discrimination and superparasitism may have on the reproductive efficiency of this species.

In the years prior to this study, the systemic insecticide imidacloprid ($C_9H_{10}ClN_5O_2$) was the most common method of controlling *E. spurius* in Calgary. Imidacloprid can be extremely and immediately effective at reducing *E. spurius* populations, with the effect lasting two or more seasons (Sclar and Cranshaw 1996; Karimzadeh and Iranipour 2017). However, imidacloprid can also have non-target effects on natural enemies of their target pests, including parasitic Hymenoptera (Rogers and Potter 2003). As large numbers of Calgary's elms had been treated with imidacloprid in the years preceding my study, examining *C. gossypariae* life history in the absence of imidacloprid effects was both impractical and undesirable. I therefore included in my study a basic evaluation of residual effects of imidacloprid on *C. gossypariae* populations.

This project seeks to answer several questions about basic *C. gossypariae* life history and the *E. spurius*-parasitoid complex, specifically the sex-ratios of *C. gossypariae*, geographic range of establishment in Calgary, the rate of parasitism in *E. spurius*, and whether *C. gossypariae* have tendencies to superparasitize at high densities. The central goal of this study is to establish a baseline of life history information to guide more detailed studies of *C. gossypariae*'s influence on the populations of its host.

Materials and Methods

Sampling scheme

I selected 46 sites, each consisting of one public American elm tree, to include in this study. All trees were within the Calgary city limits (Figure 2.1). I collected eight rounds of data at each tree, revisiting each tree every two weeks from mid-May to mid-September 2015. During each round, I collected adult parasitoids that had been caught in emergence traps over the previous two weeks, as well as adult and late-instar scale insects for later lab dissection.

Tree selection

Visual appraisals of tree condition and scale infestation were deliberately ignored during the selection process to obtain a near random assortment of *E. spurius* infestation level within the studied trees. Twenty-one of the study's trees had been treated with imidacloprid insecticide from 2005 to 2010, according to the City of Calgary's records, and the remaining 25 had no history of imidacloprid injections. I deliberately chose trees with a range of diameters at breast height (DBH), representing a range of old and young trees. For each untreated tree, I attempted to find a treated tree of similar DBH, in a similar setting (park or boulevard). The purpose of these pairings was to reduce the chance of location or age affects being conflated with imidacloprid effects in my analyses. Both trees in a "pair" were consistently visited on the same day during sampling rotations. I used allometric equations provided by McPherson et al. (2016)

to estimate tree ages, and chose trees ranging from 8.6 cm DBH (estimated 9 years old) to 64.5 cm DBH (estimated 59 years old).

Sampling *Eriococcus spurius* and parasitoid specimens

I set up emergence nets on each study tree, designed to capture adult parasitoids as they emerged from their hosts. Each tree had emergence traps set up on four branch sections. Each trap covered 10-20 cm sections of branch with fine netting (No-See-um Nylon netting; maximum hole width 0.6mm, purchased from BioQuip, Rancho Dominguez, USA). These branch sections were randomly selected, but were all between 3 and 5 cm in diameter at their widest points and contained at least a few adult *E. spurius*. I secured the netting using staples and electrical tape to ensure that study organisms could not enter or exit the enclosed area. Prior to enclosure, the area was inspected for arthropods other than *E. spurius*, including any adult parasitoids. Any other arthropods present were removed. This was to ensure all parasitoids later recovered from the net had emerged from the *E. spurius* already present in the netted area, and that no other entomophagus arthropods could predate the emerged *C. gossypariae*.

After setting these nets up in mid May 2015, I returned to each site every two weeks, and collected adult parasitoids that had been caught in emergence traps. At any given time, between one and three of the four enclosures were in place on each tree, and all sites followed the same pattern in each round (Figure 2.2). A rotating trapping scheme was necessary because when a branch enclosure is in place, it excludes parasitoids from the environment from ovipositing into the scale insects inside the trap. Using just one or two trapping locations could have inadvertently excluded parasitoids that were laid as eggs during these times from the study. The timing of the four branch enclosure locations was arranged to capture all possible timings of parasitoid oviposition and emergence. Branch enclosures were colour coded with electrical tape to distinguish branch sections 1 to 4 on each tree. Electrical tape marking the extent of the branch enclosure was left on the branches during rounds when the enclosures were removed, to ensure that enclosures could be replaced covering the same branch extents in each round. As adult female *E. spurius* are sessile, repeated measures in the same locations captured parasitoid emergences from the same group of adult female *E. spurius* every subsequent round, with the exception of individuals that fell off, or late instar nymphs that had migrated to the area between trapping sessions.

When samples were collected, the enclosures were carefully cut from the branches, and placed in plastic bags in such a way as to minimize the opportunity for any living parasitoids to escape during collection. In the lab, my assistants and I counted all *C. gossypariae* recovered from the emergence netting, using a Fisher Scientific dissecting microscope at up to 45x magnification power. I separated the data by round, site, and trap.

During sample collection in each round, I collected up to 10 adult or late-instar nymph *E. spurius* from the branches of each site's study tree. To avoid interference with the other sample collections, I did not remove these scales from the branches on which the crawler tape traps were placed, or from within the sections of branch used in emergence trapping. Samples were attempted at all trees at all sites at each collection period, but if 5 minutes elapsed without finding an adult or late-instar *E. spurius*, I halted collection at that time. All samples were frozen at -20°C to until processing.

Sample Processing

All scale insects and wasps recovered from the emergence netting were counted using a dissecting microscope. Sex ratios of all wasps recovered from all rounds at two of the sites were determined using descriptions of genitalia and sensilla length provided by Viggiani (1998, 1999). To confirm the species as *C. gossypariae,* I measured diagnostic characteristics using a compound microscope. For one male and two female specimens, I compared their colouration and morphological measurements of body length, head width, thorax width, scape length, scape width, F1 antennal segment length and width, and F1 sinsilla length to the descriptions of the species by Viggiani (1998, 1999).

In the laboratory, scale insects collected from the field were cleared in 10% KOH solution for 7-14 hrs, depending on the length of time required to view developing parasitoid larvae and *E. spurius* eggs within the scales' bodies. For each round at each site,10 adult or late-instar nymph scale insects were cleared, although for some sites, fewer scales were consistently available, due to low population densities in the field. Each of the cleared scales was dissected to count and determine the development stage of any parasitoid larvae, pupae or eggs they contained.

Descriptive statistics

To document the species assemblages and sex ratios, I used simple descriptive statistics. For species assemblage information, I counted the number of emerged parasitoids that morphologically appeared to be *C. gossypariae*, as well as parasitoids of other species that I keyed only to superfamily. I reported the proportion of *C. gossypariae* among all recovered parasitoids as a percentage. For sex ratios, I recorded the mean percentage of males and females for each round, as well as in total. I also used the variation of male-female ratios between site-round combinations to calculate a 95% confidence interval for the total sex ratio of the population.

Analysis of superparasitism

Using R software (R Core Team 2015), I used simulated data to estimate the expected frequency of multiple parasitism if parasitoids were choosing their hosts randomly and not discriminating against previously-parasitized scale insects. For each combination of site and round, I took the number of scales dissected (S), and the number of parasitoids recovered in the dissections (P). For each site-round combination, I then created a vector of length P, for which each element was a randomly generated number between 1 and S. Elements in these vectors each represented an oviposition event, and the number assigned to it represented the host that the female parasitoid “chose”. Repeats within a site-round vector therefore represented a multiple parasitism event, or two female parasitoids “choosing” the same scale insect. The total number of repeats for all site-round combinations represented the number of superparasitism events in one iteration of the model. I ran the model 10,000 times to determine the expected frequency of superparasitism in our dataset given indiscriminate oviposition. I compared this distribution to the number of superparasitism events detected to our field data to determine the probability of the observed level of superparasitism occurring by chance.

Analysis of prolonged effects of imidacloprid on percent parasitism

The 46 focal trees in this study can be divided into two groups: those that had never been treated with imidacloprid, and those that had been treated five to ten years prior to the sampling period.

The City of Calgary's tree maintenance records showed if the trees had been treated in this 2005-2010 period, this presence of historical treatment was confirmed in the field, as imidacloprid injections are filled with arborists' wax that is visible on the trunk for seven or more years afterwards. Trees treated with Imidacloprid less than five years prior were not included in this study. I constructed a mixed effects logistic regression model to test whether historical imidacloprid treatment at a site was a predictor of likelihood of parasitism of individual scale insects. These models used site data, as well as parasitism presence/absence data obtained from dissections of adult scale insects collected from 12 treated and 12 untreated sites. Sampling round and imidacloprid treatment were included in the model as fixed effects, and site was included in the model as a random effect, while the binary responding variable was the presence or absence of parasitism of individual *E. spurius*. I then performed a Wald Chi squared test of the coefficients of this model to determine the significance of the fixed effects.

Results

Parasitoid emergence rates and species assemblage

Parasitoids were widespread throughout our sampling area, but varied in abundance among sites (Fig. 2.3). In total, my assistants and I recovered 1057 adult parasitoids from our emergence traps. Of these, 1031 (98%) met the general morphological descriptions of *C. gossypariae* and 26 (2%) were other chalcidoid wasps that I did not key beyond superfamily. I did not recover any dipteran parasitoids. Other species of chalcidoids were never found in the absence of *C. gossypariae. Coccophagus gossypariae* adults were present at 41 out of the 46 sites where emergence trapping took place. The most productive site for *C. gossypariae* yielded

187 adults in the season. Mean and median individual *C. gossypariae* yields for each site were 22.4 and 6, respectively (Standard deviation 40.7).

Coccophagus gossypariae sex ratios

I determined sex of 147 wasps from all emergence samples collected on two trees, and recovered a total of 137 females and 10 males. The specimens represented a cumulative sex ratio of 93.2% females and 6.8% males (95% C.I. ±3.7%). Female emergence rates visually reflect the two-peak emergence pattern shown by the whole population (examined in Chapter 4), with a smaller peak during the third round of sampling, and a larger peak during the seventh round of sampling (Figure 2.4).

Parasitism and superparasitism rates

Only 24 out of the 46 sites were used in analyses of parasitism and superparasitism rates, because *E. spurius* populations were insufficient at 22 of the sites to consistently collect scales for dissection. These analyses therefore represent only the 52% of sites with the highest scale populations. Although second instar nymphs were collected in the field, they were frequently damaged during the chemical clearing process. Second instar nymphs that were not damaged in clearing never contained parasitoid larvae, so were excluded from these studies. Statistics therefore represent rates of adult *E. spurius* parasitism. Parasitoid larvae were recovered from living adult scales at all 24 of these sites, but percent of scales parasitized varied between sites from 6.8% to 81.0% (mean: 50.0%, S.D: 21.0%) (Figure 2.5).

Comparisons of simulated datasets to actual data demonstrate that the probability that *C. gossypariae* selects its prey at random is less than 0.0001%. In the 777 cleared and dissected scales at 24 sites, 306 were parasitized with 307 parasites. There was only one instance of superparasitism, in which an adult *E. spurius* contained both a parasitoid pupa and a parasitoid egg. This superparasitism event occurred at the site with the highest observed percent parasitism (81%). Preserving the ratios of immature parasitoids to scales at each of the 24 sites and assuming random selection of scales for oviposition an instance of 1 or fewer oviposition events did not occur; our simulations predicted a mean superparasitism frequency of 63.01 (S.D. 5.4). Given these predictions, the rarity of superparasitism in field populations suggests that *C. gossypariae* employs a highly effective strategy to avoid superparasitism.

Effect of imidacloprid on hymenopteran parasitism

I did not find evidence of long-term effects of imidacloprid injections on parasitism rate of scale insects feeding on treated trees (Figure 2.6). Five to ten years following imidacloprid injection, mean percentage of parasitism of *E. spurius* was 45.3% (S.D. 24%) across all treated trees, which was not significantly different from the observed rate of parasitism of 46.0% (S.D. 19.0%) in *E. spurius* feeding on trees with no imidacloprid treatment history (Fig. 2.6). The conclusion of non-significance was based on the results of the mixed effects logistic regression model (Table 2.1), and Wald Chi square test of the coefficients (Imidacloprid treatment coefficient: Chi squared = 0.5317, DF= 1, p = 0.466). Parasitism rate varied widely between sites regardless of treatment history.

Anecdotal field observations

During field studies, I observed several aspects of *C. gossypariae* behaviour that were not deliberately studied, but are worth noting here due to a lack of other thorough field studies on the species. Whenever I observed *C. gossypariae* in the field, they were walking quickly among *E. spurius,* and seemed to stop only to probe the adults with their antennae, or to remain motionless for several seconds at a time when they encountered pools of *E. spurius* honeydew. Ants (Hymenoptera: Formicidae) were abundant in some of the focal trees, and on one occasion, I observed a searching *C. gossypariae* fly away when approached by an ant. In early March 2016, I collected about ten second instar *E. spurius* from a focal tree with a known high rate of parasitism, and dissected them in the lab. I recovered parasitoid larvae from several of them, but the data was lost due to software issues.

Discussion

Parasitoid species assemblage and geographic distributions

The widespread recovery of large numbers of *C. gossypariae,* with few other parasitoids from the emergence traps indicates that it is well established as the dominant parasitoid of *E. spurius* in Calgary. This finding alone eliminates the consideration of classical biocontrol using *C. gossypariae,* as this method is reserved for the introduction of a predatory species not currently established in a location (Van Driesche et al. 2010; van Lenteren 2012). Augmentative or conservation biocontrol is best suited for situations where a natural enemy is present, not exerting satisfactory control over pests, but capable of exerting a desirable level of control with significant increases to its populations (van Lenteren 2012). I did not assess the effects of *C. gossypariae* parasitism on *E. spurius* population in this chapter, but as *E. spurius* populations were above levels of economic damage in 2015 despite the widespread occurrence of *C. gossypariae*, one can assume that on its own it is unable to effectively suppress *E. spurius* below pest status in Calgary. The effects that *C. gossypariae* does have on *E. spurius* are examined and discussed in Chapter 4.

The dominance of *C. gossypariae* within *E. spurius*'s associated parasitoid complex greatly simplifies the analyses and conclusions of the rest of the project. As only an estimated 2% of the parasitoids preying on *E. spurius* were not *C. gossypariae*, I deemed these largely negligible in the system. I assume that the larval parasitoids recovered in host dissections follow a similar species distribution, and attribute fecundity and population effects associated with larval scales to *C. gossypariae* when they are discussed in Chapter 4. If future research into this system attempts to modeling and predict population changes, it will be far simpler in a single-host, single-parasitoid system than if multiple parasitoids are present (May and Hassell 1981).

In the broader context of assessing the feasibility of augmentative or conservation biological control in this system, a single-species parasitoid assemblage may be favorable. In a review of the effectiveness of multiple and single agent biocontrol projects, Denoth et al. (2002) found that multiple natural enemies do not increase the success of insect pest control. In systems that used multiple agents successfully, control was usually only attributed to one agent that established dominance. Furthermore, Briggs et al. (1993) found that intraspecific competition between parasitoids sharing a host can decrease the overall control of the pest populations, although this idea has been both supported and refuted in biological control literature (Kakehashi et al. 1984).

Coccophagus gossypariae sex ratios

The retrieval of both male and female *C. gossypariae* from emergence nets supports Viggiani's (1999) laboratory observations that both males and females of this species are capable of completing their development on *E. spurius*- a trait that cannot be assumed in the *Coccophagus* genus, as many species require different host species for the different sexes (Walter 1983). Sex-ratio is not fixed in hymenopteran parasitoid species, nor are the reproductive advantages of a female-biased sex ratio universal (West and Rivero 2000). However, as this is the first study of wild populations of *C. gossypariae,* it provides a reference sex ratio for future work on the species.

Long-term imidacloprid effects on parasitoids

While I did not find that previous systemic imidacloprid treatments had detectable effects on parasitization rate five to ten years post treatment, it is important not to conclude from these results that imidacloprid does not affect natural enemies in this system. Studies of shorter-term non-target effects of imidacloprid have demonstrated that it can have negative effects on natural enemy populations, including hymenopteran parasitoids (Hill & Foster 2000; Rogers & Potter 2003). Furthermore, I did not test for sub-lethal effects on parasitoids, such as reduced fecundity, longevity, or dispersal ability, which can be caused by broad-range insecticides (Desneux et al. 2007). For the purposes of this study, assessing residual imidacloprid effects was to evaluate whether historical treatment groups should be separated in further analyses of host-parasitoid interactions in this system. Imidacloprid degrades within months in the environment (Mullins 1993; Miranda et al. 2011), so I did not expect my results to show a treatment effect upwards of five years post treatment. As the difference in means between groups was extremely small and not statistically significant, I deemed it permissible to combine the groups in the analyses of host-parasitoid interactions discussed in Chapter 4.

Rates of parasitism and superparasitism

My model simulated distributions of parasitism in accordance to the Poisson distribution. Rogers (1975) applied these assumptions to a parasitoid host discrimination system as follows: a) All hosts counted in the sample were available for parasitism, b) encounters between parasitoids and hosts are random, c) encounters between parasitoids and hosts are independent, d) eggs are laid singly whenever a host is discovered, and e) hosts can be reparasitized in subsequent encounters. Rogers (1975) speculated that the deviations of the observed rates of parasitism from similar models were due to a violation of one of these assumptions. The recovery of only one doubly parasitized host from the samples suggests that violations of the Poisson distribution occur because the presence of one parasitoid larva of egg in a host makes subsequent ovipositions less likely. Given the model's consistent prediction of much higher superparasitism frequencies, the observed rarity of superparasitism in the field suggests that *C. gossypariae* employs a highly effective host discrimination strategy.

Superparasitism rates depend heavily on host density, even given the ability of most parasitic hymenoptera for host discrimination. Rate of superparasitism is highest when host to parasitoid ratios are lowest (Rogers 1975; Weisser & Houston 1993; Mackauer & Chow 2016; Tunca et al. 2016). The single occurrence of superparasitism in my samples took place at the site where overall parasitism rate was highest (81%). This may represent a relaxation of *C. gossypariae*'s discrimination against superparasitized hosts at this density due to the decreasing likelihood of finding more suitable ones if they continue to search. However, no conclusions can be drawn from a single occurrence. Further study of superparasitism in *C. gossypariae* at high overall parasitism rates (greater than 80%) would be required to establish whether this species has a host density threshold at which they relax host discrimination in favour of superparasitism.

Superparasitism behaviour is nearly always studied in greenhouse and laboratory environments (Salt 1934; Rogers 1975; Blumberg & Luck 1990; González et al. 2007; Tormos et al. 2012; Chen et al. 2013; Bürgi & Mills 2013; Tunca et al. 2016). The field setting of this study precludes some of the advantages of ex-situ experiments, such as knowledge of absolute host-parasitoid ratios, consistent environments between sites, and the ability to easily observe searching and oviposition behaviour. However, this study provides rare insight into parasitoid host discrimination in natural settings, where searching females must navigate a much more complex landscape than in controlled laboratory settings.

As there is no way of knowing absolute parasitoid populations at each site, I cannot directly evaluate whether this system represents a high or low host-to-parasitoid ratio. However, percent parasitism at the most heavily parasitized site (81% of 21 dissected scales) was comparable to those observed by Tunca et al. (2016) in a laboratory study with deliberately high parasitoid-to-host ratios intended to encourage superparasitism. In their study of the solitary endoparasitoid *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), Tunca et al. (2016) observed a superparasitism rate of 13.9% when 85.6% of hosts were parasitized, steadily increasing to a 42.2% rate of superparasitism when 93.3% of hosts were parasitized. In contrast, Montoya et al. (2013) studied field superparasitism in the solitary endoparasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and found that superparasitism rates were very high (20.7% of total hosts) when total parasitism rate was only 37.7%. The differences in superparasitism rates between *O. kuvanae* and *D. longicaudata* may be explained by the differences in fitness consequences between the two species; *O. kuvanae* adults emerging from superparasitized hosts suffer reduced size and longevity (Tunca et al. 2016), whereas *D. longicaudata* do not (Gonzales and Gergel 2007). Because superparasitism is now regarded as a game of fitness and investment trade-offs (van Alphen & Visser 1990; Weisser & Houston 1993; Hamelin et al. 2007), one may conclude that *O. kuvanae* avoids superparasitism at higher rate than *D. longicaudata* because of the greater selective pressure exerted by the physiological consequences of superparasitism in this species.

Based on my observations, *C. gossypariae* seems to avoid superparasitism to a far greater degree than *D. longicaudata,* and may avoid superparasitism at a similar or greater degree than *O. kuvanae*. I would expect, therefore, that either *C. gossypariae* is among the species for which fitness consequences of superparasitism are high, or the environment of my study sites is one in which costs of continuing to search for unparasitized hosts are low. If the former is true, there may be implications for mass-rearing efficiency. While *C. gossypariae* has demonstrated that it can avoid potentially costly superparasitism consistently in single-parasitism rates up to 80%, the quality of offspring may be low in mass-rearing environments that push parasitism rate beyond this threshold and facilitate superparasitism. Studies of *C. gossypariae* in mass-rearing environments should therefore investigate the quality of adult parasitoids emerging from superparasitized hosts, as well as the overall effects on reproductive efficiency, should they occur.

Anecdotal field observations

No conclusions can be drawn from anecdotal observations of *C. gossypariae* in the field, but they provide hints into aspects of the biology otherwise unstudied. Viggiani (1999) was able to drastically extend the longevity of captive adult *C. gossypariae* by providing them with honey water. While this may mean that the adults of this species can feed on nectar of nearby flowering plants, the behaviour I observed in the field in which *C. gossypariae* adults halted for extended periods at pools of honeydew may indicate that they obtain their nutrition from this resource, and therefore may not be dependent on flowering plants for extended survival. While there is little direct evidence that ants actively protect *E. spurius* from parasitism, there is precedent for this in other systems, where ants guard scale insects from predation and parasitism in order to harvest their sugary honeydew (Burns 1973; Gaigher and Samways 2013). If ant-scale mutualism is occurring in this system, it may be an important mediating factor between the dynamics of *E. spurius* and *C. gossypariae* populations. Finally, while the recovery of parasitoid larvae from *E. spurius* nymphs does not guarantee that the parasitoids are *C. gossypariae* or that they will survive the winter, this finding provides a plausible mechanism for *C. gossypariae* winter survival.

Future directions

Although Aphelinid parasitoids are an important group in control of phytophagous pests, their small size and cryptic larval stages has resulted in a paucity of life history information on many species within this group (Gordh and Beardsley 1999). A general knowledge of a species' life history is vital for the assessment of a potential biological control agent, as well as for its successful rearing and release. Investigations in this chapter establish a baseline of information on field populations of *C. gossypariae*, contributing to the few existing studies of its biology as observed in laboratory settings. Many more questions still exist, such as the functional response of *C. gossypariae* to host densities, whether it is an opportunistic or obligate parasitoid of other host species, whether it is a recently introduced species in North America or a holarctic species, and how many generations it has per year and per host generation. Broader analyses of this tri-trophic system, including environmental factors contributing to *E. spurius* proliferation, and the effects of *C. gossypariae* on its host are also required to assess the potential for *C. gossypariae* in biological control. The contributions of this thesis project on these topics are explored in Chapters 3 and 4.

Tables and Figures

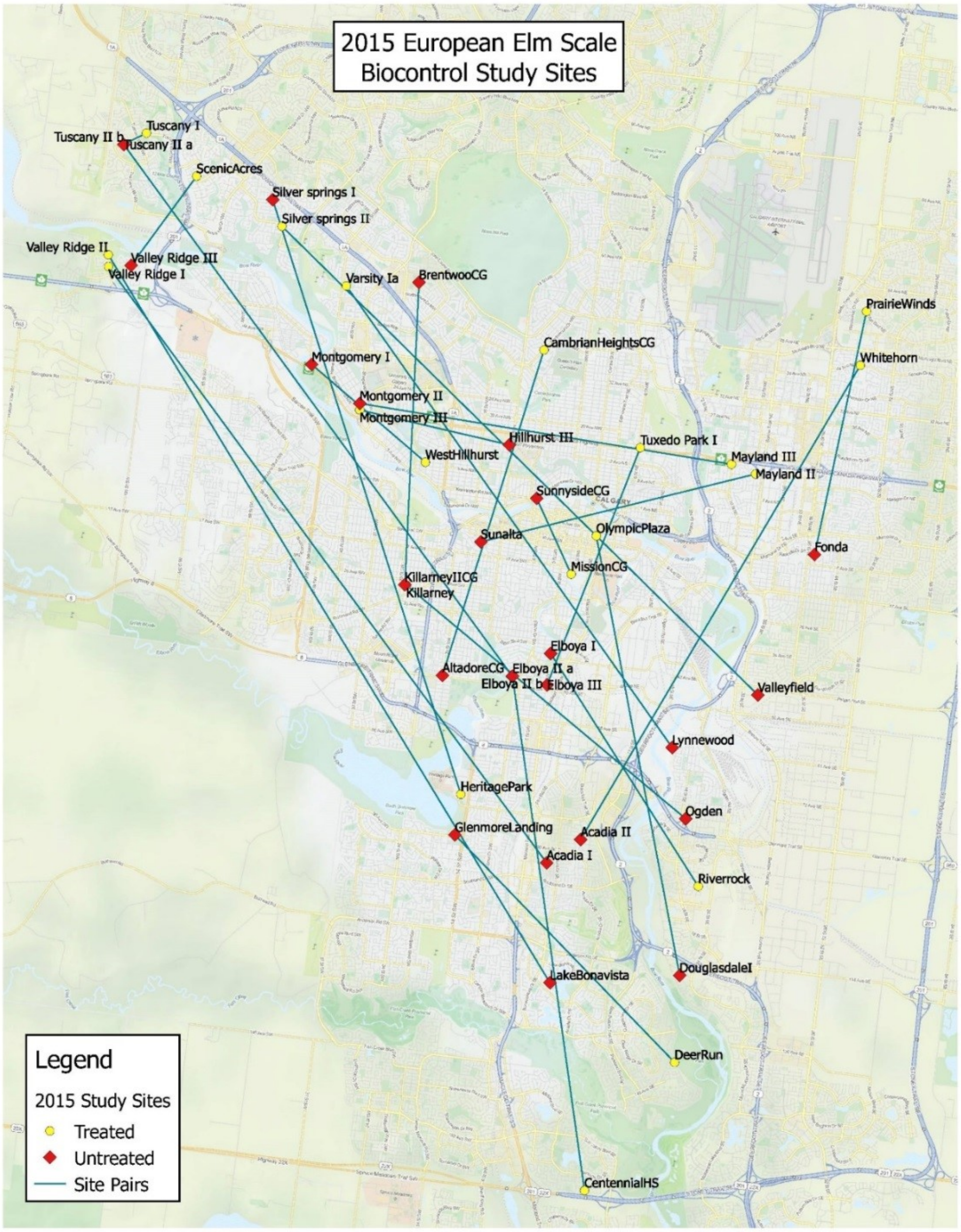
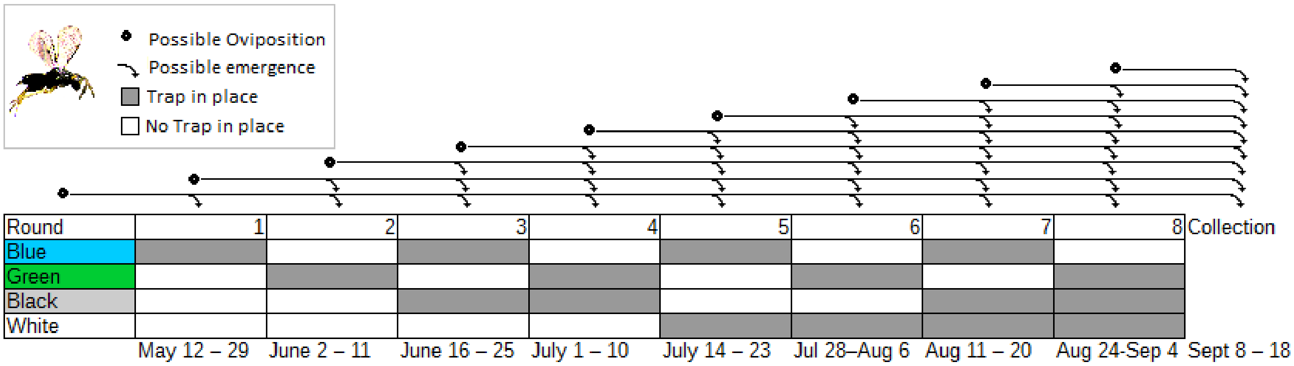


Figure 2.1: Locations of the 46 sites used in *Coccophagus gossypariae* and *Eriococcus spurius* ecology studies. Each site consists of a single American elm tree (*Ulmus americana*) that hosted parasitoid emergence traps and live *E. spurius* collection for 8 sampling rounds, which ran from May 19 to September 10, 2015. Locations in yellow denote single trees that were treated with imidacloprid between 2005 and 2010. Locations mapped in red represent trees never previously treated with imidacloprid, which were excluded from analyses in Chapter 3, but included in analyses in Chapters 2 and 4.



Round	1	2	3	4	5	6	7	8	Collection
Blue	▨		▨		▨		▨		
Green		▨		▨		▨		▨	
Black			▨	▨			▨	▨	
White					▨	▨	▨	▨	
	May 12 – 29	June 2 – 11	June 16 – 25	July 1 – 10	July 14 – 23	Jul 28–Aug 6	Aug 11 – 20	Aug 24-Sep 4	Sept 8 – 18

Figure 2.2: Schematic of net placement for parasitoid emergence trapping. Each study tree had four 10-20 cm branch sections, coded as blue, green, black and white. The locations for the traps were marked semi-permanently on each tree with electrical tape of the corresponding colour. At the end of each round, I collected and removed the trap(s) that had been in place for the preceeding two weeks, and put up traps in the assigned locations for the next sampling period.

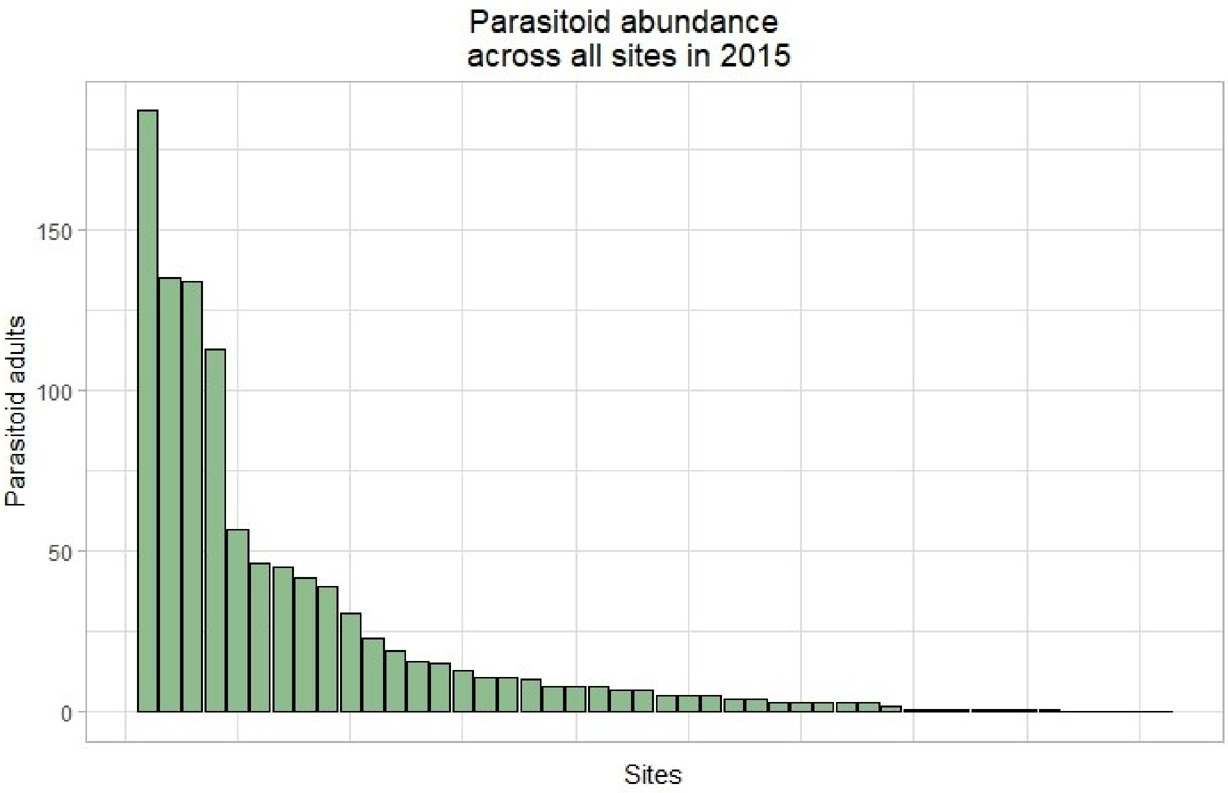


Figure 2.3: Counts of all adult parasitoids, including *Coccophagus gossypariae* and other Chalcid wasps recovered in emergence traps at each site in the Calgary study area. For each of the 46 sites (each site is a single tree), counts are cumulative for all 8 trapping rounds, representing continuous time coverage from mid May to mid September 2015.

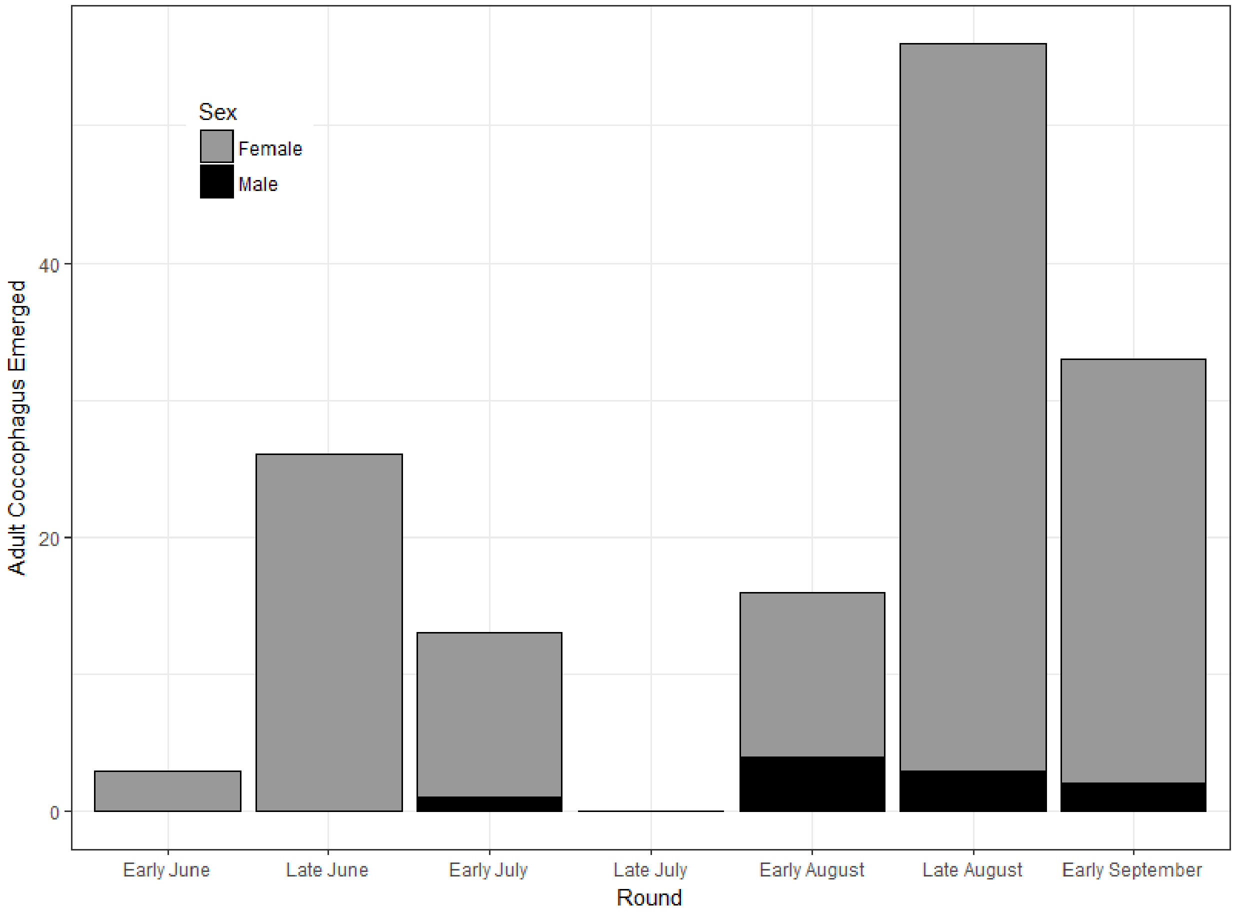


Figure 2.4: Total numbers of adult *Coccophagus gossypariae* males and females recovered and sexed from 7 rounds of emergence trapping at two locations in Calgary from June to September 2015.

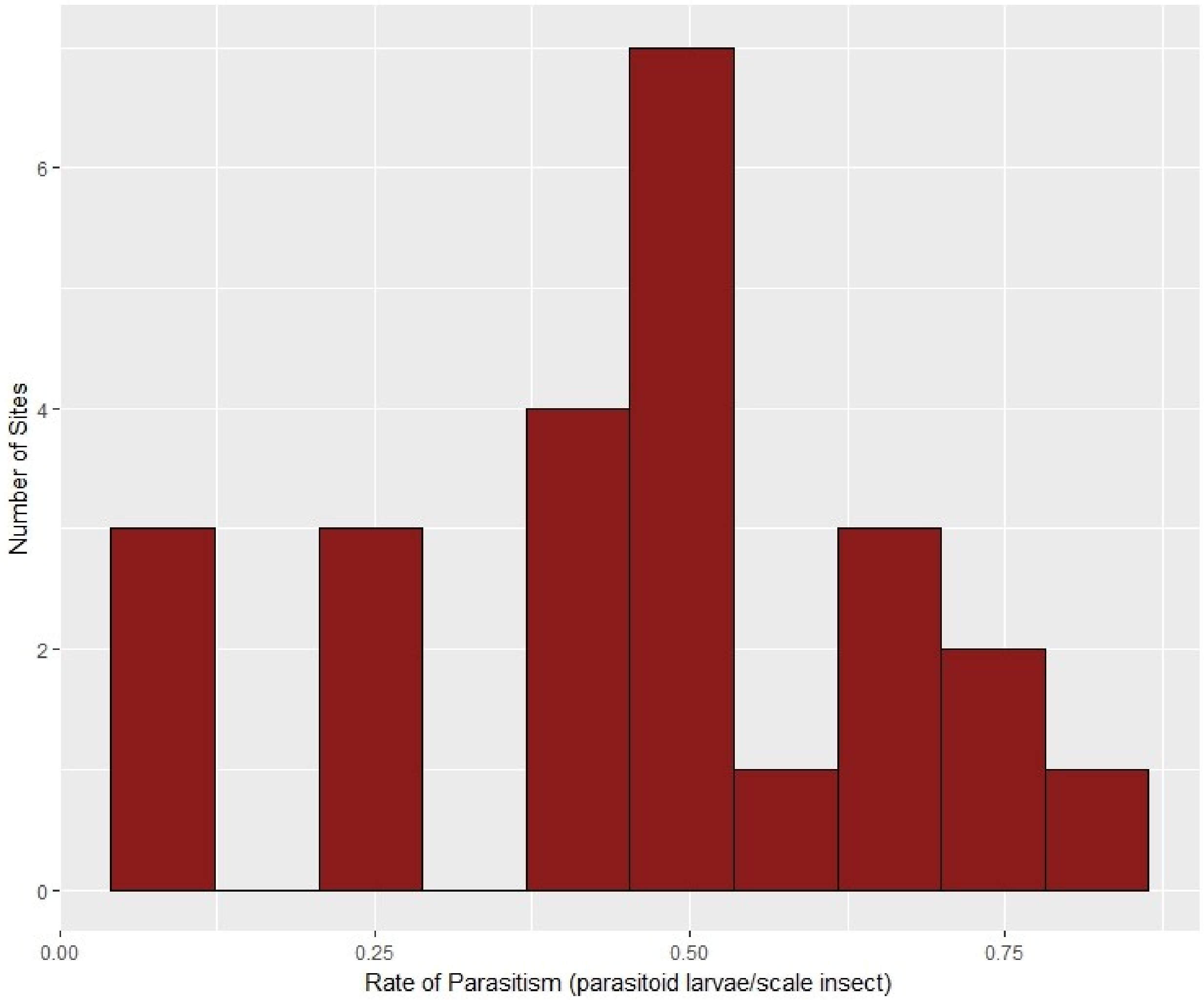


Figure 2.5: Parasitism rate of second instar nymphs and adult *Eriococcus spurius* that were collected from Calgary elm trees and dissected throughout summer 2015. Each site consisted of a single tree. Species of parasitoid larvae were not determined, but were assumed to be of a similar species assemblage as the emerged adult parasitoids that were captured (ie. 98% *Coccophagus gossypariae*).

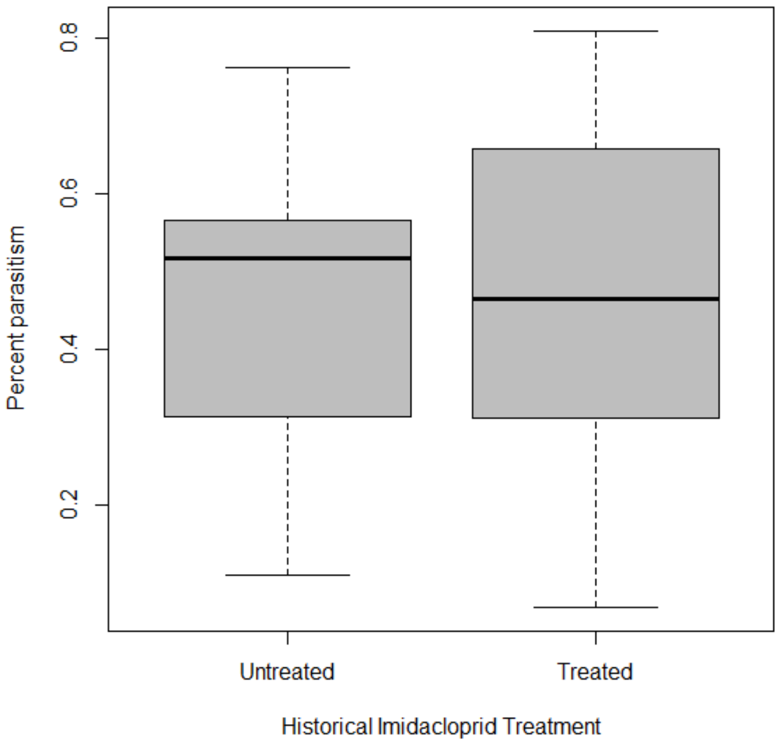


Figure 2.6: Percent parasitism by *Eriococcus spurius* estimates based on dissected scales containing parasitoid eggs, larvae, or pupae. Scales classified as "Treated" were feeding on trees that had treated with imidacloprid injections between 2008 and 2010, five to ten years prior to our study. Scales classified as "Untreated" were feeding on trees that had no previous history of imidacloprid injections.

Table 2.1: Summary of mixed logistic regression model of likelihood of *Eriococcus spurius* parasitism (binary response variable) based on historical imidaclopid treatment and sampling round. Study site was included as a random effect in the model (Variance = 0.8428, Standard Deviation = 0.9181, N= 756, groups = 24).

Fixed effect	**Estimate**	**SE**	**p value**
Intercept	-2.129	0.37	<0.001
Imidacloprid treatment (first level)	-0.308	0.423	0.466
Round	0.406	0.047	<0.001

Chapter 3: Spatial and environmental predictors of post-insecticide *Eriococcus spurius* recolonization of urban elm trees

Abstract

European elm scale (*Eriococcus spurius*) (Hemiptera: Eriococcidae) is an increasingly problematic invasive insect affecting urban American elm trees (*Ulmus americana*) in much of Western North America. Systemic insecticide injections into elms are a common current management practice. However, systemic insecticides provide only temporary suppression of insect herbivory, after which, treated trees are subject to reinfestation by *E. spurius*. This project examined four factors that potentially affected the rate at which American elms in Calgary, Canada were reinfested by *E. spurius* five or more years following injections with the neonicotinoid insecticide imidacloprid. Tree diameter at breast height, permeable surfaces over potential root area, size of urban forest patch, and total canopy area of other elm trees towards the prevailing wind direction of the reinfested tree were all significant predictors of *E. spurius* density on a per-tree basis.

Introduction

Urban forests are highly valuable, economically, ecologically and in terms of quality of life for urban residents (Tyrväinen and Väänänen 1998). American elms (*Ulmus americana* Linnaeus) are an introduced Carolinian hardwood that make up a substantial portion of the urban forests in Calgary, Edmonton, Lethbridge, and other municipalities in Alberta, Canada. European elm scale (*Eriococcus spurius* Modeer) is an invasive insect pest that occurs on various species of elms in southern Alberta, as well as many other locations in North America and the world

(Milonas et al. 2007; Kozár et al. 2009; Japoshvili and Celik 2010; Gertsson 2013). Outbreaks of *E. spurius* are currently degrading the health and aesthetic value of substantial portions of Calgary's elm trees, and have recently been detected on elms in Edmonton. Heavy infestations of *E. spurius* can cause branch dieback and severely stress the tree, making it more susceptible to other mortality risks (Burns and Honkala 1990; Dreistadt and Hagen 1994).

Both adult and larval *E. spurius* are fluid feeders on trees in the genus *Ulmus* (Williams 1985). Adult females are sessile, and feed on the fluids of young branches by inserting their mouthparts through the bark. They produce eggs in early summer, and then die the following winter. Eggs hatch into nymphs, or "crawlers", which migrate to leaves or new branches to feed. They feed for a portion of the season before migrating back to older branches where they enclose themselves in cocoon-like structures and overwinter without maturing. In the spring, nymphs that have successfully overwintered become adults and breed (Dreistadt and Hagen 1994). Like most scale insects, males are rare and winged, and females are possibly capable of parthenogenesis (Williams 1985). Because only the males are winged, and adult females are sessile, wind dispersal of crawlers is the primary method of dispersal for scale insects such as *E. spurius* (Greathead 1972; Washburn and Washburn 1984).

Neighbourhood density of elms surrounding the study trees is possibly an important predictor of pest density, as they may provide source populations for immigration to susceptible trees. Defining which elms in an urban forest surrounding a treated tree are within the range of larval wind dispersal is important for understanding this system. Wind dispersing species are most frequently deposited closest to the source of origin, with deposition rates decreasing with distance from the source (Greene et al. 2004). However, they can travel long distances, and deposition rates decrease with distance in the direction of the prevailing wind (Greene et al.

2004). This project will compare two different methods of identifying trees which may serve as source populations for reinfestation: one method using only distance, and another method which takes prevailing wind direction into account.

Calgary and surrounding municipalities have been using chemical and mechanical control to mitigate *E. spurius* outbreaks for over a decade. From 2005 to 2015, Calgary used the neonicotinoid insecticide imidacloprid, which was effective in temporarily reducing *E. spurius* populations to near zero on treated trees. Concerns over non-target effects of neonicotinoid products is currently putting their future availability in question (Health Canada 2017), and necessitating urban foresters throughout Canada to seek other products. Research is currently underway by BioForest Technologies Inc. (Sault Ste. Marie, Ontario, Canada) to evaluate the effect of azadirachtin, a non-neonicotinoid insecticide of the limnoid group, on *E. spurius*. While azadirachtin appears to significantly reduce *E. spurius* populations, it is only expected to have mortality and anti-feedant effects for up to two years, following which, the tree is expected to be recolonized by surviving *E. spurius* from the treated tree and immigrant *E. spurius* from untreated populations.

Urban foresters commonly accept the principle that increasing tree diversity and forest heterogeneity reduces the risk of healthy trees becoming infested by pests, by slowing the spread of infestation and making management more feasible (Sandalack and Marce-Santa 2002; Gulick 2014). However, as urban foresters are generally balancing the pest resilience of the total urban forest with multiple competing priorities of cost effectiveness for planting and maintenance, aesthetic appeal, and limited species available for planting, urban trees are often planted in dense, single-species stands (Conway and Vander Vecht 2015).

Despite *E. spurius* being a priority for urban forest managers in Calgary and several other North American cities, few studies exist on the spatial dynamics of this insect, in urban, rural or natural landscapes (Jalalizand et al. 2010; Karimzadeh and Iranipour 2017). This project evaluates whether the size of elm tree stands or the density of elm trees in the surrounding area can play a role in predicting *E. spurius* population densities in individual trees that have been previously treated with systemic insecticides. If so, this information can help guide planting and insecticide application practices to maximize the effectiveness insecticide treatments, by addressing risk factors that contribute to higher levels of post-treatment reinfestation. The central questions addressed by this study are: 1) Which aspects of the urban landscape are important predictors of rate of *E. spurius* recolonization following insecticide treatment? 2) Within what distance of a study site are these aspects relevant, and is this distance the same in all directions?

Because this study uses *E. spurius* density data from only one growing season, it is not possible to discern whether scale density is at equilibrium, (therefore representing total degree of post-treatment recolonization), or if populations are still recovering from previous treatments (therefore representing rate of post-treatment recolonization). However, this study assumes that *E. spurius* colonies will eventually return to their pre-treatment carrying capacities following the end of insecticide effectiveness. While there are likely many other factors determining *E. spurius* populations on different trees in Calgary, this study examines whether some of the variation in populations between trees can be explained by the spatial arrangement of potential recolonization sources.

Materials and Methods

Study area

Calgary, Alberta, Canada is located at the confluence of three ecotones in southwestern Alberta: Foothills Parkland, Central Parkland, and Foothills Fescue, less than 80 km east of the eastern slopes of the Rocky Mountains. The City covers 825 km^2, and its limits extend north-south between approximately 51°10'57.6"N and 50°50'34.9"N, and west-east from approximately 114°16'14.9"W to 113°55'8.54"W.

An important feature of Calgary's climate is its location within Canada's chinook belt, which receives warm, dry westerly winds from the Pacific region during the winter months. These winds can drastically increase temperatures in short periods of time, sometimes upwards of 20 °C in a few hours. Chinooks can have a strong negative influence on tree health in the region, because drastic mid-winter warming can induce early bud break and subsequent bud death when temperatures quickly return to their sub-continental winter normals. Losses of snow accumulation throughout the winter also detract from groundwater recharge and topsoil moisture, contributing to moisture shortage in Calgary's already dry climate (Nkemdirim 1996).

Study tree selection

The 21 trees included in this study were selected to represent a range of old and young trees (youngest estimated to be 14 years old, oldest estimated to be 65 years old). Tree condition and visual appraisals of scale infestation were deliberately ignored during the selection process. These 21 trees are subset of the 46 trees described in chapter 2, representing only those that had previously been treated with imidacloprid. All study trees were within the Calgary city limits

(Figure 2.1), but site selection aimed to maximize spacing between sites. As such, no two of these sampled trees are less than 660 m apart. All 21 trees had been treated with imidacloprid between 2005 and 2010, according to the City of Calgary's records; this selection assumes that scale populations were reduced to near zero immediately following treatment, and that scale nymphs captured in 2015 represented the degree to which recolonization had occurred.

Models predicting *Eriococcus spurius* density

I used generalized linear models to evaluate four independent variables for their ability to predict *E. spurius* densities at the individual tree level. These covariates are:

1) Trunk width at 1.3 m from the ground (also known as diameter at breast height or DBH) of the study tree,
2) area of the patch that the tree is within,
3) estimated root area beneath non-permeable surfaces, and
4) total canopy area within a specified buffer of the tree.

Figure 3.1 provides an overview of how the initial datasets were combined to create the four parameters used in the generalized linear models. The process of defining and obtaining these covariates, as well as the responding *E. spurius* density variable, is detailed below.

Field measurements of *Eriococcus spurius* density

I counted relative densities of *E. spurius* using sticky traps which intercept first instar larvae (referred to here and in general scale insect literature as “crawlers”) as they moved on tree branches. This is a commonly used method for measuring larval density of arboreal scale insects (Karimzadeh & Iranipour 2016). Traps consist of pairs of double sided tape wrapped around branches of 2 cm in diameter, at 10 cm apart. I placed traps on the same 46 elm trees used in *C. gossypariae* emergence studies, and collected and replaced them on the same two-week, eight-round schedule, for a total of 16 weeks from May 19 to September 9, 2015. Two tapes per tree were placed on the same locations on the same branches for each round, and the branches chosen were always different from branches that were being used for *C. gossypariae* emergence traps. In the laboratory, my assistants and I counted the number of crawlers on two randomly selected 1x1cm squares of each of the tapes, for a total of four squares per round, per site. I averaged these four numbers to obtain a relative density estimate for each round at each site. For the purpose of analyses in this chapter, seasonal density scores are combined into a single mean density for each site per year.

Generating canopy area estimates using Calgary's urban elm spatial data

In order to create models predicting *E. spurius* density, I needed an approximate map of the elm canopy cover within Calgary, especially those surrounding my 21 focal trees. I generated this map by adapting a dataset from the City of Calgary's urban forest database. Locations of 62,776 elm trees in within the City of Calgary were recorded in a dataset compiled between 2001 and 2015, with the aim of a complete inventory of all elm trees in the city, including those planted before and after commencement of the database compilation. The dataset was compiled from the City of Calgary Park's Departments records of planting and maintenance, and is continuously updated by its staff as trees are removed and planted. In addition to tree locations, the dataset also included DBH for most trees. DBH was determined by using calipers or a DBH tape to take the diameter of the trunk at 1.3 m from the ground. DBH scores are updated at times when City staff plant, maintain or inventory trees, and the most recent dates when these values were assessed are included in the records. While not all records are complete, 40,000 of these records that include DBH.

To make this dataset usable, an approximation of DBHs for all trees in the dataset, as of 2015 was required. I first adjusted out-of-date DBH measurements based on an estimated average growth rate of 0.25cm/yr for elm trees (Lessard et al. 2001). The mean DBH of 26.5 cm, obtained from the 40,314 complete records was applied to the remaining 22,462 records. While this method creates inaccuracies on a tree-by-tree basis, these calculations are used to estimate total canopy cover over broad areas, so I deemed applying this estimate preferable to excluding trees without recorded DBHs.

Canopy area measurements were not included in this database, and therefore needed to be inferred using DBH data, which can be a reasonable proxy (Holdaway 1986; Lessard et al. 2001). Allometric equations such as this one are often used in forest ecology. They use easily obtained tree measurements (such as DBH) to give estimates of tree dimensions that are more difficult to measure (such as age and canopy diameter) (Avery and Burkart 2015). I chose 80 elm trees for which recent (2013 or afterwards) field measurements of DBH had been recorded in the City's database, and for which Google Earth imagery was available for a similar timeframe. I measured the canopy diameters for each of these trees at their widest points using the Google Earth measurement tool. I created a general linear model describing canopy radius as a function of DBH, based on these measurements. I then applied this formula to generate a canopy diameter estimate for each tree in the City of Calgary's database, based on its own measured or estimated DBH records. These canopy diameter estimates were used to generate polygons in ArcGIS to represent elm canopy coverage within the City of Calgary.

Spatial covariates for models

1. Canopy area within circular buffers*:* To test the simplest method of quantifying canopy density surrounding each study tree, I created circular buffers of seven different radii around each site (2 km, 1 km, 500m, 250m, 125m, 62.5m, and 31.3m). I calculated the total estimated canopy area within each of these buffers. Using general linear models in R, I modeled *E. spurius* larval density as a function of canopy area at each radius. Comparing the significance of these seven models was used to determine which buffer radius yields the closest relationship between canopy area and *E. spurius* density.

2. Canopy area within wind rose buffers: A round buffer is a simple (but arbitrary) way of delineating the neighborhood surrounding a tree and the density of other trees within that area. This method does not take prevailing wind direction into account, and is therefore not ideal for wind-dispersed species such as *E. spurius*. Thus, trees may be included in the buffer area that has no influence on the target tree due to lack of favorable wind currents between the trees.

To delineate the area containing trees that are most likely to be source populations of *E. spurius*, I designed custom buffers based on hourly prevailing wind directions in Calgary for the month of July from 2012-2016 (Environment and Climate Change Canada 2017). This dataset assigns a prevailing wind direction for each hour within the specified periods. I assembled this data into counts of the month that the wind was blowing in each direction, then used this dataset to create to create wind roses, which are a circular histograms of prevailing wind direction frequencies. I created wind roses divided into twelve 30° bins (Figure 3.2) in R (R Core Team 2015), using the packages CircStats (Lund and Agostinelli 2012) and ggplot2 (Wickham 2009). July wind directions were used because this is the time of year when *E. spurius* larvae are dispersing in Calgary, (discussed in Chapter 4), and therefore the time when wind direction is most relevant to pest spread and accumulation. The choice of 2012-2016 was arbitrary, but aimed to encompass several years in which *E. spurius* dispersal into study sites might influence current populations.

As with the round buffers, I tested seven different sizes of wind rose buffers. For each wind rose buffer size the radius of the longest histogram bar corresponds to the radius of one of the seven tested round buffer classes (2 km, 1 km, 500m, 250m, 125m, 62.5m, and 31.3m). Using general linear models in R, I modeled *E. spurius* larval density as a function of canopy area at each wind rose buffer size. Comparison of the significance of these seven models was used to determine which buffer radius yields the closest relationship between canopy area and *E. spurius* density. This approach to comparison of the buffer types and sizes was used because they are slightly different methods of describing very similar data, ie. The total American elm canopy area in the surrounding neighbourhood of the focal trees. Although it is possible to use a single general linear model to evaluate which parameters are most important predictors of a responding variable, inherently correlated parameters cannot be combined in a single model. The construction and comparisons of separate models *E. spurius* density response variables allows identification of parameters yielding better model fits without the conflict of multicollinearity within a single model.

3. Patch size: Patches were defined as areas that overlapped when database trees were given buffers of two times the established radii of their canopies. This method was used because it was fairly consistent at delineating patches so that they included trees grouped during

planting by City staff. A patch area value was assigned to each study site based on the area of the patch that contained it (Figure 3.3).

4. Permeable surfaces over root area: In ArcGIS ArcMap 10.5, I created 10 m radius buffers around each of the 21 study trees. The 10-meter radius was chosen arbitrarily, but meant to approximate a reasonable area within which permeation of precipitation might reach the study tree's roots. Using ESRI's provided world imagery basemap, (with reference to more recent imagery from Google earth), I manually created polygons representing the permeable surfaces within these buffers. Ground covered with grass, garden, bare earth, and playground gravel were classified as “permeable surfaces”. All surfaces that were paved or covered by a building were classified as “non-permeable surfaces”. The permeable surface area metric used in the model was therefore the total area defined by permeable surface polygons within 10m buffers around each study tree.

Non-spatial covariates for models

Diameter at breast height (DBH) was also included in the model. I measured the DBH of each study tree during the 2015 field season, using DBH tape at 1.3 m from the ground.

Generalized linear models

Prior to other analyses, the matrix of 21 points was tested for isotropic autocorrelation in *E. spurius* density using Moran's I in R.

The four parameters were tested for colinearity using the Pearson correlation coefficient. Parameters with significant colinearity were not used in the same models.

I created generalized linear models in R describing the relationship of the four parameters (canopy area, patch area, DBH, and permeable surface area) to *E. spurius* density. A Poisson family type with a logarithmic link function was used, as the responding variable is count data, and the Poisson distribution is the most appropriate to describe this. I tested all possible combinations of the four parameters, as well as several models that included interactions between terms and ranked all models according to Aikaike's Information Criteria (Akaike 1974).

Results

Testing for spatial autocorrelation

Moran's I of the *E. spurius* density at each site did not show significant autocorrelation (Observed = -0.0169, Expected = -0.0526, SD = 0.0694, p =0.316). A spatial auto-covariate term was therefore not used in any of the linear models.

Generating the elm canopy area estimates

A general linear model of canopy diameter as a function of DBH yielded the following formula (Residual standard error: 1.742, DF:79, R^2: 0.9118 p-value: < 0.001):

$$\text{Canopy diameter} = 0.990137*(0.182)\text{DBH}$$

Visual comparison of the canopy polygons generated for Calgary's elm forest using this equation to aerial imagery of corresponding trees confirmed a satisfactory fit (Figure 3.4).

Comparing buffer sizes and shapes for canopy area metrics

The ability of elm canopy area to predict *E. spurius* density was not significant using any of the buffer sizes and shapes tested using general linear models (**Error! Reference source not found.**). In the model that came closest to significance, *E. spurius* density was plotted as a factor of elm canopy area within the 250m radius wind rose buffers. This parameter was therefore chosen as the "canopy area" measurement for the generalized linear model comparisons used later.

Generalized linear model comparisons

I tested 19 models expressing all possible combinations of the four covariates, including the null model, as well as some possible interactions between parameters. Log likelihood, and Akaike Information Criterion comparisons for all models are summarized in (**Error! Reference source not found.**). The best performing models, according to AIC rankings, included most or all of the tested parameters, including an interaction term between canopy area and impermeable surface area. In general models including more of the parameters scored higher than those including fewer, with the null model scoring lowest. McFadden pseudo R squared values for the top three models suggest that no more than one third of the between-site variation in *E. spurius* was explained using this technique.

The formula for the top model is as follows:

Scale density = 6.145e+00 + (4.911e-03)DBH + (-2.712e-04)Patch area +

$$(7.264e\text{-}04)\ \text{Upwind Canopy area} + (-2.072e\text{-}03)\text{Permeable surface area} + (-6.103e\text{-}06)\ \text{nonperm:WR250}$$

Where $p < 0.001$ for all model parameters, with the exception of DBH ($p = 0.001$). According to this model, a tree's DBH and upwind canopy area have a positive effect on post-insecticide scale recolonization, while permeable surface area above its roots, and patch size have negative effects on scale recolonization.

Discussion

After an urban elm has been treated with a systemic insecticide to reduce *E. spurius* population, a number of factors influence the rate and degree to which it will be recolonized. This paper identifies four factors that are important; the tree's diameter at breast height, the availability of permeable surfaces within a radius accessible to its roots, the canopy area of all trees in its local patch, and the canopy area of trees located towards the prevailing wind direction relative to the target tree.

Diameter at breast height had a positive influence on *E. spurius* density, but the effect was small, and less statistically significant than other parameters. This may imply that larger trees are more susceptible to *E. spurius* recolonization. While other studies evaluating the effects of DBH on scale insect susceptibility have not found it to be an important factor (Gripenberg and Roslin 2005; Wardhaugh and Didham 2006), tree age has been shown to increase susceptibility to insect and fungal pests in other systems (Amman et al. 1988; Castello et al. 1995).

The negative influence of nearby permeable surfaces on *E. spurius* density is unsurprising, given that *E. spurius* populations survive better on trees with reduced vigor

(Dreistadt and Hagen 1994), and the negative effects of impermeable surfaces on urban tree vigor are well documented (Ferrini and Baietto 2007). Reduction in tree vigor associated with permeable surfaces over tree root area is attributed to reduction of access to oxygen from the atmosphere and moisture from precipitation (Balakina et al. 2005), as well as soil compaction impeding root development (Kozlowski 1999), and heat island effects contributing to a higher temperature microclimate (Avissar 1996). The inclusion of permeable surfaces area in the highest ranked model supports the consideration of permeable surfaces when evaluating an individual tree's risk of *E. spurius* infestation. Individual tree specific estimates of adjacent permeable surface area impacts on *E. spurius* density would be improved by a more accurate representation of below-ground root coverage. However, allometric equations that would allow these estimates from above-ground measurements such as DBH are species and environment specific and I could not find any such estimates for American elms, in Calgary or elsewhere.

Trees in larger patches have lower *E. spurius* recolonization rates. This is counter to expectation, as larger single-species stands are generally more susceptible to insect herbivory, due to ease of insect dispersal between hosts, and a lower diversity of the pest's natural enemies (Hobbs 1988; Jactel and Brockerhoff 2007). A possible explanation for the lack of positive relationship between patch size and *E. spurius* density is that urban trees that are adjacent to each other are usually treated with insecticide in groups such as rows on boulevards, which often correspond with patch assignment in these analyses. This study only considered the treatment history of the 21 field surveyed trees, and assumed that all other trees within each study tree's patch were untreated potential *E. spurius* sources. However in reality, all trees in a study tree's patch were likely treated with insecticide concurrently, rendering less likely to be less significant sources for reinfesting *E. spurius* larvae. However, uniform within-patch insecticide treatment does not account for the apparent negative relationship between patch size and *E. spurius* density.

Island biogeography theory (MacArthur and Wilson 1967), provides one possible explanation for lower *E. spurius* density in post treatment elms within larger conspecific patches. If patches of urban elms behave like islands in theory of island biogeography larger patches should have greater immigration rate of *E. spurius*, but also higher species richness and immigration probability of potential natural enemies specializing on pests of American elms, which would also perceive elm patches as islands in a matrix. At least one species of parasitoid specific to *E. spurius* is present in this system, and its population interactions with *E. spurius* could account for some of these unexplained patterns in *E. spurius* density. If this is the case, the negative relationship between patch size and *E. spurius* density could be due to natural enemy benefits outweighing higher *E. spurius* immigration rates, especially if these natural enemies are more resilient to the systemic insecticide treatments. More detailed studies of the relationship between patch size and *E. spurius* density are required before any management implications can be inferred from this result.

The superior fit of models using wind rose buffers to define canopy areas in relationship to treated elms suggests that trees that are frequently upwind of treated trees are potential sources of re-infestation at longer distances than those that are less frequently upwind. This is consistent with the dispersal method of *E. spurius* larvae, as larvae originating on untreated trees have a much greater chance of being blown to a treated tree if it is more frequently downwind of it. Rigot et al. (2014) studied the predicting factors of scale insect larval dispersal, and found that wind speed and direction were the most important predictors of the speed of spread of an invasive scale insect over the island of Corsica. Rigot et al. (2014) also found evidence of the importance of surface feature induced wind turbulence in scale insect dispersal, which cannot be described by the wind rose buffers used in this study; Wind rose buffers are an oversimplification of the actual wind conditions that may affect scale dispersal, but is an easy proxy to improve estimations of areas of source *E. spurius* populations that will increase a treated tree's risk of reinfestation. Models can likely be greatly improved with more complexity, and this study indicates that such analyses of this system are worth pursuing. Models also treat all database trees other than treated study trees as untreated, when in reality, many have also been treated with insecticides, and are less likely to be *E. spurius* population sources. Improvement of City treatment records and integration into future models could also improve their fit.

Management implications

As *E. spurius* becomes an increasing problem for urban elm forests, urban forest managers area currently seeking ways of mitigating its impacts, given limited budgets, usually involving choosing trees and stands to prioritize for treatment (Lindsay Bell, City of Lethbridge Urban Forestry division, pers. comm. 2017; Mike Jenkins, City of Edmonton Pest Management Lab, pers. comm. 2017; Jim Watts, City of Calgary Urban Conservation Unit, personal communication 2017). This model demonstrates that DBH and permeable surface area values for individual trees can help identify trees at high risk of *E. spurius* re-infestation post treatment. A better understanding of the influences of wind patterns in reinfestation of treated trees from untreated trees can help managers better maximizing the longevity of treatment effects by advantageously planning the spatial patterns of their treatment programs. Reducing the treatment frequency required to control *E. spurius* populations also serves the important purposes of slowing the development of insecticide resistance in herbivore populations (Goka 1999), and reducing non-target effects in the environment, both of which are concerns with azadirachtin (Ruiu et al. 2008; Fernández et al. 2009).

Other sources of variation

As the best generalized linear model produced by these analyses explains only an estimated 27% of the variation of *E. spurius* density in recolonized elms, there are likely other important predictors not included in this study. While all study trees had been treated with imidacloprid between 5 and 10 years prior to *E. spurius* density measurements, records were not specific enough to include the exact year of treatment for each tree. Population dynamics of *E. spurius* and their natural enemies, as well as tree defenses to herbivory, and winter survival rates in relation to temperature minimums are also potentially important factors. These are still important considerations for future research on this system.

Conclusions

The degree of *E. spurius* reinfestation of American elms five or more years following treatment with imidacloprid can be partially predicted using the four parameters of: 1) Canopy area of upwind trees during *E. spurius* larval dispersal, 2) area of the patch containing the tree subject to reinfestation 2) DBH, and 3) the area of impermeable surfaces within a 10m radius of the tree. More specific investigations into any of these parameters may lead to better predictions of *E. spurius* reinfestation of treated trees. However, the top model produced by these analyses can provide insight into how to prolong the effective time of insecticide treatments by managing for these risk factors.

Tables and Figures

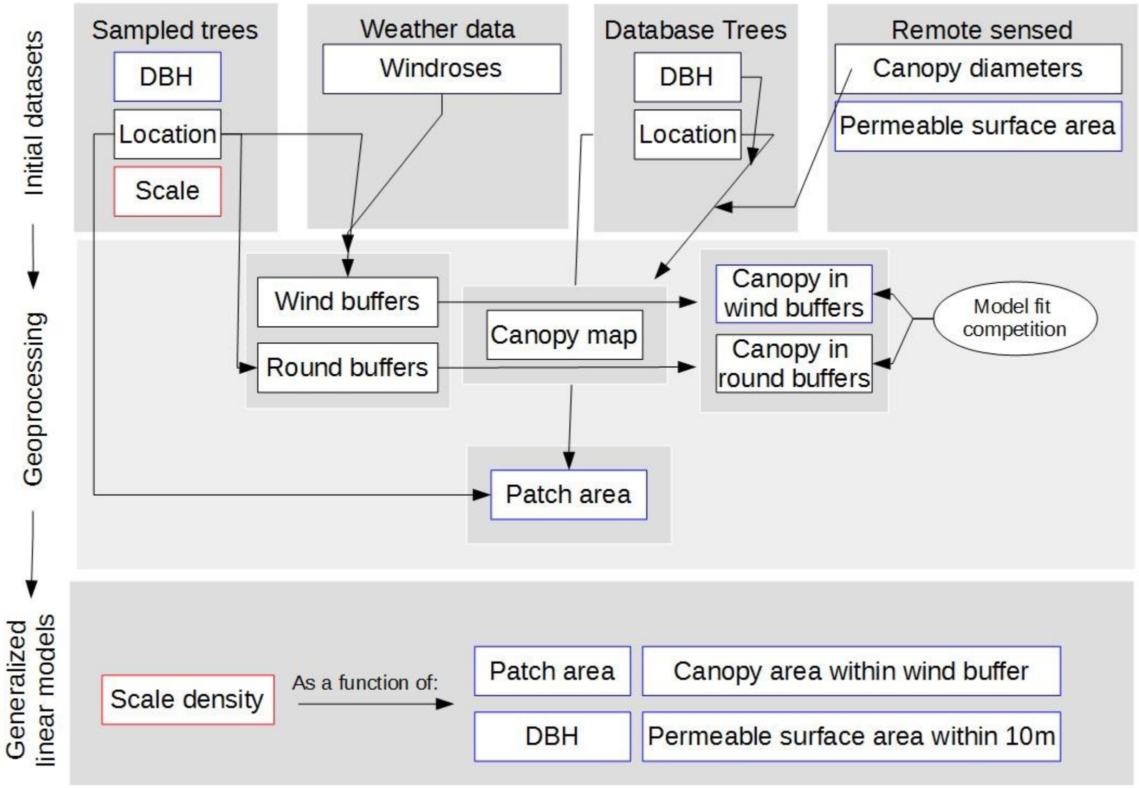


Figure 3.1: Flow diagram of the process of obtaining the values for the four parameters tested in the generalized linear models. For each of 21 study trees, four types of data were combined using geoprocessing and allometric equations to produce measurements of patch area, diameter at breast height, canopy area within a defined "neighborhood", and area of permeable surfaces within 10 m.

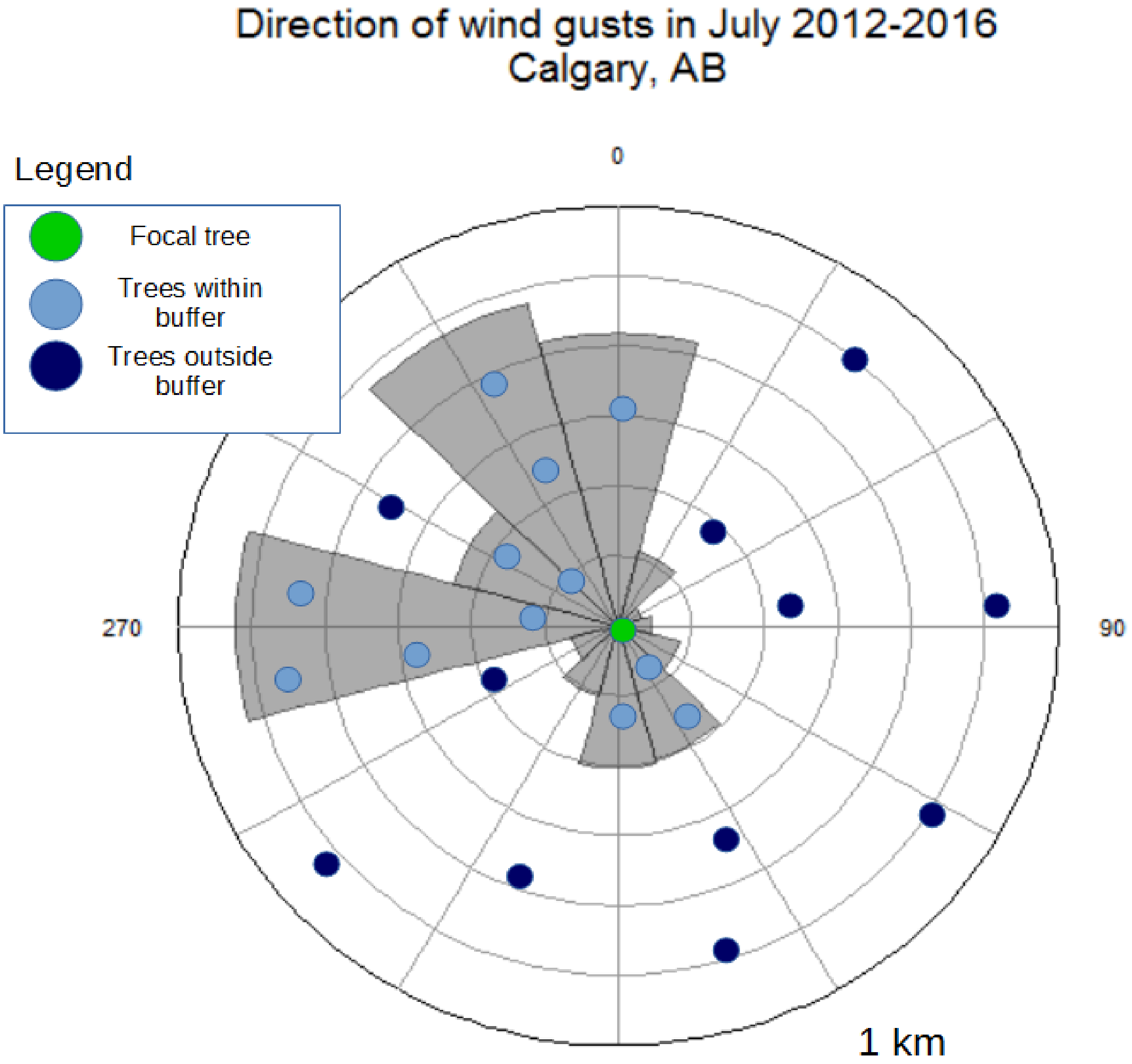


Figure 3.2: Site buffer design based on prevailing winds in Calgary during *Eriococcus spurius* larva dispersal: Trees downwind of the study site must be much closer to the focal tree to be considered part of that tree's neighbourhood, while upwind trees can be much farther away, and still considered potential sources for *Eriococcus spurius* immigration.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 3.3: An example of patch delineation for one of the study sites used to model the relationship between *Eriococcus spurius* density and patch size. The size of the patch containing each 2015 study site was estimated using the approximate total canopy area of trees in the containing stand that were close enough proximity to each other to have overlapping or near overlapping canopies.

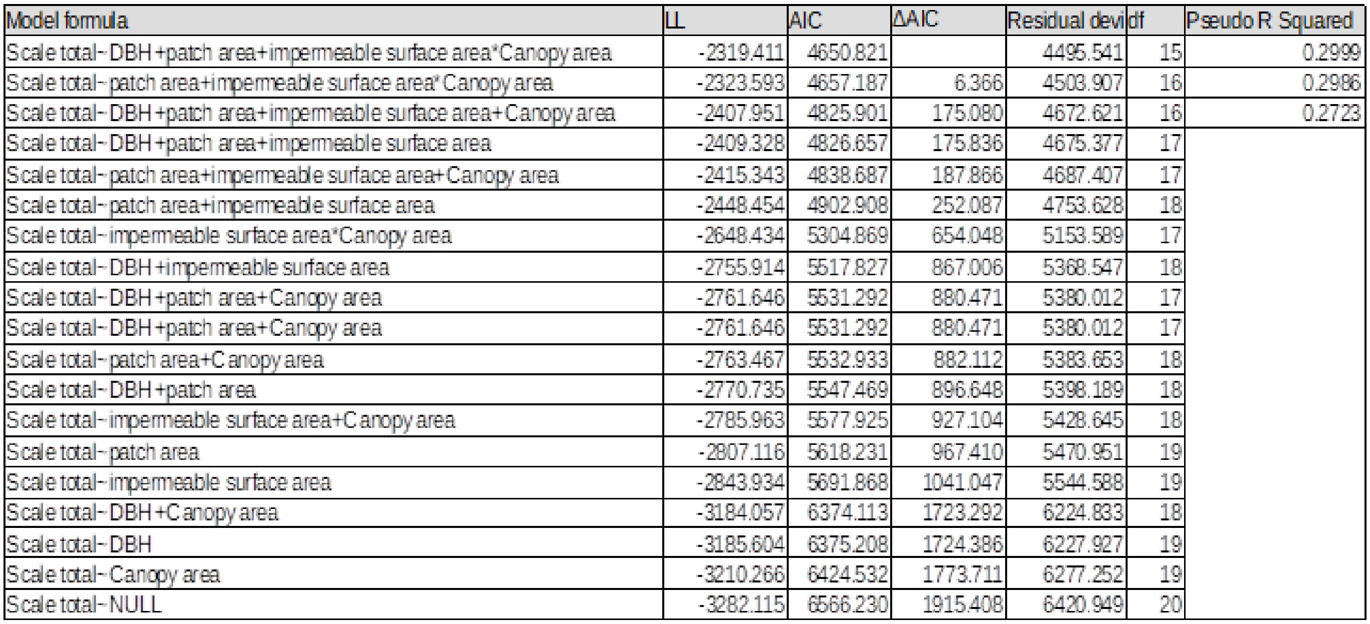
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 3.4: Automatically generated canopy polygons of Calgary's American elm (*Ulmus americana*) forest. The City of Calgary keeps records of diameter at breast height of public trees, but not of canopy diameter estimates. I generated canopy diameter estimates using a general linear model of canopy radius as a function of diameter at breast height. These diameters were used to generate polygons representing an estimate of the canopy cover of public American elms in Calgary as of 2015, which were used in the spatial analyses in Chapter 3.

Table 3.1: Comparison of significance levels of singe factor general linear models using seven different sizes and two different shapes of buffers used to define canopy area potentially influencing *Eriococcus spurius* density five or more years following treatment with insecticides.

	P values of single parameter linear models	
Radius	Round Buffers	Windrose buffers
31	0.3739	0.8847
62	0.4346	0.6433
125	0.6458	0.6206
250	0.5045	0.2337
500	0.5764	0.6523
1000	0.7723	0.3377
2000	0.8811	0.5414

Table 3.2: Summary of generalized linear models of all combinations of potential predictors of *Eriococcus spurius* density (scale total) at reinfested study sites. Models are ranked by Akaike Information Criterion score, and McFadden's Pseudo Rs are provided for the top 3 models.



Model formula	LL	AIC	ΔAIC	Residual devi	df	Pseudo R Squared
Scale total~DBH+patch area+impermeable surface area*Canopy area	-2319.411	4650.821		4495.541	15	0.2999
Scale total~patch area+impermeable surface area*Canopy area	-2323.593	4657.187	6.366	4503.907	16	0.2986
Scale total~DBH+patch area+impermeable surface area+Canopy area	-2407.951	4825.901	175.080	4672.621	16	0.2723
Scale total~DBH+patch area+impermeable surface area	-2409.328	4826.657	175.836	4675.377	17	
Scale total~patch area+impermeable surface area+Canopy area	-2415.343	4838.687	187.866	4687.407	17	
Scale total~patch area+impermeable surface area	-2448.454	4902.908	252.087	4753.628	18	
Scale total~impermeable surface area*Canopy area	-2648.434	5304.869	654.048	5153.589	17	
Scale total~DBH+impermeable surface area	-2755.914	5517.827	867.006	5368.547	18	
Scale total~DBH+patch area+Canopy area	-2761.646	5531.292	880.471	5380.012	17	
Scale total~DBH+patch area+Canopy area	-2761.646	5531.292	880.471	5380.012	17	
Scale total~patch area+Canopy area	-2763.467	5532.933	882.112	5383.653	18	
Scale total~DBH+patch area	-2770.735	5547.469	896.648	5398.189	18	
Scale total~impermeable surface area+Canopy area	-2785.963	5577.925	927.104	5428.645	18	
Scale total~patch area	-2807.116	5618.231	967.410	5470.951	19	
Scale total~impermeable surface area	-2843.934	5691.868	1041.047	5544.588	19	
Scale total~DBH+Canopy area	-3184.057	6374.113	1723.292	6224.833	18	
Scale total~DBH	-3185.604	6375.208	1724.386	6227.927	19	
Scale total~Canopy area	-3210.266	6424.532	1773.711	6277.252	19	
Scale total~NULL	-3282.115	6566.230	1915.408	6420.949	20	

Chapter 4: Relative phenologies of *Coccophagus gossypariae* and *Eriococcus spurius*, and their effects on host fitness

Abstract

This chapter describes a series of studies on *Coccophagus gossypariae* and *Eriococcus spurius* phenology, and how the two species interact over the course of a single growing season. I used repeated measures trapping throughout the 2015 Calgary growing season to trap first instar *E. spurius* nymphs as they hatched from eggs, and adult *C. gossypariae* as they emerged from their hosts, adult *E. spurius*. Using the yields of these two traps, I created a timeline throughout the season which showed that most *C. gossypariae* emerge late in the season, killing their hosts after they have reproduced. As *E. spurius* adults do not survive the winter regardless of parasitism, extra mortality caused by *C. gossypariae* late in the season is unlikely to influence *E. spurius* populations the following year. However, I also measured the effect of *C. gossypariae* parasitism on *E. spurius* egg production throughout the season, and found that early season parasitism can significantly reduce fecundity of infected *E. spurius.* If *C. gossypariae* is capable of suppressing *E. spurius* populations, it is therefore likely due to fecundity reduction rather than mortality effects. Finally, I tested the prediction that *E. spurius* populations with high rates of parasitism by *C. gossypariae* would show a lower population growth than those with low rates of parasitism. I found no evidence for this, but this may be due to limitation of the study to two host generations, or to more complicated host-parasitoid population dynamics than my exploratory study could account for.

Introduction

American elms (*Ulmus americana* Linnaeus) are an important component of urban forests in Alberta, but are often infested with the invasive soft scale insect *Eriococcus spurius* Modeer (Hemiptera: Eriococcidae). In Calgary, heavy infestations of *E. spurius* cause significant damage to the urban elm forest. The City of Calgary's Urban Conservation department currently manages *E. spurius* outbreaks using systemic injections of the neonicotinoid insecticide imidacloprid, but are currently looking for other methods of controlling *E. spurius*, including biological control.

The main parasitoid of *E. spurius* in Calgary is *Coccophagus gossypariae* Gahan (Hymenoptera: Aphelinidae). It is established throughout Calgary nearly everywhere that *E. spurius* exists, and makes up 98% of the parasitoid assemblage associated with *E. spurius* (See Chapter 2). Other members of the *Coccophagus* genus have been useful as biological control agents of scale insects and their relatives (Enkegaard and Brødsgaard 2006). *Coccophagus gossypariae* may be a candidate for augmentative or conservation biological control if it is capable of suppressing its host's populations when aided by mass-rearing and releases, or alterations in the environment to favour its proliferation over its host's. However, few studies have investigated the life history of *C. gossypariae* (Griswold 1927; Viggiani 1998, 1999), and none have conducted thorough field investigations into its interactions with *E. spurius.* In order to evaluate the feasibility of using *C. gossypariae* to suppress field populations of *E. spurius*, a thorough understanding of the system is necessary.

The relative timing of reproductive events is central to the studies in this chapter. Preliminary observations of the September emergences of *C. gossypariae* suggests that emergence events occur too late in the season to kill hosts before the onset of reproduction. If so, host mortality inflicted by *C. gossypariae* could be biologically irrelevant as adult *E. spurius* live only a single season. However, *C. gossypariae* may still reduce their host's reproduction capacities if *E. spurius* is parasitized early in the season and suffer significantly reduced egg production. In this chapter, I compare the relative timings of *E. spurius* reproduction and *C. gossypariae* emergence, to establish whether the parasitoid is likely to cause significant pre-reproductive mortality in its host populations. I also investigate whether parasitoid effects on fecundity have the potential to suppress *E. spurius* populations. Finally, I present a cursory investigation of how parasitism rate by *C. gossypariae* affects the rate of change in *E. spurius* populations between two growing seasons. The goal of these studies was to provide some base information on *E. spurius* and *C. gossypariae* phenology and interactions, as they have not been previously studied in the field.

Materials and Methods

Tree selection

Samples collected in this chapter were collected at the same, using the same sampling scheme as described in Chapter 2. Figure 2.1 provides a map of these 46 sample sites used in 2015. I attempted to use the same sites in 2016, but two of the original trees were excluded as interim winter pruning had removed all accessible branches. As the analyses in this chapter require a comparison between 2015 and 2016, I used data only from the 44 sites where sampling was possible in both years.

Emergence nets for *Coccophagus gossypariae* phenology studies

At each of the 44 elms used in these analyses, I set up a series of fine mesh nets, which captured adult *C. gossypariae* as they emerged from their hosts through out the season, allowing counts of the relative numbers of *C. Coccophagus* emerging in eight two-week blocks throughout the season. A detailed explanation of the setup of these traps is in Chapter 2.

Phenology and density of *Eriococcus spurius* larvae

To obtain relative densities of *E. spurius* larvae, as well as the timing of their hatching, I conducted larval *E. spurius* trapping using the same methods described in Chapter 3. However, as I established basic phenology using the 2015 studies, and 2016 studies were only to obtain a density estimate at peak activity times, I shortened the 2016 trapping period so that it was only 10 weeks long, running from late June to late August.

Fecundity and parasitism of *Eriococcus spurius*

Following the methods described in Chapter 2, I collected live adult and late instar nymph scales for later clearing and dissection. However, for the data used in this chapter, I also counted the number of scale eggs inside each adult female *E. spurius* to serve as a measurement of fecundity, but realized fecundity was not assessed in this study.

Data Analysis

Measurement of growing degree days

Time was measured in this study using Growing Degree Day accumulation. To calculate growing degree-day values for each date of sample collection, I used the following equation, the usage of which is described by McMaster and Wilhelm (1997):

$$GDD = [\frac{(T_{MAX} - T_{MIN})}{2}] - T_{BASE}$$

Where T_{MAX} and T_{MIN} are the maximum and minimum daily temperature daily temperatures, and T_{BASE} is a pre-determined minimum threshold, for which 10 °C is commonly used. Negative values do not accumulate, and are counted as "0". Growing degree-day accumulation for each day within the sample period represented the sum of all growing degree-day values accumulated starting on March 1, 2015. Daily maximum and minimum temperatures were obtained from Environment and Climate Change Canada's Olympic Park weather station in Calgary (Environment and Climate Change Canada 2016).

Evaluation of sampling artifacts in emergence trapping method

Each round had a unique combination of traps that was replicated across all sites. Therefore, emergence rate estimates were potentially affected by the trapping configuration used during that round. For each round, I compared means of trap yields by colour-coded trapping configuration, across all sampling trees. I used Mann-Whitney U Tests to compare trap yields within rounds three, four, five and six, which had only two traps in place per round, and Kruskal-Wallis rank sum tests on trap yields within rounds seven and eight, which had three traps in place per round. Both test types were chosen due to the non-parametric nature of the data. Mann-

Whitney U tests were best suited for comparisons of trap yields within rounds three to six because they required comparison of only two trap types, while Kruskal-Wallis rank sum tests were best suited when comparing trap yields within rounds seven and eight because they required the comparison of three trap types.

Coccophagus gossypariae emergence timelines

Regardless of differences in the effectiveness of different trapping configurations, sampling efforts were uneven in each round, as the number of traps in place at a site increased from 1 to 3 throughout the season. To account for this uneven sampling effort, round emergence rates were based on the average number of *C. gossypariae* recovered per trap.

To create an emergence timeline that reflected peak emergences for all sites, and did not over represent emergence pattern at sites with the highest density of *C. gossypariae,* I normalized the results so that each round at each site represented the proportion of the total mean of wasps recovered at that site.

Sampling was done on a two-week rotation, with half the sites visited in the first week of each round, and the other half visited in the second. This meant that traps collected in week 2 of any given round were just as temporally close to traps collected in the first week 1 of the same round as they were to traps collected in week 1 of the following round. Therefore, when performing statistical analyses and graphically representing wasp emergence, I subdivided the rounds by week and analyzed them as independent rounds.

I wanted to find out if there were significant differences in emergence rates between rounds. This would help me determine whether visual peaks represented significant, synchronous surge in emergence across all sites, or random noise. As this data was derived from count-data, non-parametric tests were appropriate to compare the emergence means. I performed Kruskal-Wallis test on the emergence rates among rounds. Following the Kruskal-Wallis test, I performed a Dunn test with a Bonferroni correction on each round-pairing to determine which rounds were significantly different from each other.

Eriococcus spurius phenology

For each measured round in 2015 and 2016, I calculated the mean and 95% confidence interval of scale crawler density (crawlers/cm^2 recovered from scale tapes), with sites considered replicates. I also calculated the value and timing of peak crawler density for each site in both 2015 and 2016.

Eriococcus spurius fecundity and parasitism by Coccophagus gossypariae

To estimate the effects of parasitism on *E. spurius* reproductive output, I used the number of eggs developing within a female scale insect as a measure of fecundity. To avoid pseudo-replication, summary statistics were based on fecundity means for each site at each round. Sites were treated as replicates, rather than individual dissected scales within sites. For each round, I calculated the mean, median, 95% confidence intervals, maximum and minimum, for fecundities of both parasitized and unparasitized female *E. spurius*.

Population effects

Using data from the scale crawler density tapes, I compared the mean crawler densities at each site's peak emergence round for 2015 and 2016. Yearly changes by site were normalized to account for overall changes in scale populations between the years due to environmental conditions. Proportion change between *E. spurius* populations at each site were then modeled in relation to each site's percent parasitism using general linear and quadratic models.

Results

Coccophagus gossypariae phenology

Evaluation of sampling artifacts in emergence trapping method

Different trapping configurations did not yield significantly different numbers of *C. gossypariae* (Table 4.1). However, the difference in means between the green and white traps in round 6 was near significance in a Wilcoxon rank sum test (W= 838.5, p=0.0582).

Temporal analyses

Coccophagus gossypariae were recovered in both weeks of all sampling rounds, but not at all sites. Different sites had different temporal distributions of wasp emergence, and the timing of peak emergence was not consistent across all sites. When emergence proportions for all sites are combined, we see a pattern of emergence rates that oscillates between rounds, but slowly builds over the season, reaching its peak in week 2 of round 6, which corresponds to 96-99 accumulated Growing Degree Days (Fig. 4.1).

When emergence proportions were compared between rounds, a Kruskal-Wallis test identified a significant difference between ranked group means (Kruskal-Wallis chi-squared = 63.988, df = 15, p-value = 5.136e-08). A Bonferroni corrected Dunn test revealed significant difference only between emergence rates during round 6, week 2 (the time period with the highest proportion of emergence), and Round 2, week 2 (the time period with the lowest proportion of emergence). However, without the Bonferroni correction, round 6, week 2 is significantly different from all other rounds, while no other rounds are significantly different from each other.

Eriococcus spurius density and phenology

Scale density (crawlers /cm^2) varied widely between sites (min= 0.09, max = 75.47, mean= 15.82, median = 4.77). Sites varied far less in their temporal patterns of scale activity, with 35 out of the 46 sites reaching their peak measured crawler density in rounds 3 to 5 (Fig. 4.2). Peak scale density was recovered in round 4, which was sampled between June 30 and July 10, 2015, and spanned 245-346 growing degree days.

Fecundity and parasitism of *Eriococcus spurius*

Only 24 out of the 46 sites were used in analyses of parasitism and superparasitism rates, because *E. spurius* populations were insufficient at 22 of the sites to consistently collect scales for dissection. These analyses therefore represent only the 52% of sites with the highest scale populations. I dissected a total of 778 scales, of which 306 were parasitized. Although some samples were damaged during KOH treatment, in most samples developing parasitoids and *E. spurius* eggs were clearly visible within adult *E. spurius* (Figs 4.3 & 4.4), and easily identified and counted. Host fecundity was significantly reduced by parasitism in all sampling rounds sessions (Table 4.2; Fig. 4.5). Although I collected second instar nymphs from the field, this was uncommon, and few of these survived the clearing process. Those that survived were never parasitized, nor did they contain developing eggs. They were therefore not included in these analyses.

Effects of *Coccophagus gossypariae* parasitism on *Eriococcus spurius* populations

Two of the 46 study trees used in 2015 were excluded from the studies in 2016, as their accessible branches were pruned off over the intervening winter. Furthermore, I was able to obtain percent parasitism estimates for only 22 of these sites due to specimen availability in the field and losses in the KOH clearing process. As a result, year-to-year comparisons represent only 22 trees. The overall scale population decreased between the two years, with 2016 scale densities representing only 74.6% of 2015's densities. I adjusted 2016 density estimates at all sites by a factor of 1.34 to account for this decrease. Although some sites decreased in density between the two years and others increased, there was no clear pattern between parasitism rates and change in scale density between the two years (Fig. 4.6). Neither linear nor quadratic models reached near significance in explaining trends between the two variables: (Linear model: multiple R-squared = 0.042, F = 0.923, p= 0.348; Quadratic model: Multiple R-squared = 0.091, F= 0.9967, p= 0.3867).

Discussion

The relative timings of peak adult *C. gossypariae* emergence to peak scale hatching demonstrates that most parasitism-induced host mortality occurs after adult *E. spurius* have laid most of their eggs. As adult *E. spurius* will die over the winter regardless of parasitism, this late-season mortality due to parasitism likely has little influence on *E. spurius* populations the following year. Although there is some parasitoid emergence, and therefore host mortality, throughout the season, any parasitoid-related population influences are likely driven by early-season fecundity effects rather than mortality effects. This study provides a starting point for further research into the multi-generational interactions between *E. spurius* and *C. gossypariae.*

The recovery of *C. gossypariae* from all sampling rounds indicates that they emerge throughout the season, and that their yearly activity likely extends longer than the mid-May to mid-September period sampled in this study. In 2015 I saw only one major peak, which culminated at the accumulation of around 97 10°C base growing degree-days, from August 4-6. The Kruskal-Wallis test serves to provide statistical validation that this pattern did not occur at random, and the Dunn test supports this peak being the season's only major peak in emergence.

The decrease in *E. spurius* fecundity associated with parasitism was consistent through time and between sites. As there was no near overlap between 95% confidence intervals of the two groups in any round, I did not deem further statistical testing necessary to classify these differences as significant. The effect of parasitism on host fecundity is not surprising, as Campbell and Mackauer (1975), and Kaiser and Heimpel (2016) have both noted that parasitism by other families of hymenopteran parasitoids sufficiently reduces fecundity in aphids to cause population reduction in the resulting generation. However, Kaiser and Heimpel (2016) noted that while the offspring of parasitized aphids were fewer, compensatory increases in fecundity of the surviving offspring caused an overall increase in aphid populations two generations after parasitism. We must therefore understand multi-generational effects of parasitism before concluding how these fecundity effects are likely to drive *E. spurius* population changes.

If parasitism by *C. gossypariae* is having an effect on *E. spurius* populations, I could not detect it based on crawler density changes between 2015 and 2016. This is not unexpected, as host-parasitoid population dynamics are more complicated than I was likely to explain in a small exploratory study. Studies that seek to build phenomenological or mechanistic models of host-parasitoid population dynamics generally require a study duration of more than two generations of host population, and a method of accounting for other factors that influence population cycles (Bellows and Hassel 1999). This brief exploration of how a single year's population change in *E. spurius* relates to parasitism rate by *C. gossypariae* should be treated as a starting point for more detailed work on the system. It demonstrates that *C. gossypariae's* influence on *E. spurius* populations requires more detailed studies such as the construction and analysis of life tables.

The comparisons of trap arrangements revealed no significant differences between the four colour-coded trap configurations. However, the near-significance of the differences in means between the "white" and "green" trap locations used during round 6 suggests that previous trap placement at a location may influence future parasitoid emergence rates. I would therefore caution against using a simple trapping scheme such as alternating between two trap locations, as such as only the "blue" and "green" traps. If these two trap types had been the only ones used in this study, the peak levels of *C. gossypariae* emergence would have been missed, as only a small proportion of emerged *C. gossypariae* were recovered from round 6 by the "green" trap. However, the lack of statistical significance, especially considering that multiple comparisons of means were made without p-value correction, implies that staggered emergence trapping design may not have been necessary. As any arrangement seemed to work equally well, this method may have needlessly complicated analyses.

This sampling design may be useful for detection of rare species, or species with highly synchronous emergences. Although this sampling design is somewhat cumbersome, it has a potential usefulness in deductively identifying peak times in oviposition rates in the field based on peak emergence times corresponding to periods of availability to oviposition. There are some precedents for exclusion-based field experiments in biological control, as reviewed by Luck et al.

(1999), although they are generally used to observe the effects of total parasitoid exclusion, rather than exclusion only during a portion of the season. This may be a useful strategy for field-based life history studies of otherwise difficult to study aspects of parasitoid life history.

Tables and Figures

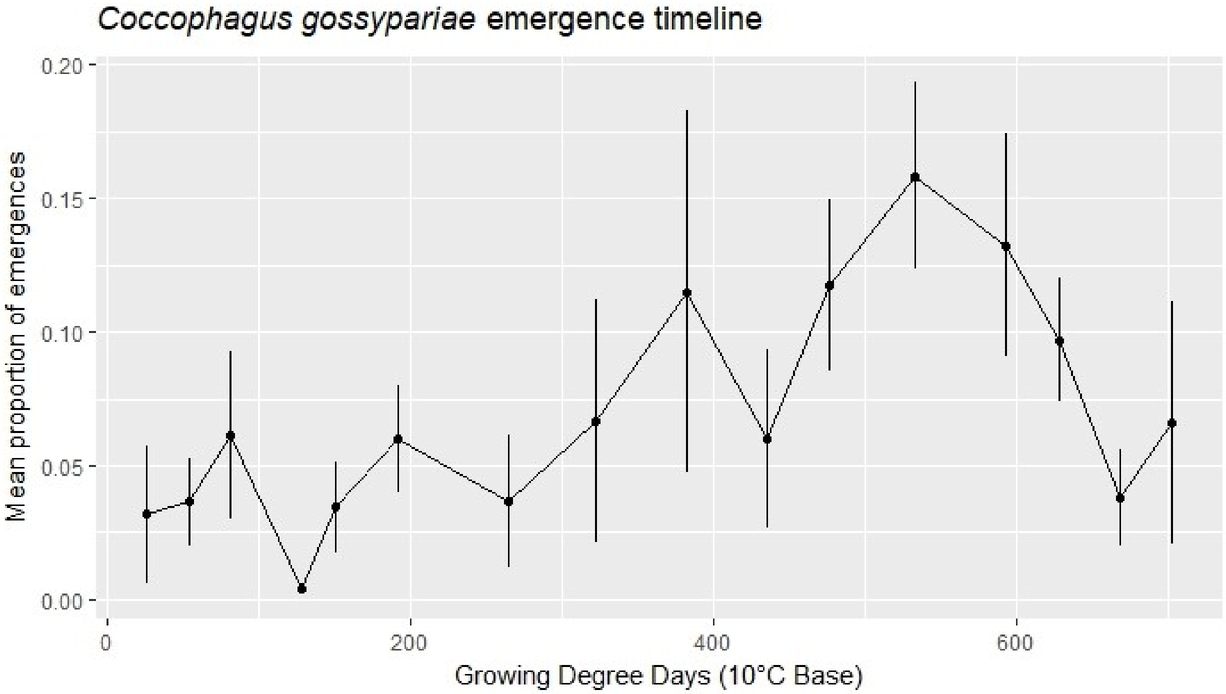


Figure 4.1: Proportion of *Coccophagus gossypariae* emergence per sampling round, averaged across all sites. *Coccophagus gossypariae* were trapped in mesh nets as they emerged from their hosts, *Eriococcus spurius.* Sampling took place in 8 two-week rounds from May 19 to September 10 2015, but the rounds are subdivided by week in this figure. Error bars represent 95% confidence intervals of means.

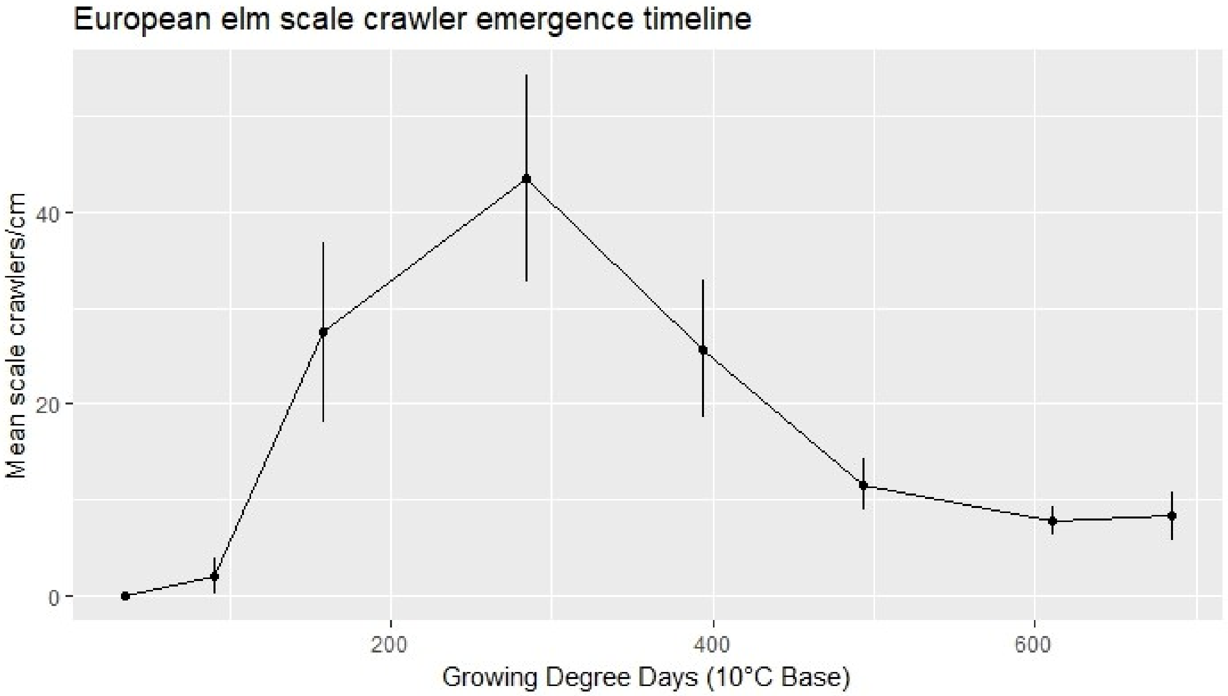


Figure 4.2: Density of first instar *Eriococcus spurius* larvae per cm^2, averaged across 46 American elm trees (*Ulmus americana* L.) in Calgary, Alberta. Larval density was sampled using sticky tape traps left in place for 8 two-week rounds on each tree, representing a continuous sampling period from May 19 to September 10, 2015. Error bars represent 95% confidence intervals of means.

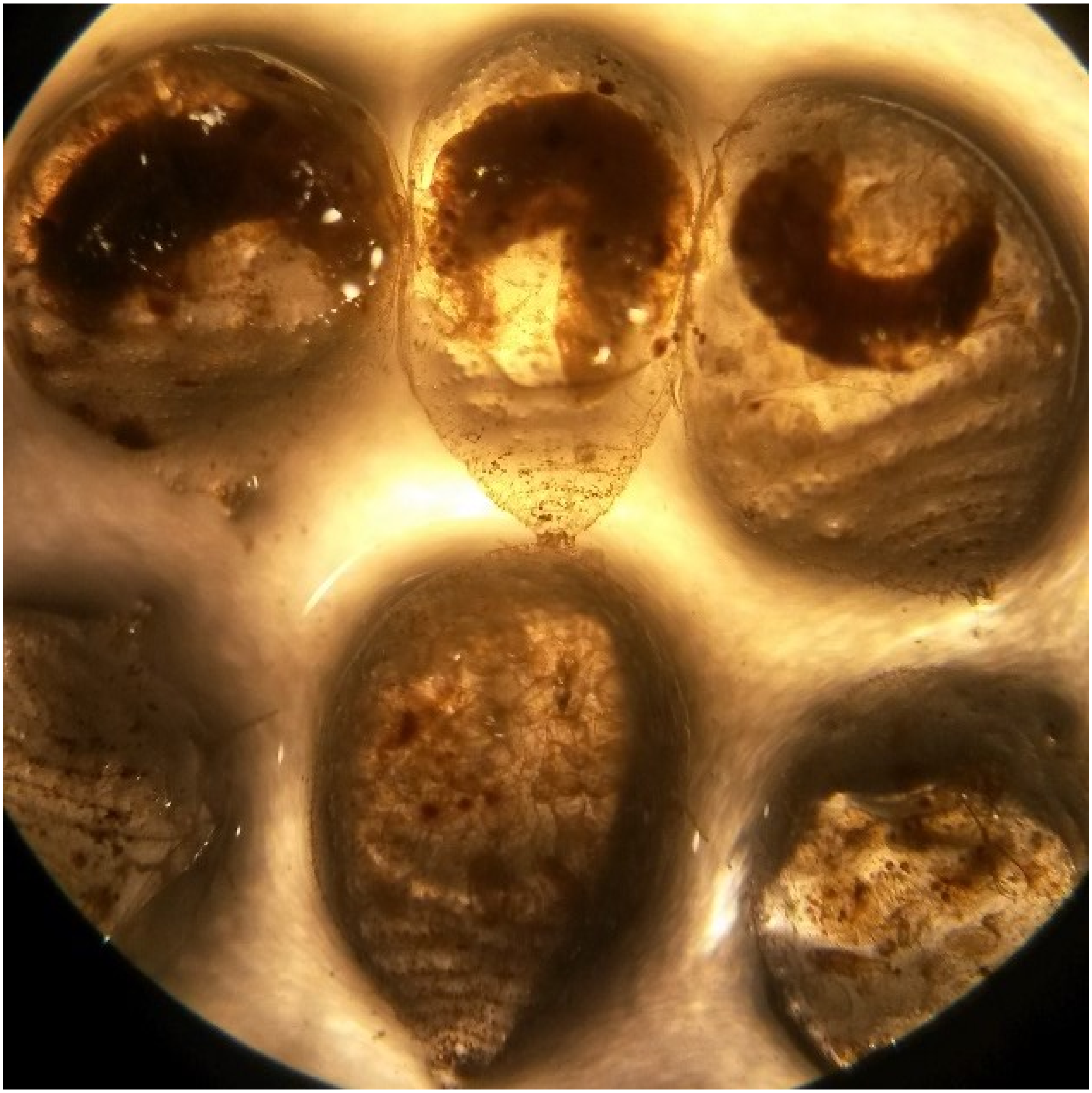


Figure 4.3: Adult female *Eriococcus spurius* after clearing in 10% potassium hydroxide. *Coccophagus gossypariae* larvae are visible inside the upper three scale insects, while developing *E. spurius* eggs are visible inside the bottom three. Each scale insect is approximately 4 mm in length.

Figure 4.4: An adult female *Eriococcus spurius*, cleared in 10% potassium hydroxide. Pale, oval *E. spurius* eggs are visible developing within the female, as is a single, darker parasitoid egg, likely of *Coccophagus gossypariae.*

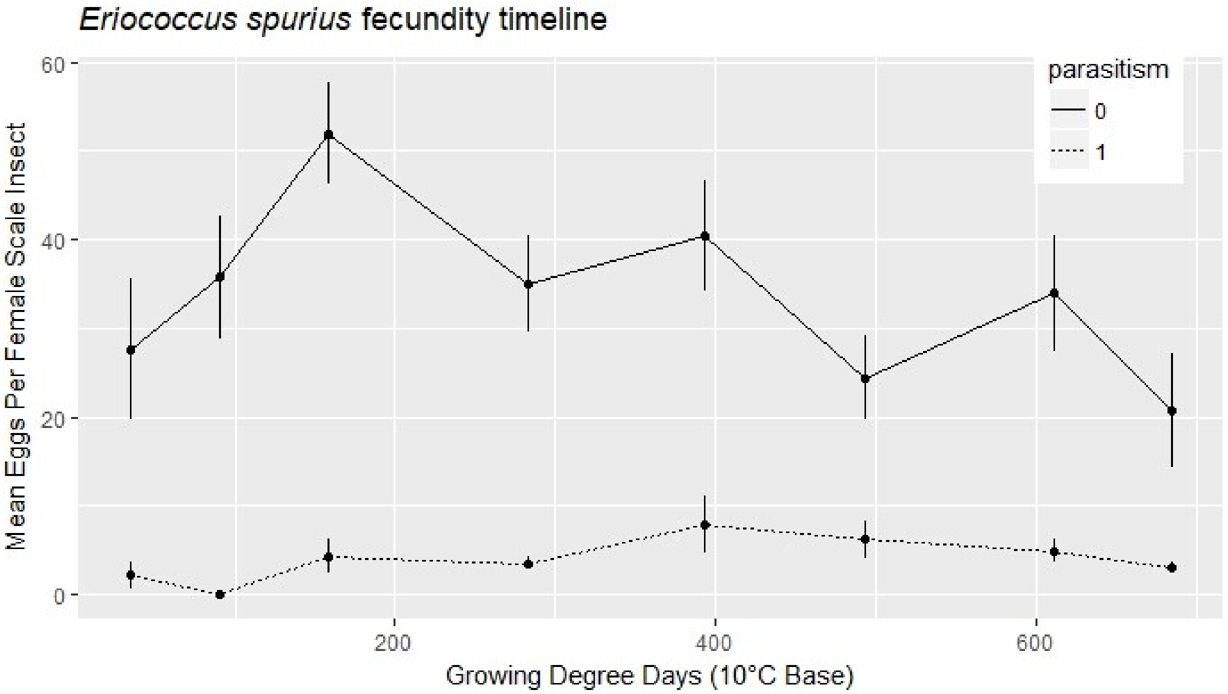


Figure 4.5: Number of eggs per live adult female was used as a means of comparing fecundity between parasitized and non-parasitized *Eriococcus spurius*. Data points represent average egg count across all sites for each of 8 rounds, sampled every two weeks from May 19 to September 10, 2015. Error bars represent 95% confidence intervals of means.

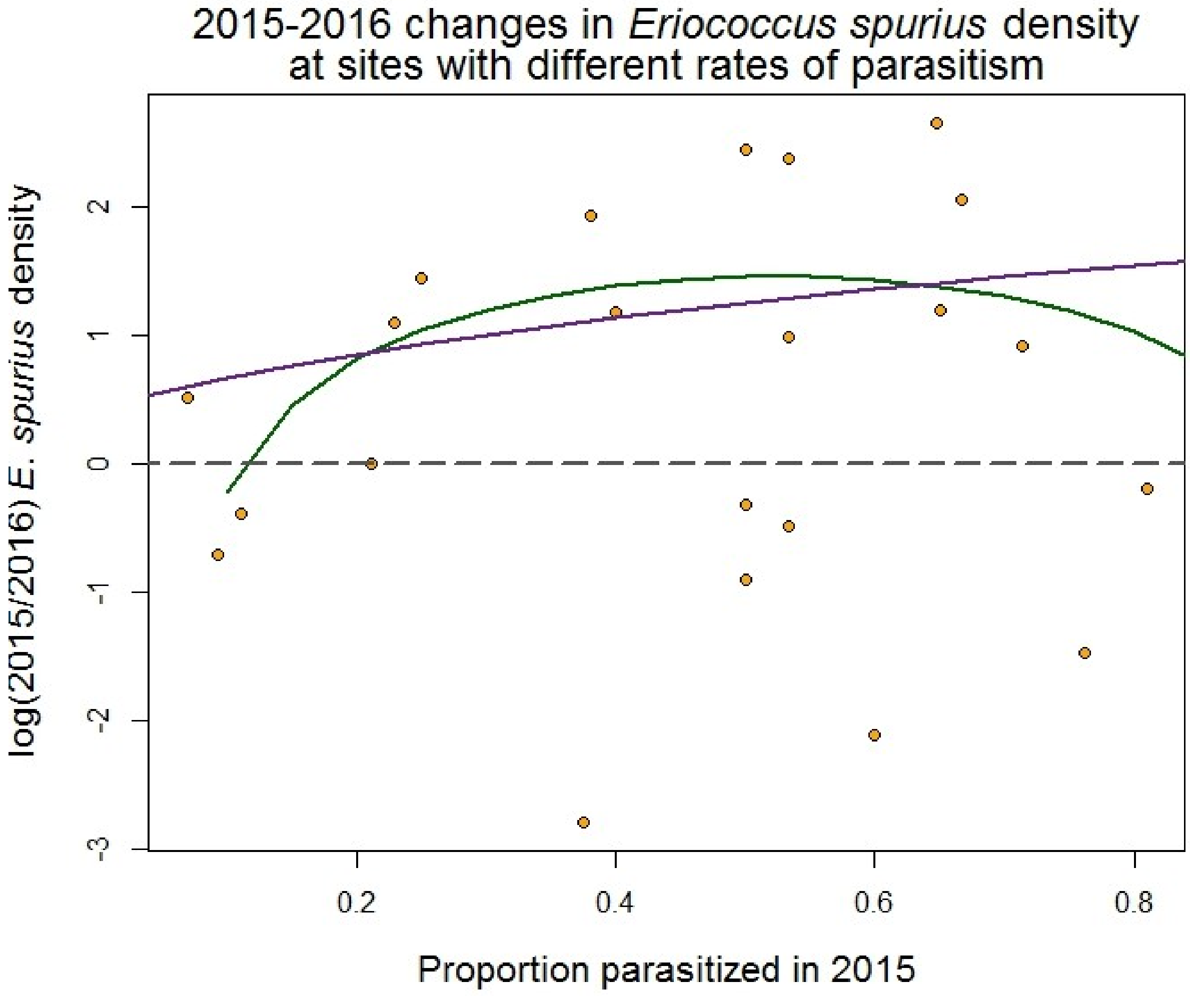


Figure 4.6: Estimated site-by-site changes in *Eriococcus spurius* population density, based on peak first instar nymph densities at 21 sites sampled in both 2015 and 2016. 2016 density estimates were adjusted to account for an overall cross-site decrease in scale densities between the two years, and better characterize the differences density changes between sites. Changes between years are modeled by proportion of *E. spurius* parasitized by *Coccophagus gossypariae* at each of 22 sites. Linear and Quadratic models are depicted, but are only for reference as they poorly fit the data (Linear model: multiple R-squared = 0.042, p= 0.348; Quadratic model: Multiple R-squared = 0.091).

Table 4.1: Summary of statistical comparisons of means between the traps in place at each round, throughout the 2015 season. Trap means represent the mean number of *Coccophagus gossypariae* recovered across all sampling sites for a given trap configuration and round. Each trap colour represents a colour-coded location on the study tree, which was consistent throughout the season, despite a trap not always being in place. This trapping configuration was replicated in 46 sites across Calgary. Each trapping round represents a two-week period in which traps were in place and collecting *C. gossypariae* as they emerged from their hosts, *Eriococcus spurius.*

	Trap means						
Round	**Blue**	**Green**	**Black**	**White**	**Test for difference of means**	**Test summary**	p value
1	0.5				NA	NA	NA
2		0.37			NA	NA	NA
3	0.717		1.826		Wilcoxon rank sum	W = 1135.5	0.459
4		0.674	0.652		Wilcoxon rank sum	W= 1009.5	0.616
5	2.565			0.717	Wilcoxon rank sum	W = 1126	0.456
6		1.587		4.85	Wilcoxon rank sum	W = 838.5	0.0582
7	1.967		1	3.217	Kruskal-Wallis rank sum	KW $X^2 = 2.72$, df= 2	0.246
8		0.63	0.565	0.609	Kruskal-Wallis rans sum	KW $X^2 =1.568$, df=2	0.457

Table 4.2: Descriptive statistics for fecundity parasitized and non-parasitized live female *Eriococcus spurius.* Means represent numbers of developing eggs found per female scale in each of 22 sites in Calgary Alberta, averaged across the 8 sampling rounds collected in 2015.

	Mean	**Median**	**Min**	**Max**	**SD**	**SE**	**N**
Parasitized	5.17	2	0	71	9.90	0.975	103
Not parasitized	33.24	21	0	240	41.13	3.5	135

Chapter 5: Discussion and future directions

This thesis was designed around the question "Is *Coccophagus gossypariae* capable of significantly suppressing *Eriococcus spurius* populations". It provides insight into the life history and interactions of these two species that have not previously been studied in the field. This information will be a useful basis for future investigations into biological control of *Eriococcus spurius*.

Ecology and host-parasitoid interactions of *Coccophagus gossypariae* and *Eriococcus spurius*

Coccophagus gossypariae Gahan is established throughout Calgary as the primary parasitoid of *Eriococcus spurius* Modeer. The widespread presence of *C. gossypariae* while *E. spurius* remains at economically damaging levels suggests that its naturally occurring rate of parasitism may not suppress the pest populations sufficiently to meet management goals. If *C. gossypariae* is to be used in the biological control of *E. spurius,* then it must be through either augmentative releases, conservation biological control, or a combination of both approaches. Either method would require a thorough understanding of *C. gossypariae's* life history and interaction with its host. The basic *C. gossypariae* life history studies in this project provide some first steps, such as establishing naturally occurring sex-ratios, emergence timing, likelihood of superparasitism, and that males and females can reach maturity on the same host. Future studies can improve understanding of how to efficiently mass-rear *C. gossypariae* in captivity or support its proliferation in the field. For example, efficient mass-rearing requires knowledge of optimal sex-ratios for efficient reproduction, as well as nutrition requirements for the health of phytophagous hosts and adult parasitoids (Thompson 1999; Thompson and Hagen 1999). This project did not focus on environmental factors associated with higher *C. gossypariae* populations beyond the lingering effects of imidacloprid treatments. Research to support conservation biological control in this system would need to investigate what makes an environment favorable to *C. gossypariae,* especially in relation to the use of azadirachtin-based insecticides, which are currently in development to replace imidacloprid in *E. spurius* control.

This project has demonstrated that *C gossypariae* parasitism is likely to have the greatest fitness impacts on individual *E. spurius* though fecundity effects, rather than mortality, but did not find evidence that individual effects of parasitism translate to the population level. The lack of discernable association between parasitism rate and *E. spurius* population suppression the following year may be due to parasitism effects being overshadowed by 'bottom-up' population constraints such as host-plant defense or herbivore competition, or by environmental constraints such as weather and insecticide treatment history. However, phytophagous terrestrial insect populations are nearly always mediated more heavily by 'top-down' effects such as predation and parasitism (Gurevitch et al. 1992; Vidal & Murphy 2018). While it is also possible *E. spurius* populations are mediated by predators rather than the parasitoids, a review of top-down mortality effects across phytophagous insects by Hawkins et al. (1997) found that parasitoids generally have far greater effects on their hosts than any predators in the system. It is most likely that *C. gossypariae* does have some effect on *E. spurius*, but these host dynamics are too complicated and varied to be captured in my study due to few sample sites, and a study length that incorporated only two generations of *E. spurius.*

Future investigations into this system must look for an effect of parasitism over a longer time span or an artificially high rate of parasitism. Quantifying these effects will be vital for determining whether augmentative or conservation biological control are likely to be effective, and the thresholds of parasitoid population density required for desirable *E. spurius* population suppression. Reference to the emergence timelines of *C. gossypariae* and *E. spurius* established in Chapter 4 of this project can increase efficiency of future studies. The timing of surveys can be targeted to portions of the season based on research questions, rather than sampling densities of both species throughout the season. For example, this study shows that in order to capture population densities at peak emergence rates, the best time to survey for *C. gossypariae* and collect them for captive breeding is between 400 and 700 Growing Degree Days, and the best time to survey for *E. spurius* populations is between 150 and 400 Growing Degree Days.

Urban landscape effects on *Eriococcus spurius* populations

The project's investigation of how spatial patterns of the urban landscape influence *E. spurius* populations deviated from the central research question, but provides important context required to understand the system. The spatial patterns in Chapter 3 explain some of the variation in *E. spurius* populations that is not explained by parasitism rates. Understanding these models can help future studies isolate host-parasitoid dynamics from density independent effects such as landscape influences. Chapter 3 also provides some of the most immediately applicable information for management of *E. spurius*. Although these models would benefit from improvements, they can be applied without much further research to help identify high-risk trees for *E. spurius* infestation, and identify probably areas as sources for reinfestation post treatment. For example, treating areas in blocks according to wind-induced reinfestation risk could help reduce the required frequency of insecticide treatments. However, an improved model would weight the degree of influence of urban elm patches on each other based on wind data, rather than categorically assigning them as 'in' or 'out' of an area of influence.

Conclusion

There is no simple fix to the issue of *E. spurius* invasion. Despite the urban setting of this system, it is a complex ecological community influenced by temporal, spatial, and interspecific dynamics. Attempts to alter aspects of this system to meet human objectives require a thorough understanding of the drivers of community interactions. Biological control of *E. spurius* still requires a great deal more research. While this study has not produced any results that exclude the possibility of integrating *C. gossypariae* into biological control, two essential questions remain: What levels of parasitism are required to significantly reduce *E. spurius* populations, and are achieving these levels in the field possible and economically feasible? Such research requires further investment of time and resources with no immediate solutions, while chemical management is a simpler and more immediately cost-effective option for managers. It is important, however, to consider long term costs of environmental impacts and insecticide resistance when weighing management options. Despite its challenges, incorporating multiple approaches to achieve long-term, sustainable management goals is at the heart of Integrated Pest Management. Management of *E. spurius* will likely change as the availability of neonicotinoids is restricted, but this project provides a scientific basis for the investigation of new options.

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