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Development of Cerceis fumipennis for Biosurveillance of the Emerald Ash Borer in the Northeast

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DEVELOPMENT OF *CERCEIS FUMIPENNIS* FOR BIOSURVEILLANCE OF THE EMERALD ASH BORER IN THE NORTHEAST

by

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A THESIS

Submitted in Partial Fulfillment of the
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Master of Science
(in Entomology)

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Advisory Committee:

Dr. Eleanor Groden, Professor of Entomology, Advisor

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DEVELOPMENT OF *CERCEIS FUMIPENNIS* FOR BIOSURVEILLANCE OF THE EMERALD ASH BORER IN THE NORTHEAST

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Thesis Advisor: Dr. Eleanor Groden

An Abstract of the Thesis Presented
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One method being utilized for detection of the invasive emerald ash borer, *Agrilus planipennis* (EAB) involves monitoring aggregations of the wasp *Cerceris fumipennis* for the presence of EAB in their collected prey. Currently there is little information published on its nest structure, phenology, and prey fidelity. The objectives of this research were to: i) monitor *C. fumipennis* male and female emergence, female prey collection, and number of colonies relative to the accumulation of degree-days (DD) throughout the summer; ii) better understand nest structure, phenology, and prey requirements of *C. fumipennis*; and iii) investigate *C. fumipennis* prey selection and fidelity.

Field studies were conducted on *C. fumipennis* aggregations located at six central Maine sites in 2012, 2013, and 2014. Soil temperatures were monitored continuously and daily degree-day accumulations (base 10°C) were calculated. Sites were monitored for wasp emergence, number of nests, number of wasps, and initiation of mating and prey capture. We excavated nests and measured depth of cells and the number of new cells per nest. The contents of cells were examined for *C. fumipennis* immature stages and prey.

We estimated the mass of beetle prey provisioned and measured the weight of pre-pupae in cells. We identified prey beetles excavated from nests and made comparisons among prey species provisioned within cells.

Mean accumulated DD at first wasp emergence across all sites and years was 594 ± 21 (SE). Male wasps emerged before female wasps 40% of the time. Prey capture was initiated 7-17 days (115 – 287 DD) post emergence, and the peak number of nests occurred in mid-July/early August. There was variation in the number of peak nests at sites between years. Our emergence data demonstrates that the period of flight activity for *C. fumipennis* and EAB in Maine would overlap.

We excavated 171 nests. The number of new cells per nest and depth of cells varied between sites. The proportion of cells containing eggs generally decreased over a field season, while the proportion of cells containing pre-pupae increased over the field season. There were no significant differences between the numbers of beetles provisioned per egg between sites, but there were differences between the estimated weights of beetles provisioned per egg between sites. Finally, we found that as the estimated provisioned weight increased, the weight of the resulting pre-pupae also increased. Twenty different prey species were identified and we found that 50% to 84% of excavated cells were provisioned with a single genera of beetle prey.

The data presented highlights *C. fumipennis's* ability to find and to utilize several different hosts as prey items which makes it a good candidate for monitoring EAB and other invasive beetles in the family Buprestidae. We also present data that will aid in the development of rearing techniques for the wasp laying the groundwork for future development of mobile colonies.

DEDICATION

I dedicate this thesis to Dr. Edith Marion Patch; scientist, environmentalist, writer, feminist and lover of all things entomological.

"If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos."

E. O. Wilson

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I could not have done anything without the support of my family. Thanks mom (Linda Lund) and dad (Bob Lund) for birthing me and putting up with my years of insect enthusiasm. Thank you Sharon Lund and Michael Lund for being great siblings. Lastly, I would like to thank my amazing husband, Dr. Stephen Coghlan and my wonderful fur-children (Nerka, Hyla, Lenok, Beatis, and the cats) who made this possible with their understanding, patience, support, and love.

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CHAPTER 1

THE EMERALD ASH BORER INVASION AND A NOVEL APPROACH FOR SURVEILLANCE

The Problem

The emerald ash borer (*Agrilus planipennis*, EAB) is an invasive beetle in the family Buprestidae. The beetle was first identified as a pest of ash trees in 2002 in Michigan (Haack et al. 2002). To date, EAB has been detected in 25 U.S. states and two provinces in Canada, where it is responsible for the death or decline of tens of millions of ash trees (EAB Info 2015). Originally from Asia, EAB is thought to have arrived on wood packing materials used for shipping containers, and is considered a minor pest of members of the genus *Fraxinus* in its native range (Yu 1992). In North America, EAB readily attacks and kills all species of *Fraxinus* found in the United States. (Haack et al. 2002).

In Northern climates, adult beetles emerge from sapwood in May and feed on ash foliage (Bauer et al. 2004). Following feeding, adults mate and oviposit eggs under the bark throughout June and July (Bauer et al. 2004). Larvae hatch and feed in the phloem from late summer and into autumn. It then overwinters as a pre-pupa (Bauer et al. 2004). Larval feeding in the phloem deprives the tree of vital nutrients and can lead to crown dieback, bark splitting and death of the tree. Since EAB are small and spend most of their time feeding under the bark of trees, they are difficult to detect. It has been estimated that nearly 100 percent of the ash resource in eastern North America could be killed by EAB by 2050 (DeSantis et al. 2013).

Over the past 60 years several diseases and insect pests have caused the loss of major tree species in our urban environments. These include elms from Dutch elm disease, beeches from beech bark disease, oaks from gypsy moth, and chestnuts from chestnut blight. Ash are typically fast-growing trees that are tolerant of poor soil conditions. Hence, they have been heavily used in reforestation projects and urban settings following the loss of the aforementioned species (Dozier 2000, Liebhold et al. 1995).

De Santis et al. (2013) estimates that the three major species of North American ash total nearly 9 billion trees and saplings, with an estimated volume of over 724 million m^3 in forests throughout the United States. Within their ranges in the United States, black, green and white ash account for nearly 14% of all woody species stems. Kovacs et al. (2010) estimates that 37.9 million ash trees grow on developed land in the Midwest and Northeastern United States.

The EAB infestation of ash is resulting in negative economic consequences for the lumber and nursery industries, as well as urban communities. The 10-year cost for removal and replacement of dead trees in urban communities has been estimated at approximately $10.7 billion (Kovacks et al. 2010, Aukmana 2011). It has been estimated that EAB's total impact to the national urban landscape is in the range of $20-60 billion (Buck 2015). Not included in either of these estimates are the higher residential cooling costs, diminished aesthetics, and negative effects on property values from tree loss.

In urban settings, trees mitigate some of the environmental impacts of urban development (Nowack and Dwyer 2007, McPherson et al. 2007). Urban trees can moderate the local climate and reduce building energy use. It has been estimated that the establishment of 100 million trees around residences in the United States saves about $2 billion annually in reduced energy costs (Akbari et al. 1992, Donovan and Butry 2009). Urban trees can reduce air pollution and in the US are responsible for removing approximately 784,000 tons of air pollution annually, valued around $3.8 billion (Nowak et al. 2006). All trees store carbon in their tissues, one of the major drivers of climate change. Urban trees in the United States currently store 770 million tons of carbon, valued at $14.3 billion (Nowak and Crane 2002). Trees and soils can improve water quality. In urban areas this can reduce the need for costly storm water treatment facilities by intercepting and retaining/slowing the flow of precipitation reaching the ground. Finally, proper planting and maintenance of urban trees can reduce noise pollution (Anderson et al. 1984).

With about 80% of the US population living in cities, the cultural and societal losses of ash are equally concerning. The presence of urban trees and forests can make the urban environment a more aesthetically pleasing and emotionally satisfying place in which to exist (Ulrich 1984, Dwyer et al. 1991, Taylor et al. 2001a, Taylor et al. 2001b). The negative repercussions from the loss of shade trees in cities and suburban communities has been well documented (Dilley and Wolf 2013). Recent research indicates associations of trees with reduced cardiovascular disease, less risk of negative infant birth out-comes, less ultraviolet radiation exposure, faster hospital recovery times, and reduced crime rates (Ulrich 1984, Heisler et al. 1995, Donovan et al. 2011, Donovan and Prestemon 2012, Donovan et al. 2013).

The assessments by Kovacks (2010) and Buck (2015) also do not take into account the ecological impacts of widespread EAB infestations. These impacts include altered forest composition and structure as well as negative effects on associated wildlife and ecosystem functions (Gandhi and Herms 2010a, 2010b).

The Response

In response to the current and predicted damage that EAB is expected to cause, foresters and entomologists throughout the eastern United States and Canada are working to develop effective means to monitor, slow the spread, and manage this invasive pest. Initially, detection of EAB relied heavily on visual surveys and trap trees. Visual surveys rely on the presence of symptoms of EAB attack (woodpecker damage, bark splitting, crown dieback, etc.) as indicators of infestation. Unfortunately, trees with small or early infestations often show no symptoms and are easily missed. Besides potentially missing infestations, ground surveys are also labor and time intensive, as they require bucket trucks and large crews to carry out.

Trap trees have been used as another detection method for EAB with mixed success. As damaged trees are attractive to wood boring insects, trap trees are established by removing a band of bark from the ash tree and letting it stand for the full flight season. In the winter the tree is felled, the bark is removed, and the exposed phloem is examined for the presence or absence of EAB. Marshall et al. (2009) found that the diameter of the damaged tree influences its attractiveness and larger diameter trap tree are more likely to attract and detect beetles in areas with low EAB densities. This technique has been adopted in many municipalities. However, banding, felling, and stripping trees are monetary and labor intensive, and it also sacrifices the tree that is banded and often several trees surrounding it.

A significant amount of money and other resources have been applied to the development of traps to aid in detection of infestations. Research on optimization of trap design, including the development of the purple panel trap, has increased the detection capability for EAB (Lelito et al. 2008, Francese et al. 2009, Marshall et al. 2009); however, this trap has some limitations at low EAB population densities (Francese et al. 2009). The major drawbacks of using traps are that they do not appear to detect beetles during early stages of infestations (often populations are not located until 2 years after the initial infestation), traps cost anywhere from a $10 to $40 each, and to be most effective they need to be hung mid canopy, requiring bucket truck and/or other equipment that adds to their overall cost (Francese et al. 2009, Contech Enterprises 2013).

The key component missing in most of the techniques currently used to locate EAB infestations is the ability to detect small, new populations. Detecting infestations early is especially important because small infestations can be quarantined and possibly eradicated, but large outbreaks are nearly impossible to control.

An Alternate Solution

Cerceris is a genus of wasps in the family Crabrionidae. It is the largest genus in the family, with over 850 described species. Members of this genus can be found on every continent. Most Nearctic species of *Cerceris* nest in bare soil in aggregations. The females often stay with a single nest during her lifetime and are considered solitary. Several tropical species are communal nesters where multiple females live together in one nest and share in nesting activities. In Nearctic species, nest and cell construction are variable.

Cerceris fumipennis is a solitary species that provisions its nest with beetles from the family Buprestidae. It occurs along the East coast of the U.S. from New England to Florida (Evans 1971, Kurczewski and Miller 1984, Careless 2009). *Cerceris fumipennis* utilize open, well-drained, hard-packed sandy soils to make their nests and are commonly found in gravel pits, baseball fields, picnic areas, camp sites and parking lots (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Since they require wood boring buprestid beetles as a food source, their nests are also found in close proximity to woody habitats (Evans 1971, Kurczewski and Miller 1984, Careless 2009).

Cerceris fumipennis is a medium sized wasp with blue/black, iridescent wings, and a single yellow band around their otherwise black abdomen. The species exhibits sexual dimorphism with the females being larger than the males and having three yellow patches between their eyes. The smaller males have two yellow patches between their eyes. Adult wasps emerge in early July in Maine. Following emergence, a female *C. fumipennis* will dig a nest that consists of a single entrance hole into a solitary tunnel that descends below the soil surface (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Off of this central tunnel, the female will dig individual cells that she provisions with paralyzed buprestid beetles for her young that she captures from the surrounding landscape (Evans 1971, Kurczewski and Miller 1984, Careless 2009). *Cerceris fumipennis* is a mass provisioner so, following the addition of the last beetle to a cell, the female will lay a single egg and then back-fill the completed cells (Evans 1971, Kurczewski and Miller 1984, Careless 2009). After consuming the prey and completing development, the larva forms a pre-pupa within its cells before overwintering

(Kurczewski and Miller 1984). The wasps pupate in the early spring and emerge as adults later in the summer.

The wasp is being used as a biosurveillance tool to detect of small infestations of EAB. Biosurveillance is where one organism is used to locate populations of another organism. In EAB infested areas in Ontario, EAB has been found to make up to 86% of a wasp colony's daily capture (Careless 2009). During the summer of 2012, infestations of EAB were detected by *C. fumipennis* in Connecticut, Illinois, and New York, while forest managers and nearby traps failed to detect beetles (Rutledge et al. 2013). With the recent success of *C. fumipennis* in detecting new infestation sites and the reduction of funds available for EAB monitoring, many New England states are increasing their reliance on *C. fumipennis* for detecting EAB. Participants in the biosurveillance programs have included professionals with states' Forest Services, USDA APHIS, and a large network of community volunteers from Maine to Virginia. Recent studies have determined the most important environmental factors influencing wasp flight and foraging activities, and identified the most productive times to monitor *C. fumipennis* colonies for EAB (Virgilio 2012).

Biosurveillance with *C. fumipennis* may be the most cost-effective means available for early detection (no specialized equipment is necessary and the wasps do the majority of the work). Currently the major limitation to using existing nesting colonies for detecting EAB, is that naturally occurring wasp populations require specific soil types for nesting which can be limited and localized in the landscape, and may not be present in areas most at risk for EAB. The ability to rear wasps in a laboratory and the development of mobile colonies would allow local, state, and national agencies to use *C. fumipennis* biosurveillance virtually anywhere. Currently, rearing techniques for the wasp are hampered by a poor understanding of conditions required for growth and development of the immature wasps in their ground nests. Studies of the immature wasp development in the nests will improve predictions of their emergence and timing for biosurveillance programs, and will also aid in the development of rearing techniques for the wasp laying the groundwork for future development of mobile colonies.

CHAPTER 2

ABOVEGROUND PHENOLOGY OF *CERCERIS FUMIPENNIS* IN CENTRAL MAINE

Abstract

Accurate predictions of insect development, adult emergence, and initiation of prey foraging behaviors is essential for effective use of *Cerceris fumipennis* as a biosurveillance tool for the invasive emerald ash borer, *Agrilus planipennis*. The objectives of this study were to monitor *C. fumipennis* male and female adult emergence, female prey collection, and number of colonies relative to the accumulation of degree-days (DD) throughout the summer. Field studies were conducted on *C. fumipennis* aggregations located at six sites in central Maine in 2012, 2013, and 2014. Soil temperatures were monitored continuously and daily degree-day accumulations (base 10°C) were calculated. Sites were monitored for adult wasp emergence, number of nests, number of adult wasps, and initiation of mating and prey capture.

Mean accumulated soil DD at first adult wasp emergence across all sites and years was 594 ± 21 (SE). Male wasps emerged before female wasps 40% of the time. Prey capture was initiated 7-17 days (115 – 287 DD) post emergence, and the peak number of nests occurred in mid-July/early August. There was variation in the peak nests at sites between years. These data demonstrate that the period of flight activity for *C. fumipennis* and EAB will overlap in central Maine. This insect continues to be a good candidate for use as biosurveillance for EAB in Maine.

Introduction

With recent successful detections of the invasive emerald ash borer (*Agrilus planipennis)* in New York, Connecticut, and Illinois by the native predatory ground wasp of *Cerceris fumipennis,* more emphasis has been placed on utilizing the wasp as a biosurveillance tool (Rutledge et al. 2013). Along the east coast *C. fumipennis* occurs from New England to Florida utilizing open, well-drained, hard-packed sandy soils to make their nest, often in human disturbed areas such as gravel pits, baseball fields, picnic areas, camp sites and parking lots (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Because female wasps provision their young with wood boring beetles in the family Buprestidae, their nests are found in close proximity to woody habitats that support these prey (Evans 1971, Kurczewski and Miller 1984, Careless 2009).

Adult wasps emerge in early July in Maine (Virgilio 2012). Emergence dates have been shown to vary within colonies, between colonies and across *C. fumipennis'* North American distribution (Careless 2009, Hellman 2010, Virgilio 2012, Rutledge et al. 2015). Careless (2009) found that while the majority of adult individuals in colonies in Ontario, Canada emerged in mid-June, there was a portion of the population that emerged as late as early August. In *C. fumipennis*' southern-most colonies, the wasps emerge earlier and have a bivoltine life cycle, completing two generations in one year (Careless 2009).

Cerceris fumipennis exhibits sexual dimorphism with the females being larger and having three yellow patches between their eyes (Careless 2009). Males are smaller in size and have two yellow patches between their eyes. Male wasps emerge before females and spend their time patrolling the colony and mating with females (Evans

1971). No courtship behaviors have been observed. Previous researchers have suggested that males simply wrestle females to the ground to copulate (Evans 1971).

Following emergence, a female *C. fumipennis* digs a nest that consists of a single entrance hole into a solitary tunnel that descends below the soil surface (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Off of this central tunnel, she digs individual cells that she provisions with paralyzed buprestid beetles, captured from the surrounding landscape (Evans 1971, Kurczewski and Miller 1984, Careless 2009). After provisioning a cell, she lays a single egg on the ventral side of one of the prey items and then back-fills and seals the cell before moving onto the next (Evans 1971, Kurczewski and Miller 1984, Careless 2009). After consuming the prey and completing development, the larva forms a pre-pupa within its cell before overwintering (Kurczewski and Miller 1984). In early spring, the immature wasp metamorphoses into a pupa and then emerges 3-4 weeks later as an adult (Careless 2009).

Accurate predictions of insect development, emergence, and initiation of behaviors is essential for effective use of *C. fumipennis* as a biosurveillance tool. Since insects are exothermic, meaning their development is dependent on external temperatures, monitoring degree-day accumulation can be a valuable tool for predicting activity. A degree-day (DD) is a measure of the amount of heat accumulated above a specified base temperature, during a 24-hour period. One DD accumulates for each degree the average temperature remains above the base temperature over the 24 hours, and several DDs can be accumulated in one 24-hour period.

Rutledge et al. (2015) reported that the mean accumulated soil degree-days needed for emergence of *C. fumipennis* adults in monitored colonies in Ontario Canada, New York, Maine, and Connecticut was between 696.2 ± 16.8 (SE) at base 10°C and 1,504.51 ± 5.97 (SE) at base 0°C. The objective of this study was to monitor *C. fumipennis* male and female emergence, female initiation of prey collection, and the number of colonies relative throughout the season in central Maine and determine if this soil DD model will successfully predict these events between sites and years.

Methods

Field studies were conducted on *C. fumipennis* aggregations (hence forward referred to as colonies) located at six sites in central Maine in the towns of China, Dedham, Madison, Newport, Skowhegan and Veazie. The *C. fumipennis* colonies in China, Dedham, Newport, and Veazie were located in baseball fields maintained by local school districts. The colony in Madison was located in a grassy hillside area surrounding a town maintained baseball field. The colony in Skowhegan was located in sandy disturbed soils outside a local sportsman club.

Soil temperatures at each of the colony locations were monitored continuously through this study using Thermochron^{TM} iButton temperature probes (Embedded Data Systems, Lawrenceburg, KY). In the fall of 2011, four probes were placed in 2.5 cm by 2.5 cm zipper style plastic bags and buried at each site with two probes buried at 8 cm and two at 20 cm. The iButtons were programmed using the iButton Viewer 32$^{®}$ software (Maxin Integrated Products, Sunnyvale, CA) to monitor temperature every 60 minutes from early May to early November and every 180 minutes for the remainder of the year.

Temperature data was downloaded from the probes regularly. Soil degree-days (DD) were calculated using the average temperature readings at each site (averaged over all probes at all depths) for each day and the development threshold for *C. fumipennis* of 10°C (Rutledge 2015) (DD = AveTemp – 10°C). Degree-days were accumulated starting on January 1 for each of our study years. These calculations were compared to the initial timing of adult wasp emergence and the date of maximum wasp emergence.

Starting in late June, sites were visited on a 3-5 day rotation to monitor for initial wasp emergence. We confirmed emergence by locating active *C. fumipennis* nests or the visual confirmation of a wasp. Active nests were identified by the size of the entrance hole and the presence of a tumulus (a small mound of soil excavated by the wasp) outside the entrance hole. After emergence was observed, regular observations of wasp activity were made at each site. Monitoring occurred during daylight hours and multiple sites were monitored per day. Data were collected to determine the number of active nests, the number of male and female wasps over time, and the initiation of mating and prey capture by females. All active nests within a field site were counted on each sample date. If two baseball fields were present at a site, both were counted to estimate the total number of nests at the site. Wasps flying about the nests were observed for mating encounters between males and females while nests were being counted. On each sample date, two 10-minute sweep samples of wasps were conducted during peak flight periods (late morning to early afternoon). During that time interval an attempt was made to capture any wasp flying in the area. The number of males, females, and prey beetles carried by the females were recorded. Wasps were released immediately after the 10-minute sampling period. Sweep net sampling occurred from initial wasp emergence until females were collected with prey. Initial wasp emergence, number of nests, mating activity, and prey captures were plotted relative to soil accumulated degree-days.

Results

The mean degree-day accumulation at first emergence across all sites and years was 594 ± 21 (SE). The earliest date of first emergence across all sites and years was 2 July (2012) at the Skowhegan site and the latest was 21 July (2014) at the China site (Table 2.01). The range in date for first emergence over the three years within a site, differed from 7 days at the Veazie and Dedham sites to 12 days at the China site. Mean accumulated soil degree-days at first emergence between sites and years ranged from 525 at the Veazie site to 690 at the Madison site. The difference in the range of accumulated soil degree-days at first emergence within a site was 27 DD at the Veazie site to 142 DD at the Skowhegan site.

Sweep net samples were conducted at six sites in 2012, three sites in 2013 and five sites in 2014 in central Maine (Figure 2.01-2.03). Male wasps were collected prior to female wasps at the Dedham, Madison, and Skowhegan sites in 2012 and at the Dedham site in 2013. Mating was observed at the Dedham site on 7 July (583 DD) in 2012 and on 15 July (759 DD) at the Skowhegan site in 2013. In 2012, the first females collected with prey was on 9 July (735 DD) at the Skowhegan site, 19 July (786 DD) at the Dedham site, and 27 July (1008 DD) at the Madison site. In 2013, females with prey were collected on 22 July (820 DD) in Dedham, 7 July (640 DD) at the Skowhegan site and 18 July (711 DD) at the Veazie site. In 2014, female wasps with prey were first collected on 17 July at the Dedham and Madison sites (669 and 659 DD respectively) and 21 July at the Skowhegan and Veazie (698 and 714 DD respectively) site.

Colony growth dynamics throughout the season showed some variability between years and sites (Table 2.01 and Figure 2.04). Colonies peaked later on average in 2012 when compared to the following two years and the decline in colony activity following peak (colony decay) was more extended in 2013. Across all sites, active nests were observed from 7/2 to 8/27 (620 to 1470 DD) in 2012, from 7/5 to 8/25 (547 to 1314 DD) in 2013 and from 7/10 to 8/25 (557 to 1180 DD) in 2014 (Figure 2.01).

The peak number of active nests were variable at sites between years (Table 2.01). At the China site, *C. fumipennis* populations increased from 88 in 2012 to 185 active nests in 2013, followed by a decrease in number of wasps to 86 in 2014. The densities at the Dedham and Veazie sites showed some variability over the study period, ending with slightly more active nests in 2014 than previously. Active nests declined over the years at the Skowhegan site, and both the Newport and Madison sites, which had small colonies in the first year of the study and no active nests in 2013. Colony activity was seen at the Madison site in the third year, but had not resumed at the Newport site.

Table 2.01: First emergence and peak *C. fumipennis* nests during the 2012, 2013 and 2014 field season.

		2012			**2013**			**2014**		
Site	**Emergence**	**Date**	**Open Nests**	**DD**	**Date**	**Open Nests**	**DD**	**Date**	**Open Nests**	**DD**
China	1st	7/9/12	1	616	7/15/13	3	675	7/21/14	18	716
	Peak	7/27/12	88	914	7/30/13	185	889	8/4/14	86	901
Dedham	1st	7/3/12	2	524	7/5/13	3	547	7/10/14	5	506
	Peak	7/26/12	284	897	7/17/13	265	731	7/21/14	301	699
Madison	1st	7/9/12	3	721	X	X	X	7/17/14	5	659
	Peak	7/27/12	32	1008	X	X	X	8/5/14	54	917
Newport	1st	7/11/12	1	646	X	X	X	X	X	X
	Peak	8/2/12	17	977	X	X	X	X	X	X
Skowhegan	1st	7/2/12	1	620	7/7/13	6	640	7/10/14	3	498
	Peak	8/2/12	343	1136	7/19/13	189	831	7/21/14	156	671
Veazie	1st	7/11/12	6	539	7/7/13	1	523	7/14/14	2	512
	Peak	8/2/12	201	965	7/18/13	275	711	7/30/14	245	673

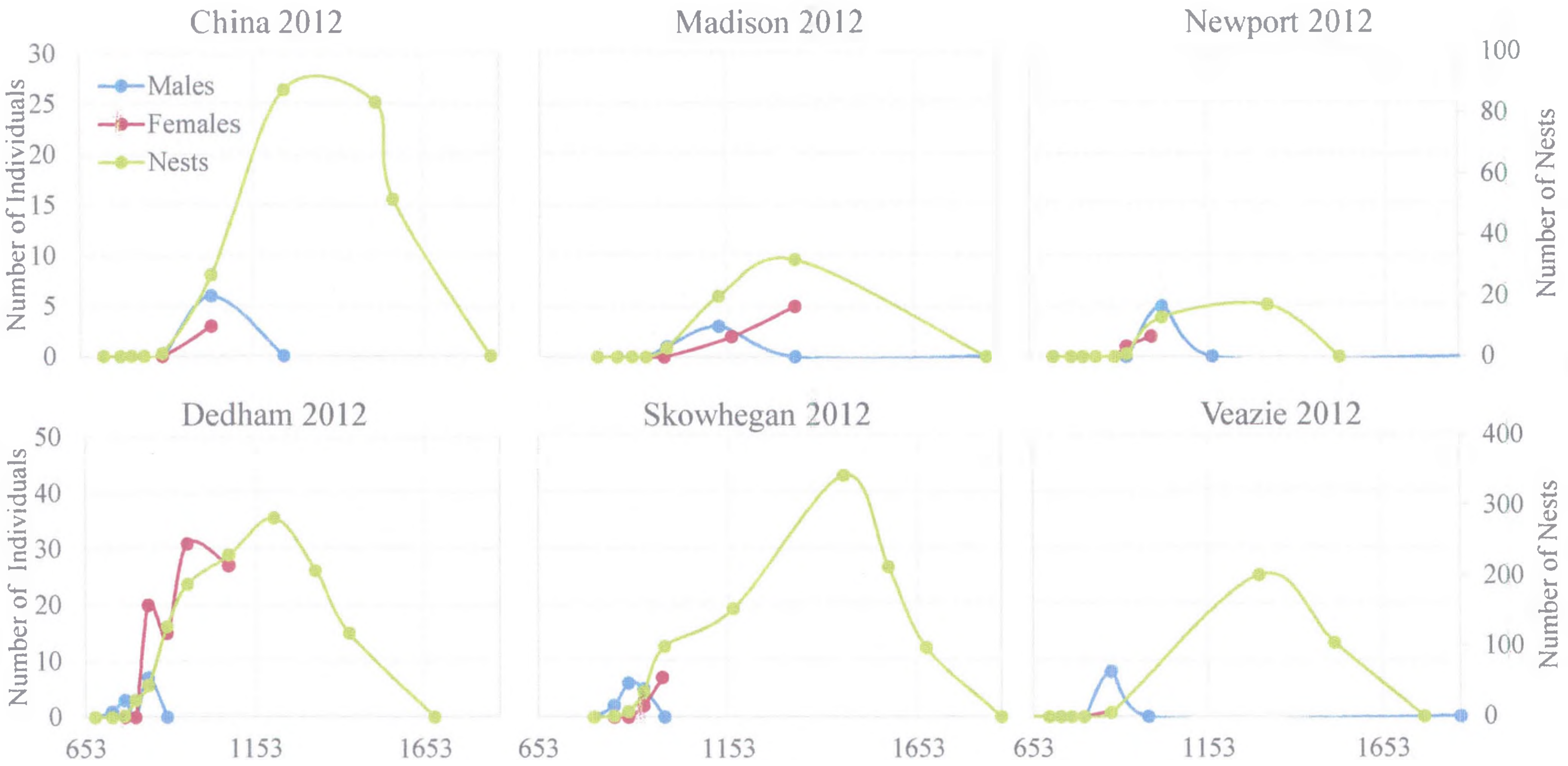

Figure 2.01: Sweep net sampling counts of *C. fumipennis* adult wasps and number of nests over the flight season at six sites in central Maine in 2012.

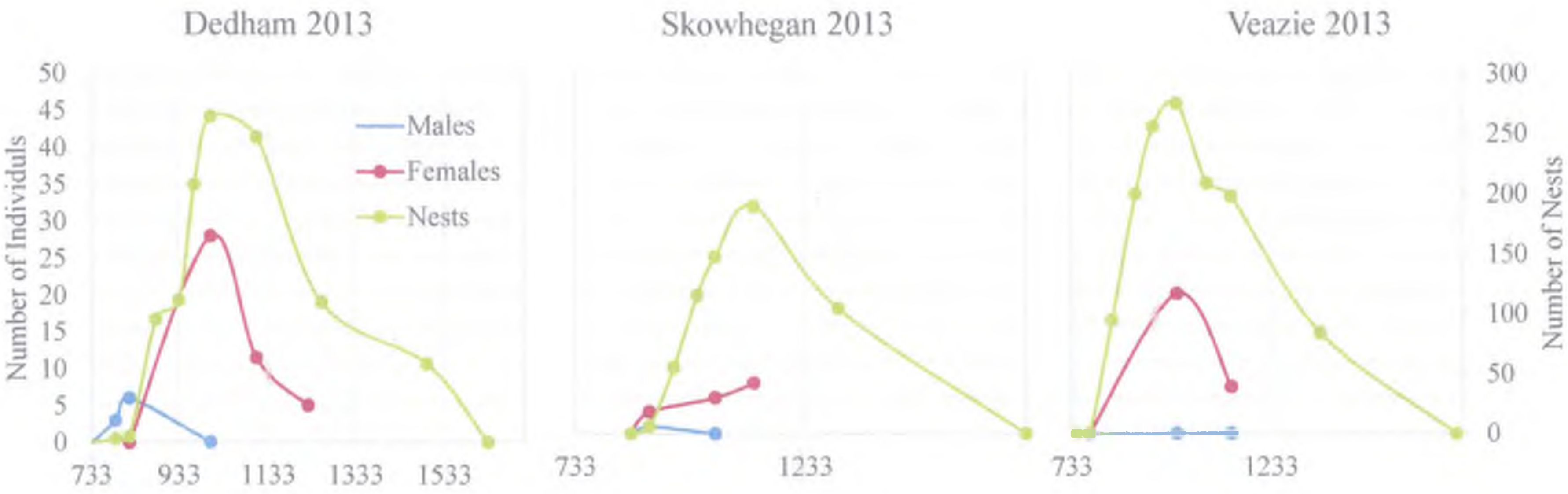

Figure 2.02: Sweep net sampling counts of *C. fumipennis* adult wasps and number of nests over the flight season at three sites in central Maine in 2013.

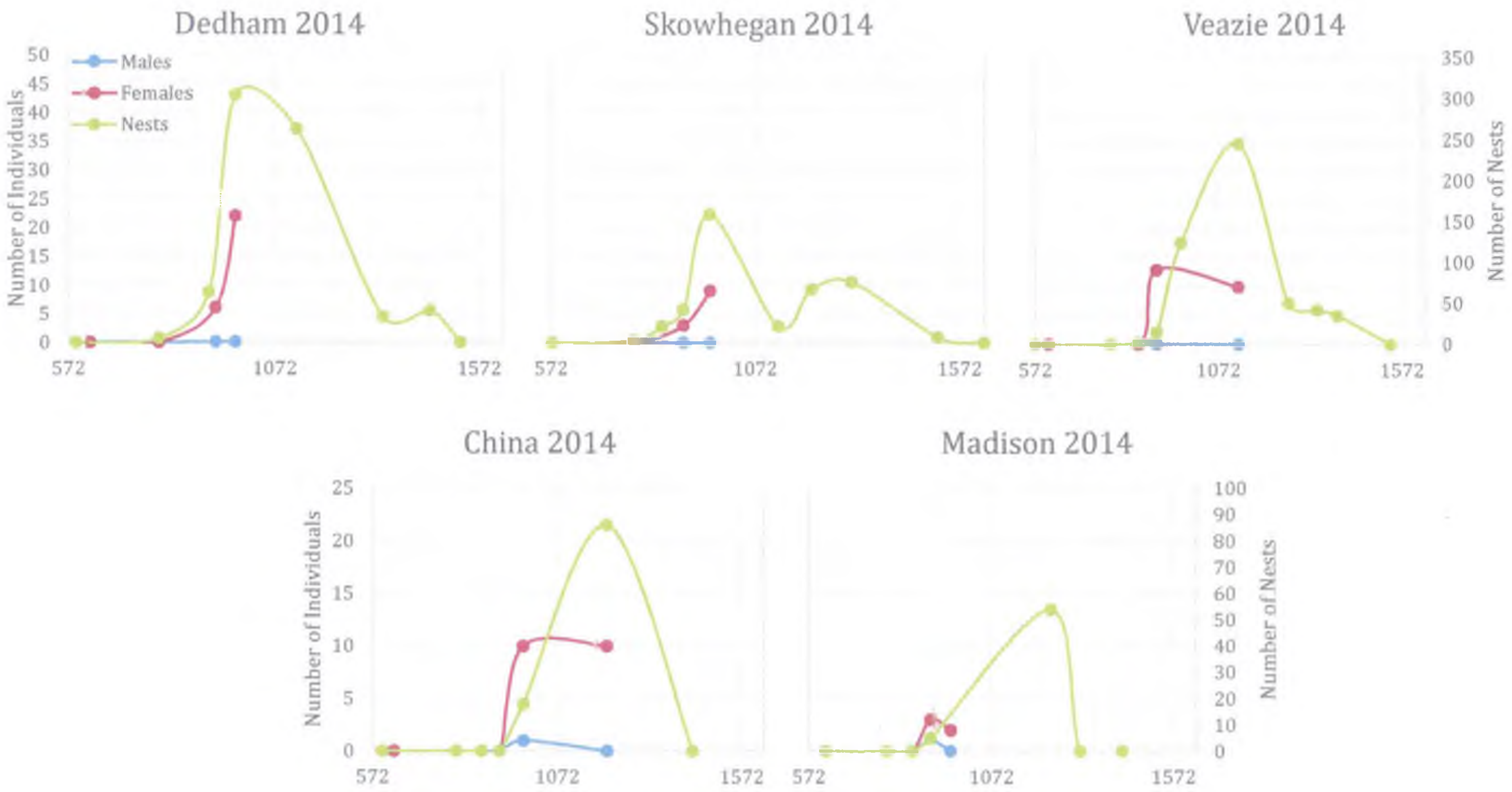

Figure 2.03: Sweep net sampling counts of *C. fumipennis* adult wasps and number of nests over the flight season at five sites in central Maine in 2014.

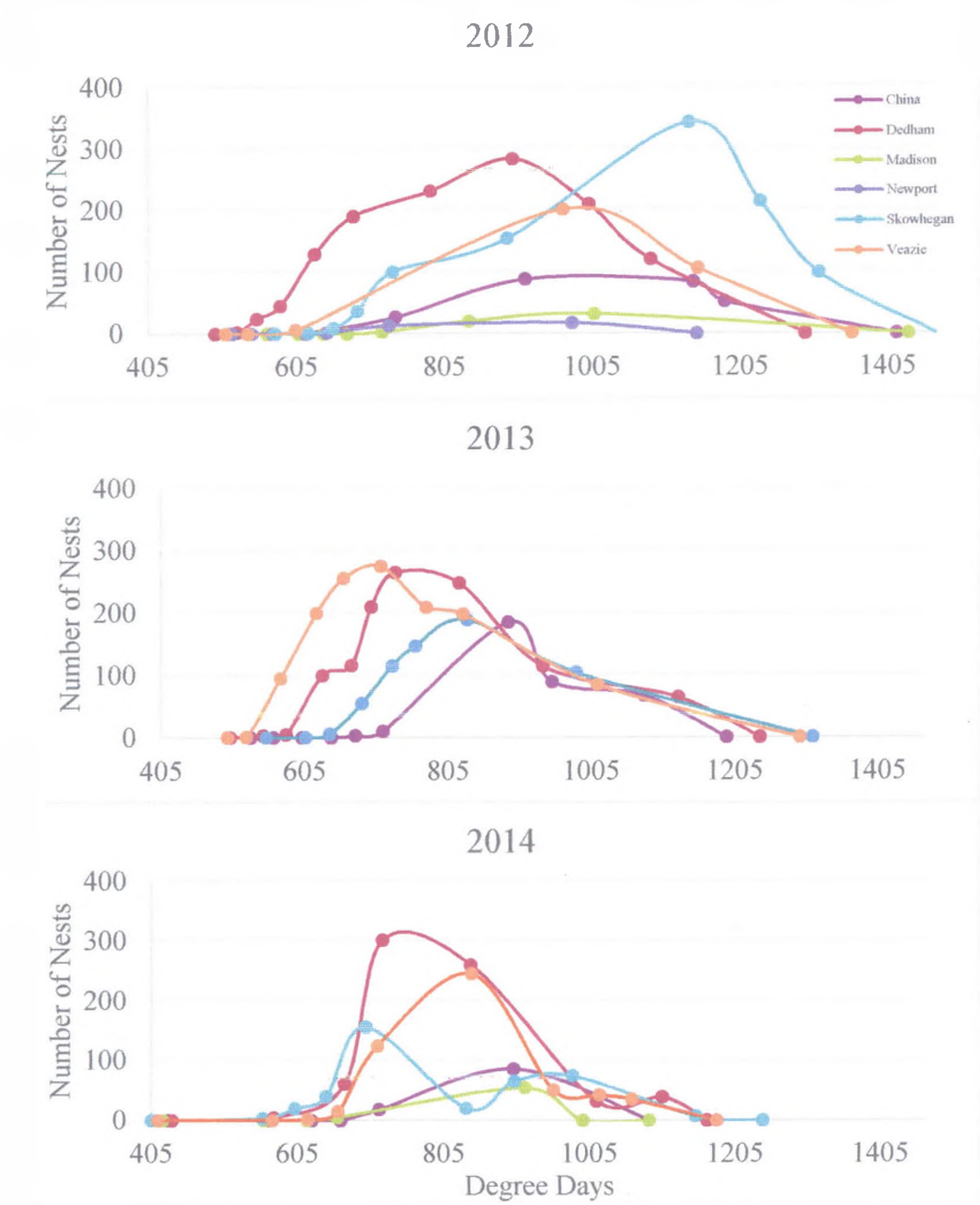

Figure 2.04: Number of *C. fumipennis* nests observed during the 2012, 2013, and 2014 field season at six sites in central Maine.

Discussion

We found that the mean accumulated soil degree-days for first emergence of *C. fumipennis* across all sites and years was 594 ± 21 (SE), base 10°C, which is lower than recently published findings. Rutledge et al. (2015) estimated the mean accumulated soil degree-days needed for wasp emergence to be 696 ± 17 (SE) base 10ºC. We observed a 7-12 day range in the date and a 27 to 142 range in accumulated soil degree-days at first emergence within sites among years. Variation within a site among years could be explained by abiotic conditions other than temperature. Rutledge et al. (2015) found no correlation between cumulative rainfall at a site and emergence dates, whereas Careless (2009) found that the first emergence of adult wasps often coincided with the days following a heavy rain in late June in Ontario. If emergence occurs following heavy rain events, it could account for variation in emergence times among years within a site.

We also found differences in first wasp emergence among sites. First emergence occurred over 9 days (201 DD) at sites in central Maine in 2012, over 10 days (152 DD) in 2013, and over 11 days (218 DD) in 2014. The wasps at the Dedham and Skowhegan sites emerged within a day of each other, however there was a 60 degree-day difference in mean accumulated soil degree-days at emergence (526 vs 586 DD). The Veazie site had a mean accumulated degree-day at emergence of 525, similar to Dedham (526 DD) but wasps emerged at Veazie 6-11 days after the Dedham site. China was often the latest emerging site and had one of the highest mean accumulated degree-day at emergence across sites (670DD). Some of these differences may be explained by the difference between sites and abiotic factors associated with them (shading, soil moisture, etc.). However, it is also possible that genetic differences exist among colonies. *Cerceris* *fumipennis* has very specific nesting site requirements (dry, sandy, compacted soil). In Maine, these are scarce and geographically isolated from one another so differences among colonies is not unexpected. Unique selective pressures on wasp populations could be experienced at sites and lead to differing patterns of wasp development among sites.

We observed male adult wasps emerging in colonies before female adult wasps 40% of the time across sites and years, partially contradicting speculation by other investigators that male wasps always emerge before females (Evans 1971). They emerged 4-10 days (67 – 152 DD) earlier than females. Investigations by Evans (1971) and Kurczewski and Miller (1984) demonstrated that the sex ratio of offspring in a single nest was 1:1, indicating that males and females should be as equally represented in a colony. We did not collect or observe male wasps at any sites after 10 days post emergence. Careless (2009) and Evans (1971) have suggested that immediately following emergence, males start searching for mates. Some male wasps patrol the colony, mating with emerging females, but many spend their time away from the colony and patrol flowers where adult female can be found feeding on nectar (Evans 1971, Careless 2009).

We collected females with prey beetles 7-17 days (115 – 287 DD) following first emergence. Following emergence, female wasps spend time excavating nests and defending those nests from other wasps which could account for the delay in foraging (Evans 1971, Kurczewski and Miller 1984, Mueller et al 1992, Careless 2009).

We attempted sampling on a regular schedule (3-5 days) but other portions of research and weather sometimes made it impossible. As a result, as much as a week would pass between sampling periods. Our sweep net sampling also varied in the time of day they were taken. Careless (2009) and Virgilio (2012) demonstrated Julian day, time of day, air temperature, wind speed, humidity, barometric pressure, and solar radiation can impact flight and foraging behavior.

Cerceris fumipennis colonies are often found in human disturbed areas, which experience frequent changes resulting in somewhat ephemeral habitats (Evans 1971, Kurczewski and Miller 1984, Careless 2009, Virgilio 2012). During our study the colony at the Newport site was lost. A few years prior to the start of our study, the field at Newport had been resurfaced. Typically, when a baseball field is resurfaced a machine tills up the top 8 to 15 centimeters of soil. A heavy clay soil is mixed into to the tilled soil and then packed down with a steam roller. *Cerceris fumipennis* nest depth range from 7-22 cm therefore resurfacing could have had a large impact on the survival of the buried wasps in the colony (Evans 1971, Evans and Rubink 1978, Kurczewski and Miller 1984, and Careless 1999). If the majority of individuals that were disturbed died, the remaining wasps may not have been enough to sustain the colony.

The Skowhegan site is another example of where human caused changes in the colony site impacted *C. fumipennis* populations. We found a decrease in peak number of nests over the three years sampled. In 2011, Virgilio (2012) counted 423 nests at this site and in 2014 there were 156. For many years prior to 2012, the area at the Skowhegan site where the colony is located had been used as a parking lot for a car racing speedway located a short distance from the sportsman's club. Every weekend cars would park on the site which reduced plant growth and compacted the soil. The speedway's last season was 2011 and since the closing, grasses and other plants have re-established and the populations of wasps have decreased. I recovered pre-pupa cells during the spring that had grass roots growing into the cells and appear to have killed the developing wasps (Careless 2009 and personal observation).

One further example of the ephemeral nature of *C. fumipennis* nesting sites, is demonstrated at a site that was ultimately dropped from this study. Originally, I was monitoring 7 colonies for this study, six sites previously presented in this chapter and one in the town of Norridgewock. The Norridgewock colony was found on top of a hill next to a gravel pit that is a favorite spot for dirt bike and ATV riders. The soil is very sandy, hard compacted in spots, and because of all the off-roading, prone to erosion. This colony was small, with only 19 active nests. Shortly after wasp emergence, there was a large rain event that caused the hillside to collapse, burying the colony.

There was a considerable increase in the number of nests at China between 2012 and 2013. This is a small colony and the 2-fold increase could have been caused by favorable weather conditions or increased prey availability in 2012. Several researchers have demonstrated that Buprestidae and other wood boring insects dramatically increase the year following a disturbance event; fire, logging, or extreme weather (Werner 2002, Ulyshena et al. 2004). Following the initial increase there is an equally drastic decline in the number of wood boring insects to or below the original undisturbed forest levels (Werner 2002, Ulyshena et al. 2004). We saw a similar trend in Dedham where Virgilio (2012) observed a peak number of nests of 659 in 2011, while we observed between 280 and 300 during the following three years. If increased prey availability increases *C. fumipennis* populations, the introduction of exotic buprestids (EAB specifically) into an area may impact the establishment and success of local wasp colonies.

Even with the limitations in this study we can draw some broad conclusions about the above ground phenology of *C. fumipennis*: wasp populations are variable between years and first emergence dates are variable among colonies; male wasps sometimes emerge before females; females have a delay between emergence and start of provisioning.

Although EAB is not currently found in Maine, the USDA (2015) has estimated that first emergence of EAB in central Maine would be June 15 to June 21 (450 DD base 10° C). Our emergence data demonstrates that the period of flight activity for *C. fumipennis* and EAB would overlap. This insect continues to be a good candidate for use as biosurveillance when EAB arrives in Maine. One area that could limit the usefulness of *C fumipennis* for biosurveillance is the variability in size and persistence of colonies from year to year due to human disturbance or other factors. The further development and use of mobile colonies of *C. fumipennis* might provide a more reliable option.

CHAPTER 3

BELOWGROUND DEVELOPMENT OF THE PREDATORY WASP *CERCERIS FUMIPENNIS* IN CENTRAL MAINE

Abstract

One method being utilized for detection of the invasive emerald ash borer (EAB) involves monitoring colonies of the wasp *Cerceris fumipennis* for the presence of EAB in their collected prey. Currently there is little information published on the nest structure and belowground phenology of *C fumipennis*. Our objective was to better understand nest structure, phenology, and prey requirements of *C. fumipennis*.

We excavated nests at four sites in central Maine during 2012, 2013 and 2014. We measured depth of cells and the number of new cells per nest. The contents of cells were examined for *C. fumipennis* immature stages and prey. We estimated the mass of beetle prey provisioned and measured the weight of pre-pupae in cells.

We excavated 171 nests. The number of new cells per nests and depth of cells varied between sites. The proportion of cells containing eggs generally decreased over a field season, while the proportion of cells containing pre-pupae increased over the field season. There were no significant differences in the numbers of beetles provisioned per egg among sites but there were differences in the estimated weights of beetles provisioned per egg among sites. Finally, we found that as the estimated provisioned weight increased, the weight of the resulting pre-pupae also increased. The data presented in this chapter will aid in the development of rearing techniques for the wasp laying the groundwork for future development of mobile colonies.

Introduction

The emerald ash borer (*Agrilus planipennis*, EAB) is an invasive beetle in the family Buprestidae. The beetle was first identified as a pest of ash trees (*Fraxinus spp.*) in 2002 in Michigan (Haack et al. 2002). To date, EAB has been detected in 25 U.S. states, several parts of Canada, and is responsible for the death or decline of tens of millions of ash trees (EAB Info 2015). One method being utilized in some areas for detection of early, small infestations of EAB involves monitoring local aggregations of the predatory wasp *Cerceris fumipennis* for the presence of EAB in their collected prey.

Cerceris fumipennis is a solitary, ground-nesting wasp that provisions its nest with beetles from the family Buprestidae. Along the East coast *C. fumipennis* occurs from New England to Florida (Evans 1971, Kurczewski and Miller 1984, Careless 2009). *Cerceris fumipennis* utilizes open, well-drained, hard-packed sandy soils to make their nests and are commonly found in gravel pits, baseball fields, picnic areas, camp sites and parking lots (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Since they require wood boring beetles as a food source, their nests are also found in close proximity to woody habitats (Evans 1971, Kurczewski and Miller 1984, Careless 2009).

Adult *Cerceris fumipennis* wasps emerge in early July in Maine (Virgilio 2012). Following emergence, a female *C. fumipennis* will dig a nest that consists of a single entrance hole into a solitary tunnel that descends below the soil surface (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Off of this central tunnel, the female will dig individual cells that she provisions with paralyzed beetles that she captures from the surrounding landscape (Evans 1971, Kurczewski and Miller 1984, Careless 2009). *Cerceris fumipennis* is a mass provisioner so, following the addition of the last beetle to a cell, the female will lay a single egg and then back-fill the completed cells (Evans 1971, Kurczewski and Miller 1984, Careless 2009). After consuming the prey and completing development, the larva forms a pre-pupa within its cell before overwintering (Kurczewski and Miller 1984). The wasp pupates in the following early spring and emerge as an adult later in the summer.

Some information has been published on the nest structure of *C fumipennis*. Evans (1971), Evans and Rubink (1978) Kurczewski and Miller (1984) and Careless (1999) provided measurements of nest size and prey number, however, their data is limited (Table 3.01). Evans (1971) suggested that these wasps are mass provisioners, meaning that a wasp will completely provision a cell before laying an egg and moving onto the next cell. Evans and Rubink (1978) observed that female *C. fumipennis* captured "medium size" beetles ranging in size from 5.5 to 10.5 mm in length. Kurczewski and Miller (1984) reported individual prey weight ranging from 128-233 mg and 308 – 430 mg in Auburn, NY, and 98-259 mg and 409-441mg in Lake Placid, Fl. Although they did not statistically analyze the data, they suggested that there was a bimodal distribution based on the sex of the wasp, with males being provisioned with fewer beetle prey per individual.

In EAB infested areas in Ontario, EAB has been found to make up to 86% of a wasp colony's daily capture (Careless 2009). During the 2012 summer, infestations of EAB were first detected in Connecticut, Illinois, and New York by observers monitoring *C. fumipennis* colonies, while forest managers' visual surveys and nearby traps failed to detect the beetles. The "biosurveillance" technique utilizing *C. fumipennis* may be the most cost-effective means available for early detection as no specialized equipment is necessary.

Recent studies have determined the most important environmental factors influencing wasp flight and foraging activities; identifying the most productive times to monitor *C. fumipennis* colonies for EAB (Virgilio 2012). However, few studies have explored the conditions required for growth and development of the immature wasps in their ground nests.

The objective of this study is to: i) better understand *C. fumipennis* nest structure, ii) investigate below ground wasp development and phenology, iii) determine the prey requirements of immature *C. fumipennis* and iv) determine if the weight of provisioned beetles affects the resulting wasp weight.

Table 3.01: *Cerceris fumipennis* nest characteristics reported in previous studies.

Site	**Number of Nests Excavated**	**Depth of Cells (cm)**	**Number of Cells per Nest**	**Number of Prey per Cell**	**Source**
Arkansas Co, AR	1	10 – 17	5	3 - 15	Evans 1971
Versailles, IN	1	11 – 15	5	3	Evans 1971
Sebring, FL	1	15 – 16	No data	No data	Evans 1971
Yellowstone National Park, WY	3	7 - 12	2 - 10	4 - 8	Evans 1971
Albany, NY	1	11 - 22	6	No data	Evans 1971
Rensselaerville, NY	13	8 - 10	4 - 24	2 - 32	Evans 1971
Monahas State Park, TX	2	7 - 15	1 - 11	15 - 16	Evans Rubink 1978
Auburn, NY	7	11 - 17	No data	2 - 15	Kurczewski and Miller 1984
Lake Placid, FL	6	12 - 17	No data	2 - 5	Kurczewski and Miller 1984
Wheatley Lagoon, Ontario, Canada	1	8 - 17	4	No data	Careless 1999

Methods

Field studies were conducted on *C. fumipennis* colonies located at four sites in central Maine in the towns of Veazie, China, Dedham, and Skowhegan (previously described in Chapter 2). The China, Veazie and Skowhegan colonies were sampled in 2012; colonies at all four sites were sampled in 2013; and colonies in Dedham, Skowhegan and Veazie were sampled in 2014. The China colony was not sampled in 2014 due to low wasp density, delayed emergence, and an unforeseen field disruption. Soil temperatures at each of the colony locations were monitored as previously described in Chapter 2.

Nest excavation and prey sampling

During the summer of 2012, 2013 and 2014, four to 10 individual tunnels were sampled weekly or biweekly at field sites following the initiation of nest provisioning in mid to late July. Entrance holes to individual active nests were identified by the presence of tumulus (a small mound of soil excavated by the wasp) outside the mouth of the hole or the visual confirmation of a wasp. Excavated nests were chosen at random from active nests. Once an entrance hole was located, a 60 cm diameter circle of sod, if present, with approximately 2 cm of soil was removed. Remaining soil was carefully removed around the tunnel using a hand trowel until a cell was located. Cell depth was measured using a standard ruler and its contents were put into a 30 ml portion cup (Solo Cup Operating Corporation, Lake Forest, IL), closed and labeled.

All samples were brought back to the laboratory at the University of Maine and their contents were examined, measured and recorded. A female *C. fumipennis* will reuse portions of her emergence tunnel for her new nest, resulting in both old cells (those from the previous year) and new cells present in the excavated areas. The contents of all cells were examined for the three below-ground life stages of *C. fumipennis* (egg, larva, and pre-pupa) and their prey and prey remnants. A cell was deemed new if it contained new, intact paralyzed beetles or an immature *C. fumipennis*. Old cells often contained an empty cocoon with an exit hole and/or tattered elytra from provisioned prey. Any whole beetles plus remaining elytra from previously consumed prey were counted for both old and new cells. Elytra per cell were counted and divided by 2 and added to the number of intact beetles to estimate number of beetles provisioned in each cell. All whole beetle prey were weighed and elytra were measured at their widest and longest point. Miscellaneous beetle elytra found in cells were also measured at their widest and longest points. All *C. fumipennis* pre-pupae recovered in cells were weighed and measured at the longest and widest point on the cocoon.

Estimation of prey weights and statistical analyses

Differences in the number of new cells per nest were compared among years and sites using independent 1-way ANOVA (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015) because sampled sites differed between years. Linear and polynomal trends in the accumulation of new cells within nests throughout the season (relative to accumulated soil degree-days) were explored using regression analysis (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015). Differences in depth of all cells (both old and new cells) among sites was examined using 1-way ANOVA (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

The proportion of eggs, larvae and pre-pupae found in cells were examined over the sampling period in relation to accumulated soil degree-days. Linear trends for proportion of eggs, larvae and pre-pupae in cells in relation to accumulated soil degree-days at each site throughout the season were explored using regression analysis (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

Weighted mean age of the immatures in a nest was calculated to compare larval maturation at each site across the sampling period for 2013 and 2014. The number of cells per site on each sampling date with eggs was multiplied by 1, with larvae by 2 and with pre-pupae by 3. The resulting number was divided by the number of new cells found during the collection period. These three numbers were summed to produce a weighted mean age. The weighted mean age was plotted against degree-days and linear and polynomic trends throughout the season were explored using regression analysis (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

The mass of beetle prey provisioned for each immature wasp was calculated using the combined weights of intact live beetles when available and the estimated weights of prey based on size of remaining elytra when prey were partially or completely consume. To estimate prey weights from elytra remains, live buprestid beetle prey were collected from foraging wasps returning to their nests during the summers of 2012-2014. Each beetle was weighed and the length and width of their elytra (outer hard wings of a beetle) at the longest and widest sections was measured. A model II linear regression was performed to examine the relationship between elytra size (length*width) and beetle weight. The resulting formula was used to estimate the weight of provisioned beetle prey before it was consumed. With this method, the combined prey weight per cell were calculated for both new and old completed cells. Differences between years and populations in the mass of beetle prey provisioned for immature wasp were analyzed using 2-way ANOVA (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015). Whole beetles were identified to species and elytra were identified to genus using morphological keys (Paiero et al. 2012, Majka et al. 2011) and are reported in Chapter 4.

The relationship between *C. fumipennis* pre-pupae found in excavated cells and the estimated mass of total provisioned beetle prey was examined with linear regression (JMP SAS Institute, Cary, North Carolina).

Results

A total of 171 nests were excavated during the 2012, 2013, and 2014 (Table 3.02). During the 2012 field season, development of a reliable technique for nest excavation took several weeks, and therefore, I was unable to successfully collect data on viable cells until mid-August.

Number of new cells and depths of cells

We found no differences in the number of new cells per nest among years at each site (ANOVA, T-test, $P > 0.05$) so data were combined across years. The number of new cells per nest ranged from 0 (all sites) to 14 (Skowhegan) in 2013 and 2014 (Table 3.03), and did vary among sites. Wasps produced significantly more new cells per nest at the

Table 3.02: Number of *Cerceris fumipennis* nests excavated at four sites in central Maine during the 2012, 2013, and 2014 field seasons.

China		**Dedham**		**Skowhegan**		**Veazie**	
Date	**# Nests**	**Date**	**# Nests**	**Date**	**# Nests**	**Date**	**# Nests**
8/13/2012	5			8/14/2012	6	8/21/2012	4
7/30/2013	7	7/22/2013	2	7/30/2013	9	7/25/2013	6
8/6/2013	10	7/31/2013	9	8/5/2013	10	7/29/2013	10
8/16/2013	6	8/16/2013	6	8/21/2013	7	8/6/2013	10
						8/22/2013	4
		7/23/2014	4	7/25/2014	5	7/30/2014	6
		7/31/2014	4	7/31/2014	10	8/7/2014	5
		8/20/2014	6	8/5/2014	5	8/12/2014	3
				8/11/2014	5	8/19/2014	3
				8/21/2014	4		
Total Nests Sampled	28		31		61		51

Table 3.03: Number of new cells per nest excavated during the 2013 and 2014 field seasons across four sites in central Maine. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

		Number of New Cells Per Nest		
			Range	
Site	**N**	**Mean ± SE**	**Low**	**High**
China	12	1.67 ± 0.81 b	0	3
Dedham	22	1.68 ± 0.60 b	0	8
Skowhegan	49	4.06 ± 0.40 a	0	14
Veazie	37	3.14 ± 0.44 ab	0	9

Skowhegan site then in either the Dedham and China sites (ANOVA, Tukey's test, N=120, $F_{(3, 116)} = 4.82$, $P = 0.003$).

Nests were sampled between 7 July and 22 August in 2013. The number of new cells per nest increased throughout the season (with accumulated degree-days) at the Dedham site ($N = 10$, $F_{(2, 7)} = 9.31$, $P = 0.01$, $r^2 = 0.73$) and the Veazie site ($N = 21$, $F_{(3, 17)} = 6.01$, $P = 0.006$, $r^2 = 0.51$) (Figure 3.01). At the Skowhegan and China site we observed a slowing of new cells earlier than at the other two sites with activity peaking earliest at the China site around 970 DD (10 August). We did not observe a decline in the accumulation of new cells at the Dedham, Skowhegan, or Veazie sites. The largest numbers of new cells per nest at each site were found at the last sampling dates in late August (16 August for China and Dedham, 21 August at Skowhegan, 22 August in Veazie).

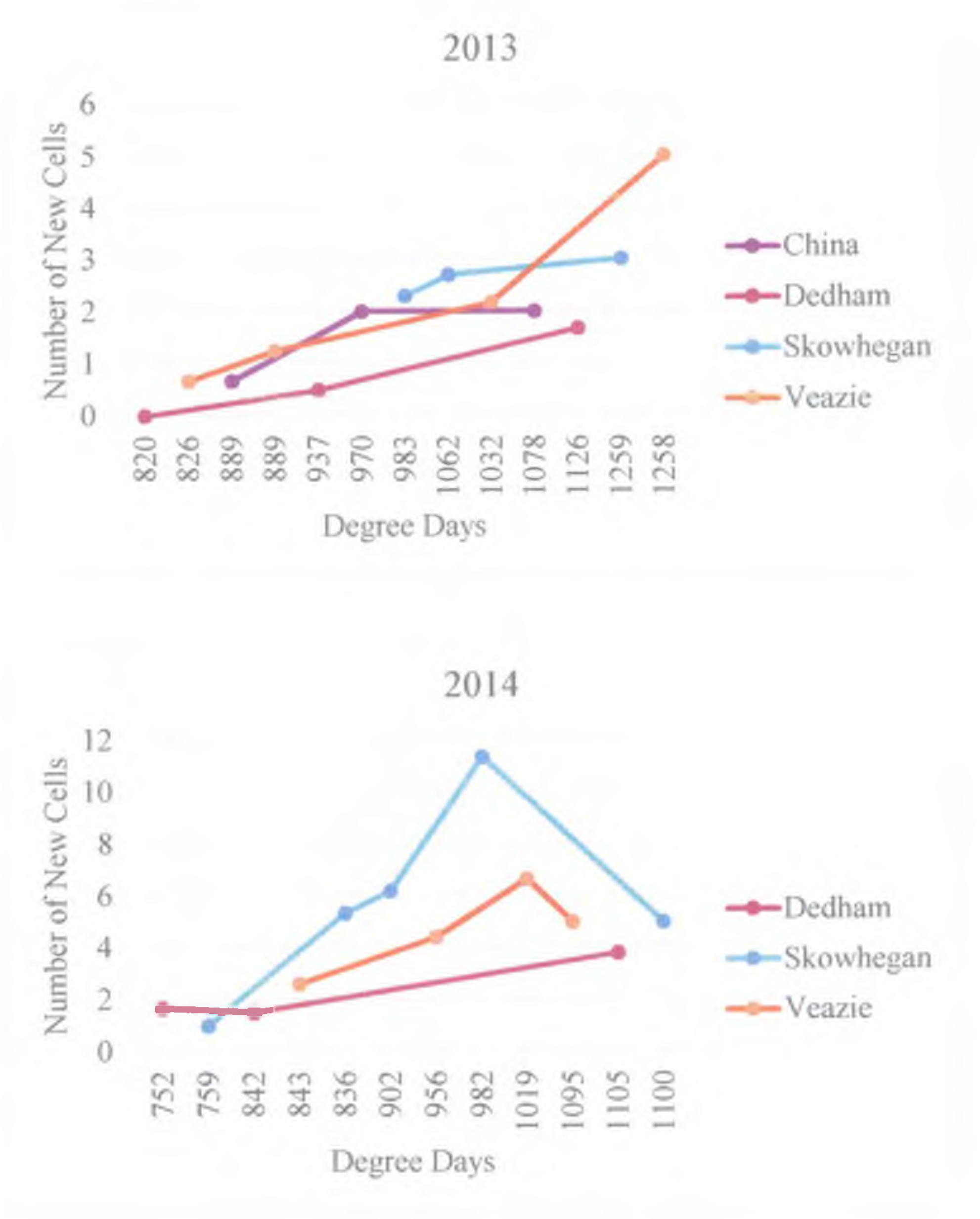

Figure 3.01: Accumulation of new cells per sample date in *Cerceris fumipennis* nests over the 2013 and 2014 field season (represented in degree-days) at four colony sites in central Maine.

Nests were sampled between 23 July and 21 August in 2014. Accumulation of new cells in nests over the season at each site differed from the previous year (Figure 3.01). The number of new cells per nest peaked at the Veazie site at ca. 1019 DD (13 August), and at the Skowhegan site at ca. 982 DD (11August). There were significantly more new cells in nests at 982 DD (11 August) in Skowhegan than at the first sampling period at 759 DD (25 July) (ANOVA, Tukey's, $N = 26$, $F_{(4, 21)} = 5.53$, $P = 0.003$). The mean number of new cells per nest was lower on the last sampling date than the previous sampling date at these two sites, whereas at the Dedham site, the new cells per nest increased to the last sample date.

The maximum depth of *C. fumipennis* cells excavated ranged from 20.32 cm to 27.94 cm. The depth of new cells within nests did not vary among years ($P > 0.05$), and therefore data were pooled across all years for subsequent analyzes. Depth of all cells (old and new) within nests were significantly deeper at the Dedham site than at other sites (ANOVA, Tukey's Test, $N = 563$, $F_{(3,559)} = 18.71$, $P < 0.001$; Table 3.04). The depth of completed cells in nests from all sites ranged between 5.08 cm and 27.94 cm.

Table 3.04: Depth and range of *Cerceris fumipennis* complete cells within nests excavated at four sites in central Maine in 2013 and 2014. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different (α = 0.05).

		Depth of Completed Cell (cm)		
			Range	
Site	**N**	**(mean ± SE**	**Low**	**High**
China	32	14.22 ± 0.60 bc	10.16	20.32
Dedham	53	16.98 ± 0.47 a	7.62	25.40
Skowhegan	254	14.84 ± 0.23 b	5.08	27.94
Veazie	115	12.78 ± 0.34 c	5.08	20.32

Belowground development

On the one mid-August sample date that I successfully excavated nests in 2012, pre-pupae were the most abundant stage present in the nests at all sites (Figure 3.02). All three life stages (egg, larva, pre-pupa) were often found within in the same nests.

The proportion of eggs decreased over the sampling period for all sites in 2013 and 2014, but eggs were present in nests throughout most of the season in both years (Figure 3.02). We found eggs in cells on the last sampling period at Dedham but not at China and Skowhegan for 2013. All sites in 2014 had eggs present at the last sampling period.

In 2013 the proportion of cells containing larvae generally increased, peaked, and then decreased towards the end of the field season (Figure 3.03). This peak occurred at 889 DD (29 July) in Veazie and 970 DD (6 August) in China. The highest proportion of cells with larvae was observed on the first sample date in Skowhegan (983 DD, 30 July), and decreased from that point to the end of the sampling period. Similarly, in 2014, the proportion of nests with larvae was highest on the first sample date in Skowhegan and Veazie, with 67% and 75% of the nests with larvae on 25 July (759 DD) and 30 July (843 DD), respectively. However, after the proportion of nest with larvae declined over the following 16 and 13 days respectively at these sites, they increased on the last sampling dates (1100 DD, 21 August and 1095 DD, 19 August respectively). Nests at the Dedham site increased from no larvae on 23 July (752 DD) to half of the nests with larvae by the second sampling date on date (842 DD). Larvae continued to be present in ca. 50% of the nests throughout the remainder of the sampling period.

In 2013, pre-pupae were first observed in nests on 22 August (1650 DD) at the Veazie site, and on 16 August (1078 DD), at the China site, and were present at the first sample date in Skowhegan on 30 July (983 DD) (Figure 3.03). The proportion of nests with pre-pupae increased at all sites over the remaining season. In 2014 pre-pupae were observed in nests earlier in the season. Pre-pupae were first detected on 30 July at the Dedham and Veazie sites (842 DD and 843 DD respectively) and on 31 July (836 DD) at the Skowhegan site. Pre-pupae peaked at 50% (30 July, 842 DD) at the Dedham site, 69% (11 August, 982 DD) at the Skowhegan site and at 63% (12 August, 1019 DD) at the Veazie site. After this the proportion of active nests with pre-pupae declined on the last sample date of the season.

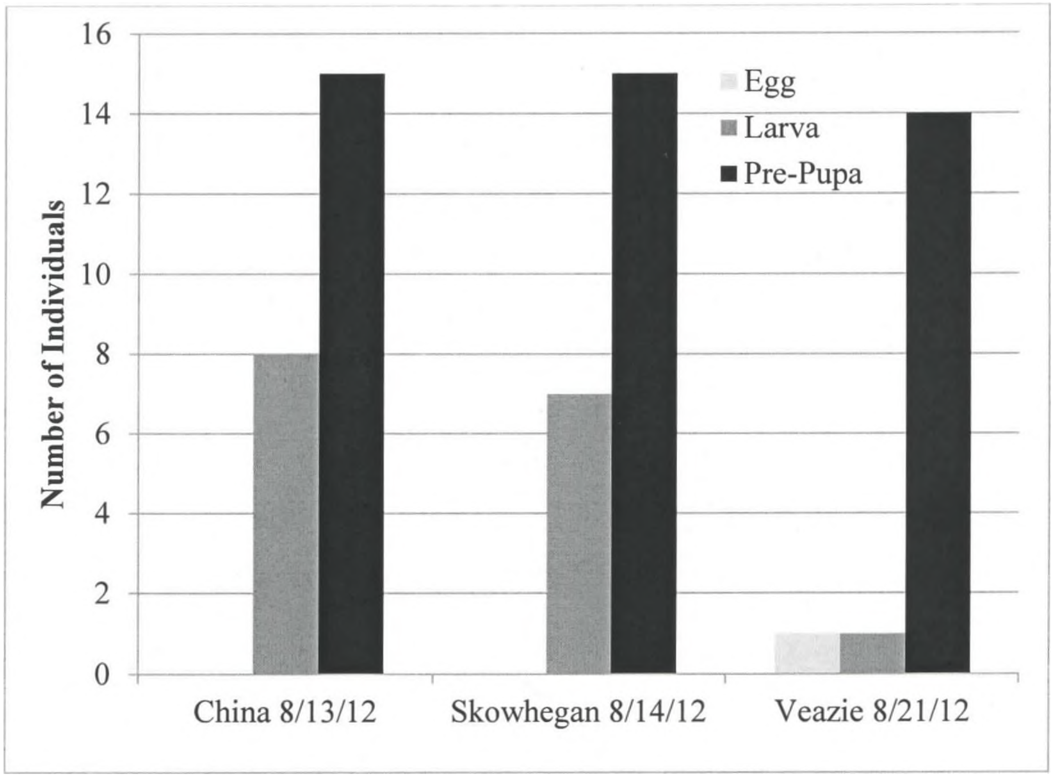

Figure 3.02: Total *Cerceris fumipennis* eggs, larvae, and pre-pupae excavated from nests in mid to late August 2012 at three sites in central Maine (China, Skowhegan and Veazie).

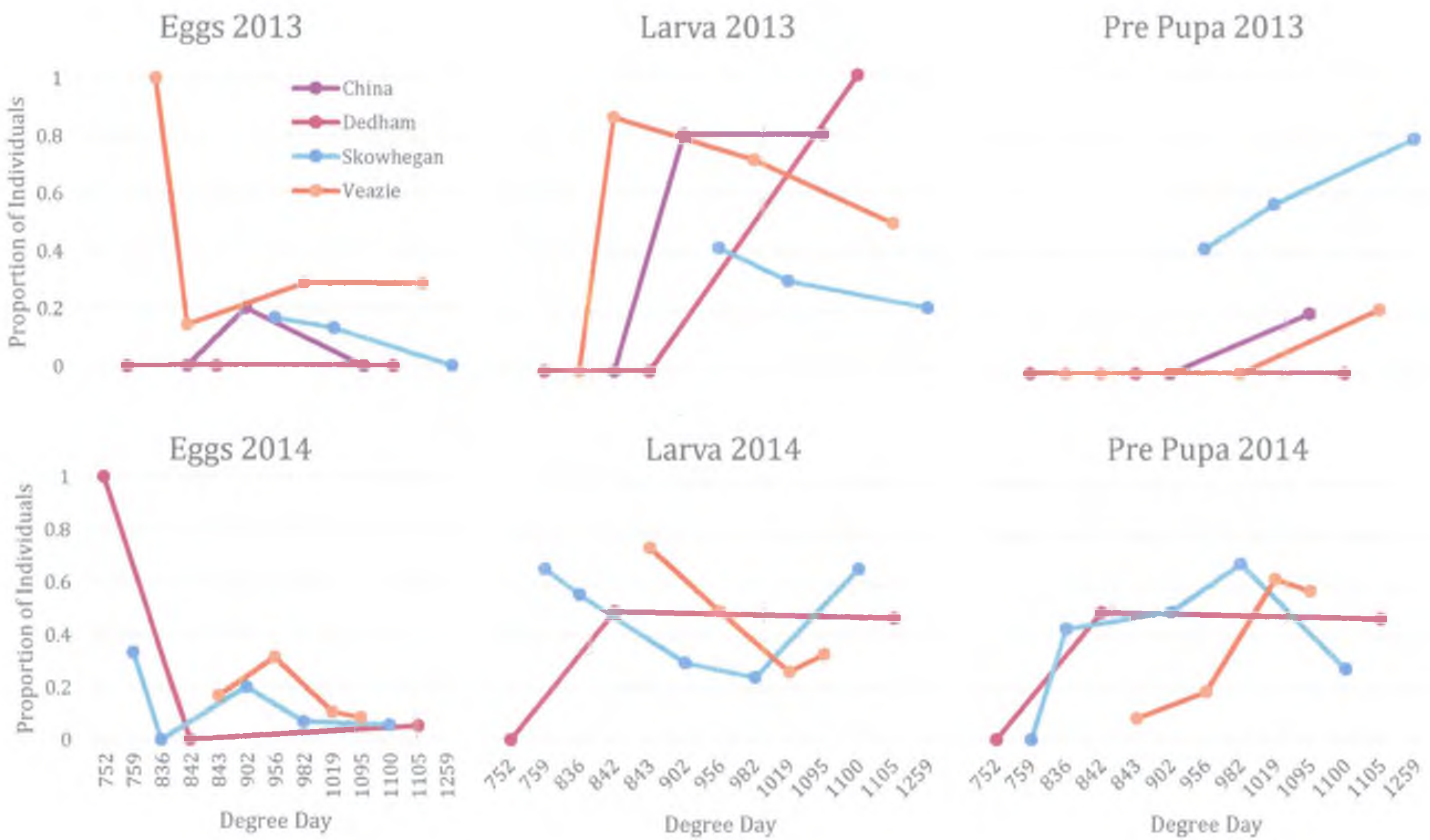

Figure 3.03: The proportion of *C. fumipennis* immature life stages in belowground nests at four sites during 2013 and 2014 sampling. There were no significant correlations between proportion of different life stages present and degree-day for all sites (all p values > 0.05)

We found that the weighted mean age of the population increased linearly with DD throughout the season at the Dedham site (N = 10, $F_{(1,8)} = 6.25$, P = 0.04, $r^2 = 0.44$) and the Veazie site (N = 21, $F_{(1,19)} = 13.05$, P = 0.002, $r^2 = 0.41$, Figure 3.06). No significant linear relationships between soil degree-days and weighted mean age over the 2013 field season were detected at the China or Skowhegan sites. Weighted men age increased at the Veazie and Skowhegan sites early in the season and leveled off at 889 DD and 1062 DD respectively. China and Dedham showed no leveling off by the last sampling dates of 16 August and 20 August, respectively. Highest weighted mean age was found at the last sampling period for each site.

In 2014, the weighted mean age of the population at the Dedham site increased linearly with DD throughout the season (N = 12, $F_{(1,10)} = 7.90$, P = 0.02, $r^2 = 0.41$) whereas a polynomial trend was observed at the Skowhegan site with age of the population peaking between 1334 and 1647 DD (N = 26, $F_{(2,23)} = 7.06$, P = 0.004, $r^2 = 0.38$, Figure 3.04). The age trend appeared to be variable throughout the season at the Veazie site.

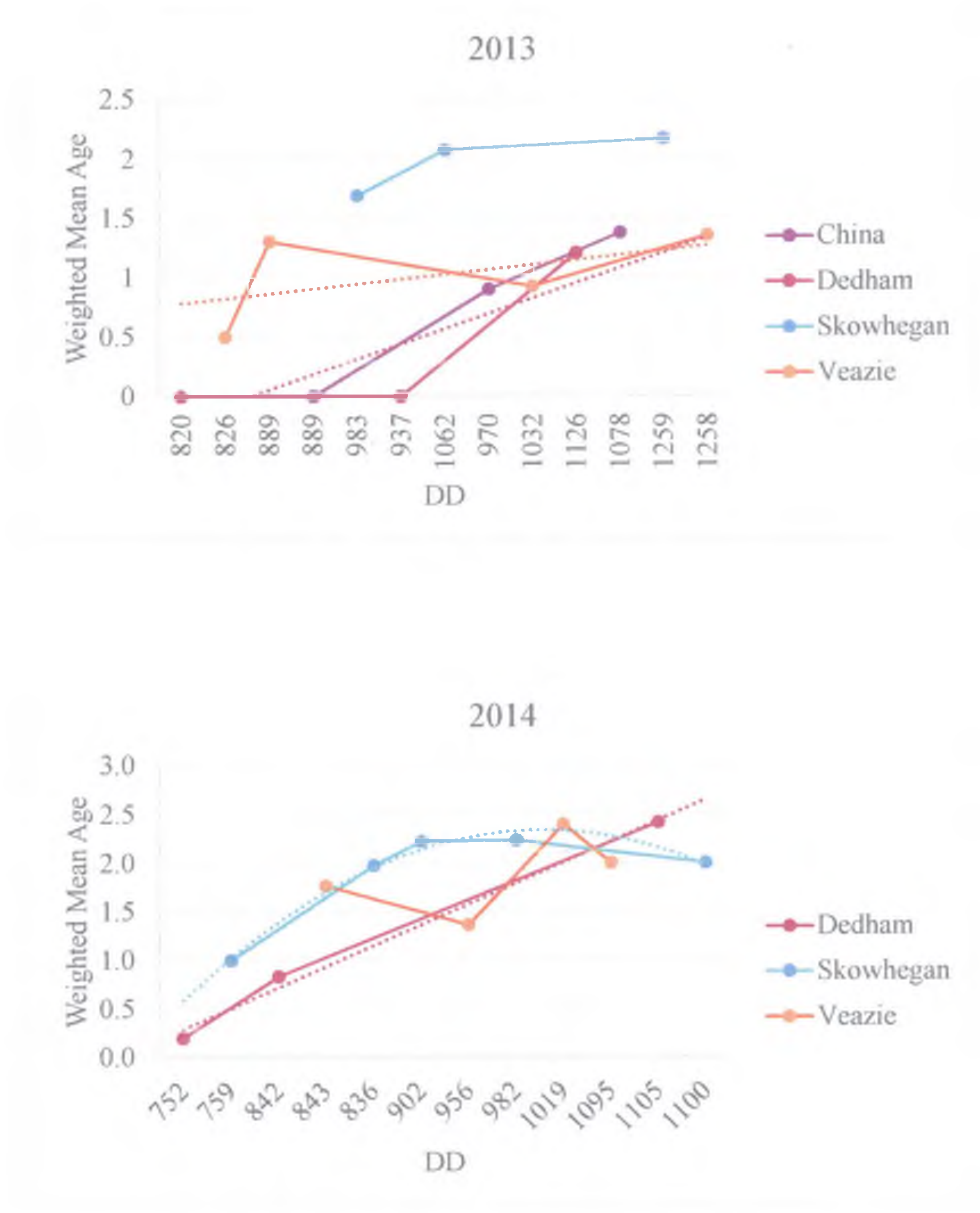

Figure 3.04: Weighted mean age of immature *C. fumipennis* in nests at four sites in central Maine in 2013 and 2014. Dotted lines represent best fit regression.

Prey requirements

Six hundred forty-five whole beetle prey and 2,283 beetle elytra were recovered from cells in *C. fumipennis* nests between 2012 and 2014. There was no significant difference between the number of beetles provisioned per cell among sites (ANOVA, Tukey's, N = 465, $F_{(3, 461)} = 0.71$, P = 0.55; Table 3.05). The lowest and highest number of beetles provisioned per cell was found at the same site, Skowhegan (0 and 16 beetles respectively). The "0" represented a pre-pupa found in a cell with no associated beetles or elytra. The next lowest number of beetles provisioned was 1 at the Skowhegan site.

Table 3.05: Number of beetles provisioned per completed cell in *C. fumipennis* nests at four sites in central Maine during the 2013 and 2014 field season. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different (α = 0.05).

		Beetles Provisioned per Cell		
			Range	
Site	**N**	**(Mean ± SE)**	**Low**	**High**
China	32	3.45 ± 0.42 a	1	7.5
Dedham	60	3.18 ± 0.30 a	0.5	10
Skowhegan	256	3.66 ± 0.15 a	0	16
Veazie	117	3.53 ± 0.02 a	0.5	11

A significant positive linear correlation was found between the product of beetle elytron measurements and weight of intact beetles collected from foraging females explaining 73% of the variation in beetle weight (($y = 0.0044x - 0.0263$, $P < 0.001$, $r^2=0.73$, Figure 3.05).

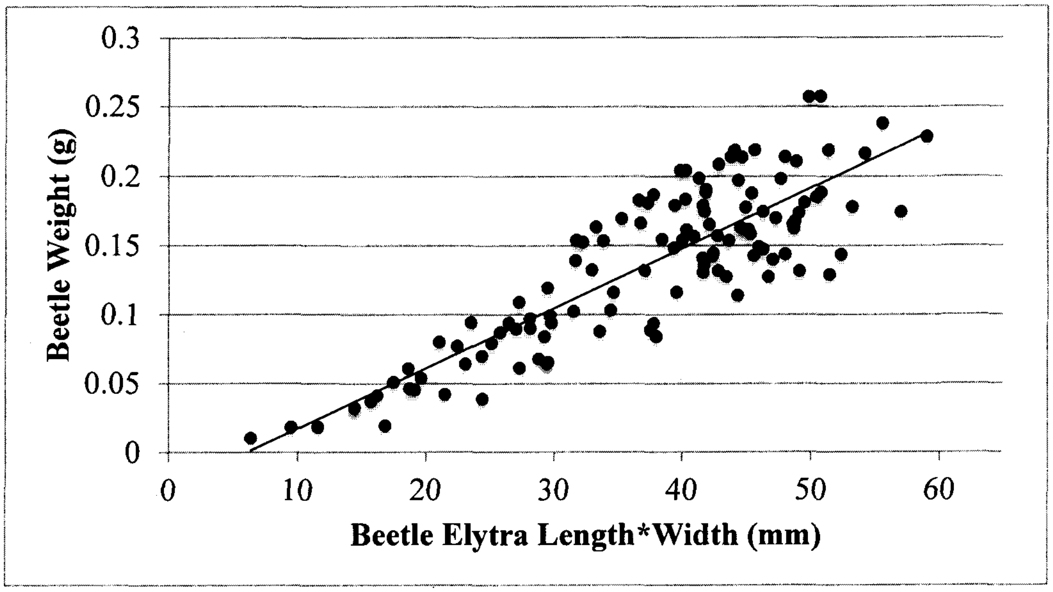

Figure 3.05: Buprestidae beetle weight in relation to the length*width of their elytra. Beetles were collected from foraging *C. fumipennis* wasps from 2012-2014 ($P < 0.001$, $r^2 = 0.73$).

The estimated total weight of beetles provisioned per completed cell did differ among sites (Table 3.06). The largest mean total estimated weight of provisioned beetles was found at the Dedham site (0.33g) and the smallest at the Veazie site (0.22g). The range of total estimated weight was 0.003g to 1.27g. Nests at the Dedham site were provisioned with significantly heavier estimated beetle prey then those at the Skowhegan and Veazie sites (ANOVA, Tukey's test, $N = 466$, $F_{(3, 462)} = 5.06$, $P = 0.002$).

We found a positive correlation between the mean estimated individual weight of beetles and their estimated cell weight ($N = 464$, $F_{(1, 462)} = 200.46$, $P < 0.0001$, $r^2 = 0.30$, Figure 3.06).

Table 3.06: Estimated weight of beetles provisioned per completed cell in *C. fumipennis* nests at four sites in central Maine during the 2013 and 2014 field season. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different (α = 0.05).

		Total Estimated Beetle Prey Weight of Completed Cells		
			Range (g)	
Site	**N**	**(Mean g ± SE)**	**Low**	**High**
China	32	0.27 ± 0.03 ab	0.075	0.58
Dedham	60	0.33 ± 0.02 a	0.041	1.11
Skowhegan	256	0.24 ± 0.01 b	0.003	1.27
Veazie	118	0.22 ± 0.02 b	0.010	0.61

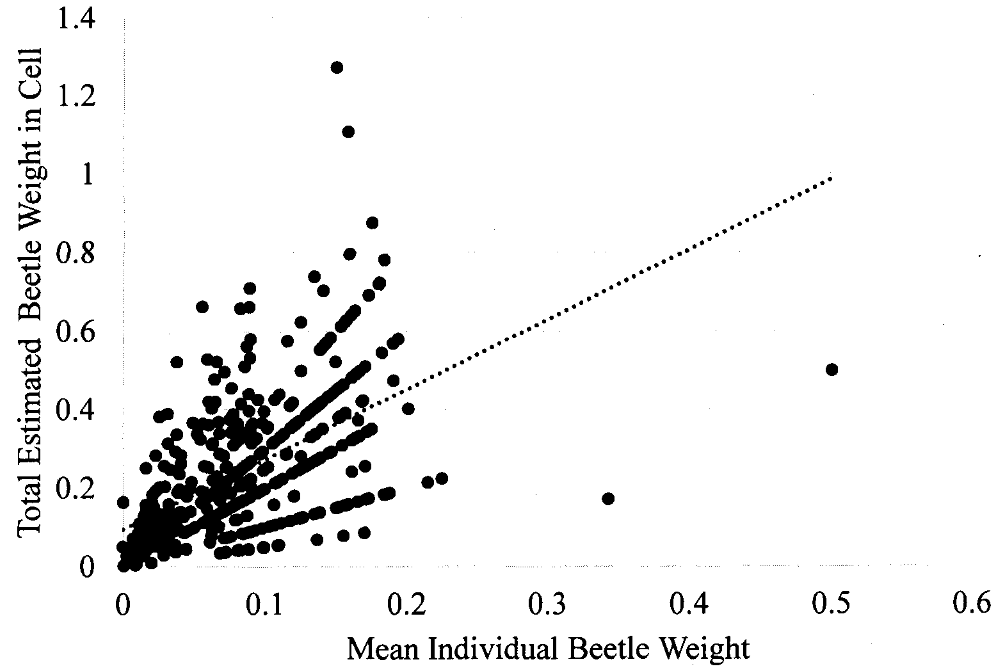

Figure 3.06: The individual beetle weight per completed cell plotted against the predicted total weight of the beetle prey at four sites in central Maine during the 2013 and 2014 field seasons.

Provisioned weight and resulting pre-pupal weight

The estimated weight of beetle provisioned in a cell was positively correlated with the weight of the resulting pre-pupae (N= 98, $F_{(1, 96)} = 46.81$, $P < 0.001$, $rF^2 = 0.33$). As the estimated provisioned weight increased, the weight of the resulting pre-pupae also increased (Figure 3.07).

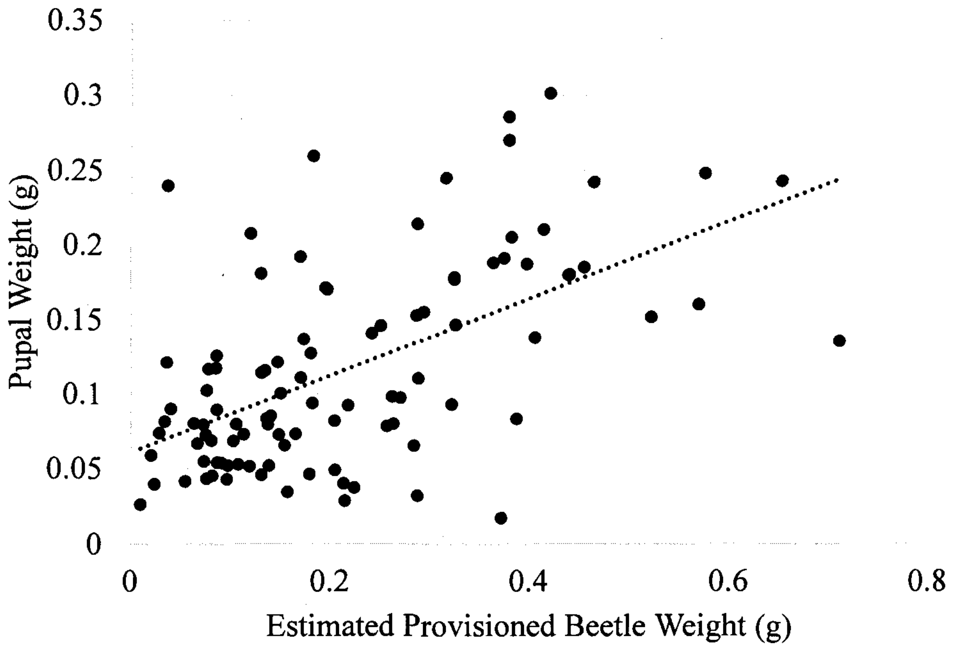

Figure 3.07: The resulting weight of pre-pupae in relation to the estimated weight of beetles provisioned per completed cell across four sites in central Maine during the 2013 and 2014 field seasons.

Discussion

Cerceris fumipennis require specific nesting sites that are limited in Maine and isolated from one another. It is likely that there is low genetic mixing which probably results in genetic drift between populations due to local conditions at each site (Slatkin 1987). Differences among populations in depth, reproductive potential, size of pre-pupae, and belowground phenology is not unexpected.

The number of cells per nest and mean depth of nests at the four sites in Maine were within the range of reported for previous studies (Evans 1971, Kurczewski and Miller 1984, Careless 2009). The differences we observed in depth of cells across the four sites sampled is likely due to soil composition at the sites. The colonies of wasps at Dedham, China, and Veazie are in baseball fields that have a base clay layer limiting the depth the wasps can excavate. The colony at the Skowhegan site is located in an abandoned parking lot and adjacent field with no clay layer allowing the wasps to dig tunnels deeper than the other sites sampled. One interesting thing to note is that we found completed cells as shallow as 5.08 cm. This is significant for future rearing protocols in regards to the depth of rearing containers that would be needed for *C. fumipennis*.

The number of new cells per female (reproductive potential) varied among sites, but was generally low. A *C. fumipennis* female exhibits a high degree of parental care; digging nests, guarding nests, and provisioning young with beetles. Gilbert and Manica (2010) found that across 275 species (16 orders), lifetime egg number was associated with evolutionary changes in parental care; provisioning species produced fewer eggs than those species with low or no parental care. They suggested a metabolic constraint on clutch size in provisioning species, resulting from the way foraging capacity (supply) scales with body size compared to brood energy requirements (demand).

Skowhegan had significantly higher number of new cells per female wasp but had a decreasing year to year populations (Table 2.01). *Cerceris fumipennis* colonies are often found in human modified areas, making these habitats unpredictable and prone to disturbance (Evans 1971, Kurczewski and Miller 1984, Careless 2009, Virgilio 2012). During our field studies we observed spraying for weeds and pest insects, refurbishing of ball fields, human landscape management, and specifically at the Skowhegan site, loss of prime nesting areas. For many years prior to 2012, the area at the Skowhegan site where the colony is located had been used as a parking lot for a racing speedway. Cars parking in the area reduced plant growth and compacted the soil. Since the speedways' closing, grasses and other plants have re-established reducing the quality of the nesting area for *C. fumipennis*.

Unfortunately, my data for the 2012 season is incomplete and I am unable to draw conclusions from the results. One interesting observation was that we found both eggs and pre-pupae together within the same site and often within the same nest at the end of the season. This confirms the speculation of Evans (1971) who suggested that these wasps mass provision and completely provision a cell before moving onto the next one.

We observed differences between years in the temporal distribution of new cells over the sampling period. The number of new cells within the nests were highest at the end of the season in 2013. In 2014 the number of new cells decreased at the last sampling period for Skowhegan and Veazie. In 2013 the late summer and early fall was unseasonably warm and wasps were observed foraging into mid-September. In 2014 it was difficult to locate active nests during the last sampling period in late August which followed several bouts of heavy rain and cool early fall temperatures.

The overall decrease in proportion of cells with eggs and increase in the proportion of cells with larvae over the two field sampling periods is expected as wasps complete their provisioning of cells. However, the observance of eggs in cells at the last sampling date for most sites indicates that a) female wasps continue laying eggs throughout their life and/or b) that female wasps do not emerge synchronously so some individuals are provisioning eggs later in the season because they emerged later than other individuals. The ability for wasps to forage as long as possible allows them to maximize the number of offspring that they can produce. Studies by Virgilio (2012) and Careless (2009) as well as data presented in chapter 2 of this thesis demonstrates that female *C. fumipennis* do not emerge synchronously, but instead the numbers of females present in a colony increases over time.

The mean number of beetles found in a completed cell was not different among sites indicating that wasps are counting the number of prey they put with an egg rather than weighing their prey. Counting is common is predatory wasps (Jayasingh and Taffee 1982, Cowen 1983, Vanderwall 1990). The mean estimated weight of prey provisioned in completed cells varied among sites with significantly more weight being provisioned in cells at the Dedham colony.

The amount of food that is provided for immature *C. fumipennis* by its mother is very important for the survival of offspring because the larvae are unable to acquire their own food. Growth of the insect stops at pupation and adult size has been found to be directly proportional to the amount of food provisioned in predatory solitary

Hymenoptera (Alcock 1979b, van den Assem 1971, Charnov et al. 1981, Cowan 1981, Cross et al. 1978, Freeman 1981, Jayasingh and Taffe 1983). We found a positive correlation between the estimated weight of provisioned beetle and the resulting wasp weight. Those eggs provisioned with a higher weight of beetle, had larger resulting pre-pupae.

We do not know if the differences in the estimated weights provisioned among sites is caused by differences in the populations of beetles in the landscape surrounding each site or some factor in the population of the wasps that limits the size of beetles with which the wasps are able to successfully carry to their nests with. These differences in weights of food provisioned may have some implications on resulting size of wasps.

Currently the major limitation to using existing nesting colonies of *C. fumipennis* for detecting EAB, is that naturally occurring wasp populations require specific soil types for nesting which can be limited and localized in the landscape, and may not be present in areas most at risk for EAB. The ability to rear wasps in a laboratory and the development of mobile colonies would allow local, state, and national agencies to use *C. fumipennis* biosurveillance virtually anywhere. However, right now, rearing techniques for the wasp are hampered by a poor understanding of conditions required for growth and development of the immature wasps in their ground nests. The data (depth of cells, reproductive potential, prey requirements for egg to pre-pupa development, etc.) presented in this chapter will aid in the development of rearing techniques for the wasp laying the groundwork for future development of mobile colonies.

CHAPTER 4

PREY SELECTION OF *CERCERIS FUMIPENNIS* IS CENTRAL MAINE

Abstract

Many studies have investigated prey selection of *C. fumipennis* but few have investigated if individual wasp prey fidelity occurs throughout the foraging period. The objectives of this study were to investigate *C. fumipennis* prey selection and fidelity, to determine abundance of Buprestidae in the landscape, and to explore how wasp size relates to prey selection.

We identified prey beetles excavated from nests at four sites in central Maine during 2013 and 2014 and made comparisons of prey species provisioned within cells. In 2013 we sampled beetles in the landscape surrounding colony sites using traps currently utilized for detecting EAB. In 2014 adult wasps were collected from sites, weighed, and mean weights were compared across sites.

A total of 156 nests were excavated containing 1,776.5 buprestids. Twenty different species were identified and we found that 50% to 84% of excavated cells were provisioned with a single genera of beetle prey. Over a 3-month trapping period, 128 Buprestids were collected, representing four species. We found difference in the mean weight of wasps among sites. This study highlights *C. fumipennis's* ability to find and to utilize several different hosts as prey items which makes it a good candidate for monitoring other invasive beetles in the family Buprestidae.

Introduction

Cerceris fumipennis is a solitary, ground-nesting wasp that provisions its nest with metallic wood boring beetles in the family Buprestidae. Recently there has been considerable interest in this species for use in biosurveillance programs for detection of Emerald ash borer, *Agrilus planipennis,* and other invasive wood boring insects. Following emergence of adult wasps in early to mid-summer, a female *C. fumipennis* dig a nest that consists of a single entrance hole into a solitary tunnel, off of which she will dig individual cells that she provisions with beetles captured from the surrounding landscape (Evans 1971, Kurczewski and Miller 1984, Careless 2009). *Cerceris fumipennis* is a mass provisioner so, following the addition of the last beetle to a cell, the female will lay a single egg and then back-fill the completed cells (Evans 1971, Kurczewski and Miller 1984, Careless 2009). Following egg hatch, the larva will consume the provisioned prey, complete development, and form a pre-pupa before overwintering (Evans 1971, Kurczewski and Miller 1984, Careless 2009).

Many studies have investigated prey selection of *C. fumipennis* (Grossbeck 1912, Cartwright 1931, Scullen 1965, Scullen and Wold 1969, Kirk 1970, Evans 1971, Evans and Rubink 1978, Kurczweski and Miller 1984, Hook and Evans 1991, Mueller et al. 1992, Marshall et al. 2005, Hellmen 2010, Rutledge et al. 2011, Virgilio 2012, Swink et al. 2013, Careless et al. 2014, Hellmen and Fierke 2014, Swink et al. 2014), and have found several new local records of native buprestid species captured by the wasps. Few studies have investigated whether individual female *C. fumipennis* utilize one species of Buprestid beetle throughout the foraging period or switch prey depending on availability or some other factor.

There are several species of predatory wasps that have demonstrated plasticity in prey utilization due to changing prey availability, ecological changes, or environmental changes (Brockmann 1985, Stubblefield et al 1993, Elger and Jebb 1999, Polidori et al. 2005, Grant 2006, Polidori et al. 2007, Polidori et al. 2009, Vargas et al. 2014). Vargas et al. (2014) found that the potter wasp *Hypodynerus andeus* can adapt to localized conditions and differing prey availability. These wasps switched the prey species they utilized in response to fluctuations in caterpillar abundances, as one species of caterpillar's population increased, the wasp increased its catches of that species of caterpillar. Brockmann et al. (1985) discovered that changes in the ecology of prey over time influenced the grasshopper species that *Sphex ichneumoneus* utilized for prey. As the season progressed and the size of individual species of grasshoppers increased, *S. ichneumoneus* changes to smaller species that were more easily handled.

Provisioned prey has been shown to influence the resulting size of predatory wasps (Cowan 1981, Chapter 3), and intraspecific variation in size of individual female wasps has been shown to influence offspring. In female wasps, a larger adult body size has been shown to result in more space for storing eggs, larger fat bodies for producing more eggs, greater food carrying capability in flight, and the ability to subdue larger prey (Byers 1978, Kurczewski and Elliot 1978, Laing 1979, Cowen 1981, Gwynne and Dodson 1983, O'Neil 2001). Cowan (1981) found that larger females of the predatory solitary eunimid wasp, *Anchistrocerus adiabatus*, had more offspring and females provisioned their offspring with larger amounts of food than smaller conspecific females. The resulting adult size was positively correlated with the weight of the provisioned food it was given as an immature.

The objectives of this study are to i) investigate *C. fumipennis* prey selection, ii) determine prey fidelity of female wasps, iii) determine the relative abundance of Buprestidae prey in the landscape surrounding *C. fumipennis* colonies, and iv) explore how the wasp size relates to prey selection.

Methods

Field studies were conducted on *C. fumipennis* colonies located at four sites in central Maine in the towns of Veazie, China, Dedham, and Skowhegan, as described in Chapter 2. Colonies at all four sites were sampled in 2013; and colonies in Dedham, Skowhegan and Veazie were sampled in 2014. Nests were excavated at each site and wasp prey beetles were collected as previously described in Chapter 3. Prey items found in cells were identified using morphological keys (Paiero et al. 2012). Many of the beetles had been consumed by wasp larva in the cells so elytra were often the sole remains available for identification. Solitary wings were often only identified to the genus level because many of the morphological features for species identification were missing. Intact beetles were identified to species. Prey species provisioned in nests were summarized by sampling date and site. Nominal logistic regression was used to examine differences in the proportion of prey genera excavated per site across all sampling dates (JMP$^{©}$, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015). The number of species identified per site and sampling effort (number of beetles collected) were plotted, and the proportion of each Buprestidae genera found at each site was calculated. Comparisons of prey species provisioned in cells within the same nest were made to determine if an individual female wasp provisioned with a single species, single genera, or multiple genera of Buprestidae. Completed cells (those with a *C. fumipennis* egg, larva, or pre-pupa) were used to compare the proportion of cells on a particular sample date with more than one prey present (prey fidelity). Comparisons were made between years at sites and between sites using nominal logistic regression (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

Landscape survey of Buprestidae

During the 2013 field season, different insect trap types and colors representing some of the current trap designs being developed for monitoring the emerald ash borer and other Buprestidae (Marshall 2010, Francese et al 2013, Domingue et al 2013, Poland and McCullough 2014, USDA APHIS PPQ EAB 2015) were used to sample beetles in the landscape surrounding four *C. fumipennis* colony sites (Table 4.01). Trap comparisons included, a) three trap designs: cross vein panel intercept traps (Contect Enterprises Inc, Victoria, BC, Canada), Lindgren funnel traps (Contect Enterprises Inc, Victoria, BC, Canada), and prism traps; b) three colors of Lindgren funnel traps: black, green, and purple; and c) green Lindgren funnel traps with and without a "green leafy volatile" lure (Z3-Hexen-1-ol, Synergy Semiochemicals Company, Burnaby, BC, Canada). Both the black cross vein panel traps and the Lindgren funnel traps were coated in Fluon (Bioquip Products, Rancho Dominguez, CA) and used ethylene glycol as a preservative and killing agent. The prism traps were purple and coated in Tanglefoot© (The Tanglefoot Company, Victoria, BC, Canada). Two sets of traps were hung at each site on two different species of trees common in the landscape. These were oak (*Quercus* sp.) and white pine (*Pinus strobi*) at the Skowhegan site, and oak and maple (*Acer sp.*) at the

China, Dedham, and Veazie sites. Traps were deployed from mid-July to early October during which time they were monitored and their contents collected every 5-7 days. Samples were brought back to the laboratory, sorted to order using morphological keys (Borror and White 1998, Borror 1989) and preserved in 70% ethanol. Buprestidae were identified to species using morphological keys (Paiero et al. 2012). Number of beetles caught per sampling date and trap were transformed to species presence-absence. Differences across sites and trap types were explored using general linear model with a binomial distribution (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

Wasp size differences between sites

During the summer of 2014 adult wasps were collected from all four sites in Central Maine and weighed. Wasp weights were compared across sites using ANOVA (JMP©, Version 12.1.0, SAS Institute Inc., Cary, NC, 2015).

Table 4.01: Traps deployed to sample relative density of Buprestidae in the landscape surrounding *C. fumipennis* colonies at four sites in Central Maine. Sampling took place from mid-July to early October in 2013.

Site	**Host Tree**	**Style of Trap**	**Color**	**Lure Presence**
Skowhegan	White Pine	Cross Vein	Black	No
		Sticky Prism	Purple	No
		Lindgren Funnel	Black	No
			Green	No
			Green	Yes
			Purple	No
	Oak	Cross Vein	Black	No
		Sticky Prism	Purple	No
		Lindgren Funnel	Black	No
			Green	No
			Green	Yes
			Purple	No
China Dedham Veazie	Maple	Cross Vein	Black	No
		Sticky Prism	Purple	No
		Lindgren Funnel	Black	No
			Green	No
			Green	Yes
			Purple	No
	Oak	Cross Vein	Black	No
		Sticky Prism	Purple	No
		Lindgren Funnel	Black	No
			Green	No
			Green	Yes
			Purple	No

Results

A total of 156 nests were excavated during the 2013 and 2014 field season (Table 3.02). We identified 2,928 beetles and wings, representing 1,776.5 total buprestids found in excavated nests during the 2013 and 2014 field seasons (Table 4.02). Twenty different species were identified in nests, all of which have been previously described in Maine (Majka et al. 2011). We found 18 species across sites in 2014 and 15 in 2013. Seventeen species were collected from Skowhegan, 16 in Veazie, 9 in Dedham, and 6 in China. We found a positive trend with the number of species identified increasing with the number of beetles excavated from cells (sampling effort, Figure 4.01). We identified 913.5 of the beetles from excavated nests to species; 23% were *Chrysobothris sexsignata*, 22% were *Poecilonota cynapipes*, and 16% were *Dicerca caudata.* The most common genera excavated in 2013 and 2014 was *Dicerca* (41%) followed by *Chrysobothris* (21%).

Several species were found in low numbers or only at one site. Two specimens of *Brachys aerosus* were collected at the Skowhegan site in 2013. *Buprestis consularis* was only identified from the Skowhegan site in both 2013 and 2014. Three specimens of *Chrysobothris neopusilla* were found only in 2013 at the Veazie site. One specimen of *Dicerca tenebrosa* was collected at the Skowhegan site in 2014. Seven specimens of *Eupristocerus cogitans* were found at the Veazie site during 2014 sampling.

Table 4.02: Buprestidae species collected from *C. fumipennis* nests excavated in 2013 and 2014 from four sites in Central Maine. Identification was made to species when able. *Note China was only sampled in 2013.

Species	**China**		**Dedham**		**Skowhegan**		**Veazie**		**Total**	
	2013	**2014***	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
Agrilus anxius	3	-	0	7	21	9	16	7	40	23
Agrilus spp.	3	-	1	0	36	89	31.5	57.5	71.5	146.5
Brachys aerosus	0	-	0	0	2	0	0	0	2	0
Brachys ovatus	0	-	0	0	0	14	0	8	0	22
Brachys spp.	0	-	0	0	9.5	0	3.5	23	13	23
Buprestis consularis	0	-	0	0	1	2	0	0	1	2
Buprestis maculativentris	0	-	3.5	6	34	41	8.5	5	46	52
Buprestis striata	0	-	1	1	3	5	1	3	5	9
Buprestis spp.	0	-	0	0.5	0	0	0.5	0	0.5	0.5
Chrysobothris adelpha	0	-	0	0	0	14.5	0	1	0	15.5
Chrysobothris azurea	0	-	0	0	1	17	2	11	3	28
Chrysobothris denipes	0	-	0	0	1	3.5	1	0.5	2	4
Chrysobothris femorata	0	-	0	0	0	0	1	13	1	13
Chrysobothris harisi	0	-	0	0	0	3	0	4	0	7
Chrysobothris neopusilla	0	-	0	0	0	0	3	0	3	0
Chrysobothris sexsignata	0	-	0	1.5	18	162	0	25.5	18	189
Chrysobothris spp.	1.5	-	0	0	27.5	37	3	14	32	51
Dicerca caudata	3	-	0	3	1	12	6	7	10	22
Dicerca divaricata	24	-	8	13.5	21	20.5	35	23	88	57
Dicerca dumolini	2	-	1	1	8	19.5	0	1	11	21.5
Dicerca tenebrosa	0	-	0	0	0	1	0	0	0	1
Dicerca tuberculata	6.5	-	0	2	0	1	0	0	6.5	3
Dicerca spp.	50	-	84	61	68.5	166.5	18.5	60	221	287.5
Eupristocerus cogitans	0	-	0	0	0	0	0	7	0	7
Poecilonota cynapipes	2	-	0	17	4.5	106.5	18.5	52.5	25	176
Buprestidae spp.	1.5	-	0	3.5	7	4.5	0	0	8.5	8
Total	96.5	-	98.5	117	264	728.5	149	323	608	1168.5

There were no significant differences among sites in the proportion of *Buprestis, Brachys, Eupristocerus,* and *Poecilonota* excavated from nests (Figure 4.02). There was a significantly higher proportion of *Dicerca* identified in cells at China and Dedham (nominal logistic regression, $x^2_{(3)}$ =160.75, $P < 0.0001$, Figure 4.02). We also found significantly higher proportions of *Chrysobothris* and *Agrilus* at Skowhegan than in China and Dedham (nominal logistic regression, $x^2_{(3)}$ =160.75, $P < 0.0001$ and $x^2_{(3)}$ =59.19, $P < 0.0001$, Figure 4.02). *Dicerca* (representing the largest beetles in the family Buprestidae) make up almost 90% of the beetles collected at the Dedham site and 80% of those collected at the China site. Beetles excavated at the Skowhegan and Veazie sites are 30% Dicerca and 70% other smaller sized genera.

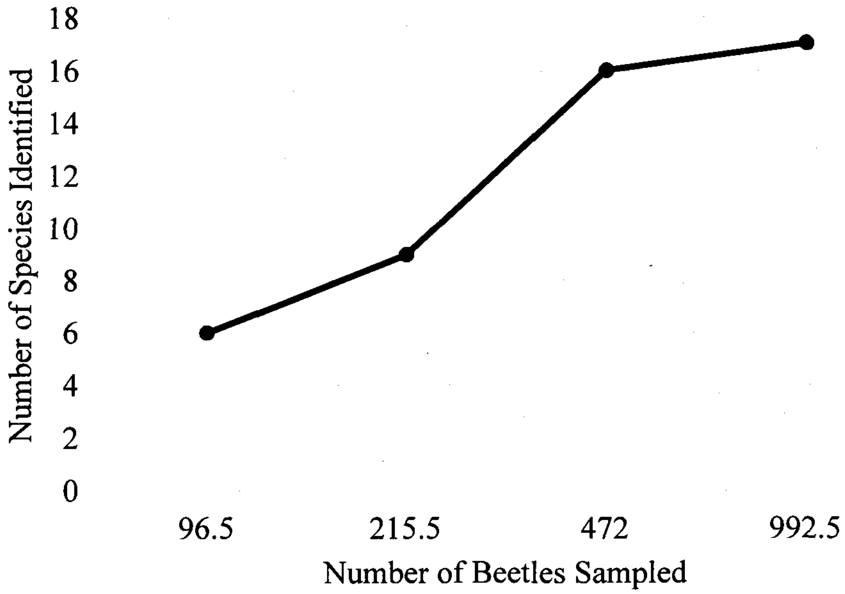

Figure 4.01: The number of beetles excavated at each of four sites (sampling effort) and the resulting number of species identified.

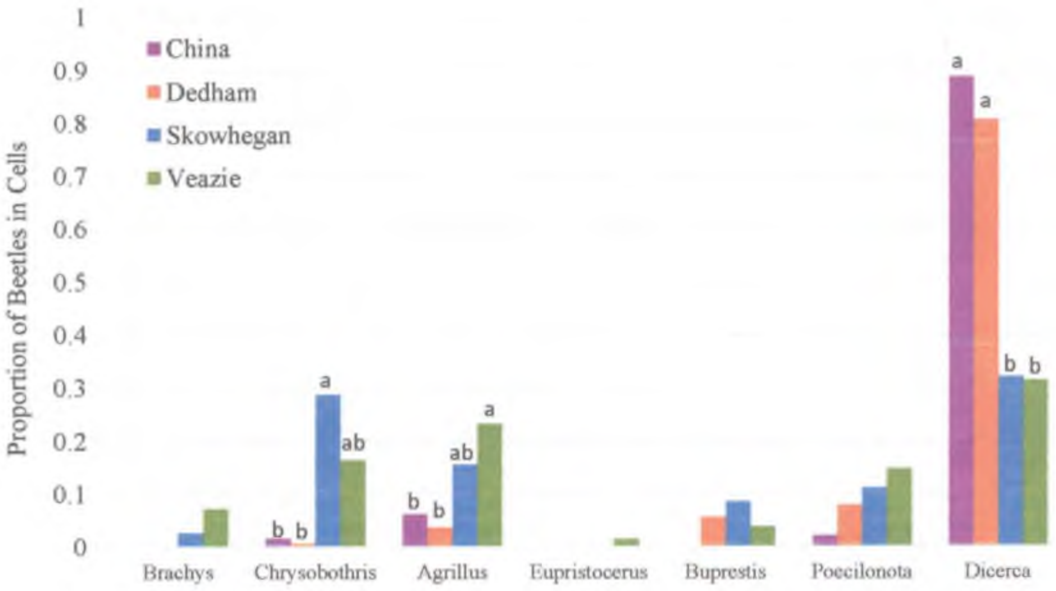

Figure 4.02: Proportion of beetle genera identified in cells of *C. fumipennis* excavated at four sites during the 2013 and 2014 field seasons. Different letters designate significant differences between proportions ($\alpha = 0.05$).

There was no difference in the proportion of completed cells provisioned with more than one genera of buprestids between years at each site (nominal logistic regression, $P > 0.05$) so data was pooled by site. A significantly higher proportion of cells contained more than one genera of beetle at Skowhegan and Veazie than at Dedham (nominal logistic regression, $x^2_{(3)} = 24.65$, $P < 0.0001$, Table 4.03).

Table 4.03: The proportion of completed *C. fumipennis* cells provisioned with more than one genera of buprestids at four sites in central Maine. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Site	**N**	**Proportion of completed cells provisioned with more than one genera of Buprestid (Proportion ± SE)**
China	17	0.24 ± 0.12 ab
Dedham	55	0.16 ± 0.07 b
Skowhegan	219	0.5 ± 0.03 a
Veazie	105	0.42 ± 0.05 a

Landscape survey of Buprestidae

Trapping in the landscape surrounding *C. fumipennis* colonies occurred from 17 July to 24 October at the Dedham site, 24 July to 10 October at the China site, 18 July to 8 October at the Veazie site and from 15 July to 5 October at the Skowhegan site in 2013. Over 7,000 insects were collected across sites during the sampling periods. Beetles comprised 1,829 of the specimens, 128 of which were members of the family Buprestidae. Four species of Buprestidae were collected. *Agrilus anxius* comprised 52%, *Brachys ovatus* 45%, *Chrysobothris adelpha* 1%, and *Dicerca divaricata* 2% of the Buprestidae caught in traps (Table 4.04). Nearly all the Buprestidae beetles (93%) were caught in the green funnel traps. However, the purple funnel traps collected the highest number of species; trapping three of the four species captured.

We found no differences in species presence-absence per trap type between sampling sites (GLM binomial distribution, $x^2 > 0.05$) so subsequent analyses were completed on data collected from the different trap types on each sample date. Buprestid beetles were caught significantly more often in green no lure funnel traps than the black funnel, purple prism, and black panel traps (GLM binomial distribution, $x^2_{(5)} = 60.48$, $P < 0.0001$). *Agrilus anxius* were caught significantly more often in green no lure funnel traps than the black funnel, purple prism, and black panel traps s (GLM binomial distribution, $x^2_{(5)} = 57.68$, $P < 0.0001$).

Table 4.04: Total and mean number beetles caught per trap type during the sampling period mid-July to early October 2013 at four sites in central Maine.

				Buprestidae		*Agrilus anxius*		*Brachys ovatus*		*Chrysobothris adelpha*		*Dicerca divaricata*	
Trap Type	**Color**	**Lure**	**Number of Traps**	**Total Catch**	**Per trap (mean ± SE)**	**Total Catch**	**Per trap (mean ± SE)**	**Total Catch**	**Per trap (mean ± SE)**	**Total Catch**	**Per trap (mean ± SE)**	**Total Catch**	**Per trap (mean ± SE)**
Cross Vein	Black	No	8	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0
Funnel	Black	No	8	1	0.13 ± 0.13	1	0.13 ± 0.13	0	0 ± 0	0	0 ± 0	0	0 ± 0
Funnel	Green	No	8	72	9.00 ± 3.41	37	4.62 ± 1.66	35	4.38 ± 1.82	0	0 ± 0	0	0 ± 0
Funnel	Green	Yes	8	47	5.88 ± 2.56	24	3.00 ± 1.38	23	2.22 ± 1.59	0	0 ± 0	0	0 ± 0
Funnel	Purple	No	8	8	1.00 ± 0.27	5	0.63 ± 0.18	0	0 ± 0	1	0.13 ± 0.13	2	0.25 ± 0.16
Sticky Prism	Purple	No	8	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0

Wasp size differences between sites

The Dedham wasps are significantly heavier than the wasps at Skowhegan, but not larger than those sampled from China and Veazie (ANOVA, Tukey's, N = 44, $F_{(3, 40)} = 3.83$, P = 0.017, Table 4.05).

Table 4.05: Mean weights of female *C. fumipennis* at four sites in central Maine collected during the 2014 field season. Only data from 2013 is presented for China. Means followed by the same letter are not significantly different (α = 0.05).

	N	**Weight of adult *C. fumipennis* (Mean ± SE)**
China	5	0.11 ± 0.01 ab
Dedham	8	0.12 ± 0.02 a
Skowhegan	15	0.08 ± 0.01 b
Veazie	16	0.08 ± 0.01 ab

Discussion

The species composition that we identified at the four sites sampled differed from a previous study conducted in 2010-2011 at six sites (including the four we sampled) in Central Maine. Virgilio (2012) found that the most commonly detected species of Buprestidae collected by *C. fumipennis* was *Dicerca divaricata*, representing 35% of the individuals collected over both years. In my sampling I found that the most common species collected from cells were *Chrysobothris sexsignata* (23%) followed by *Poecilonota cynapipes* (22%), and *Dicerca caudate* (16%). Virgilio's findings were further supported by Hellman (2010) in New York who's most commonly collected Buprestid in 2009 and 2010 was *D. divaricata* (36.8%).

One explanation for the differences between our findings and those of Hellman (2010) and Virgilio (2012) is that buprestid diversity or richness could change from year to year (Table 4.02). Another possible explanation for these differences is that both Hellman (2010) and Virgilio (2012) collected intact beetles from foraging wasps or intact beetles discarded around entrance holes, while the beetles that I identified came from below ground nests. The beetles from below ground were often partially consumed by larvae and had few parts remaining to aid in identification. Those beetles with distinctive, easy to identify wing patterns or coloration were most readily identified to species (typically found in *Chrysobothris* spp. and *Buprestis* spp.). Beetle prey identified using absent/consumed parts were usually lumped into genera identifications (typically *Dicerca* spp.). This bias towards the easier identified species (*Chrysobothris* spp. and *Buprestis* spp.) could bias conclusions about certain species over harder to identify species (*Dicerca* spp.) in our final composition calculations.

When we re-analyze Hellman (2010) and Virgilio's (2012) by combining species level data into generic level data, we observe differences between their buprestid composition and ours. Virgilio (2012) found that *Dicerca* composed 61.5%, *Buprestis* 18.0%, *Agrilus* 4.0% and *Chrysobothris* 4.0% of the beetles collected. Similarly, Hellman (2010) found that *Dicerca* comprised 55.9%, *Agrilus* 15.6%, *Buprestis* 10.8% and *Chrysobothris* 10.0% of the beetles collected. We found that *Dicerca* comprised 41%, *Chrysobothris* 21%, *Agrilus* 15.8%, *Poecilonota* 11.3%, *and Buprestis* 6.5% of the beetles collected.

Another possible explanation for the differences in composition identified, could be explained in methods that Hellman (2010) and Virgilio (2012) utilized for sampling. Collecting buprestids from foraging wasps and those discarded around entrance holes could potentially bias a sample to those beetles that are larger in size (typically *Dicerca and Buprestis spp.*). Female *C. fumipennis* search the surrounding landscape for beetle prey and upon locating one she will paralyze it and return to her nest with it grasped under her body (Evans 1971, Kurczewski and Miller 1984, Careless 2009). The beetle prey can be collected from the returning wasp two different ways, both of which can bias toward the collecting of large prey items. The first is using a sweep net. Wasps with large prey items are slower, often take several "resting/repositioning" stops, and are highly visible (Careless 2009, personal observation). All of these factors could make wasps with larger loads easier to capture. The second sampling technique developed by Careless (2009) involves placing a collar around the entrance hole of returning female that is large enough for her to enter the hole alone (without prey), causing her to abandon her prey by the entrance hole. I have observed that these collars are not completely effective in restricting the wasps from entering their tunnels with prey and smaller wasps with smaller prey items can often get through the collar without dropping her prey. Finally, in the case of discarded beetles, smaller sized beetles are potentially easier to miss when searching the ground for them. One thing to note is that the most common genera excavated in 2013 and 2014 was *Dicerca* (41%) followed by *Chrysobothris* (21%) which supports both of the explanations I provided above. I was unable to identify many of the *Dicerca* to species because they often do not have distinctive wing patterns and the second most common genera, *Chrysobothris,* include some of the smallest beetles in the family Buprestidae and appear in low numbers in Hellman (2010) and Virgilio (2012).

Skowhegan had the largest variety of species found in excavated cells and had more novel species present than the other sites. This is to be expected because more nests and prey were excavated at this site. One of the basic principles of ecology and community structure suggests that the number of species found while sampling increases in direct proportion to increased sampling because the probability of finding rare species increases (Ricklefs 2007). Virgilio (2012) also found that in 2010, the most beetles and the largest number of species were identified at the Skowhegan site and in 2011, the largest number of beetles were collected and identified from the Dedham site followed by the Skowhegan site (Virgilio 2012).

We found that female *C. fumipennis* wasps do not show fidelity in the prey that they provision for their young. Half of the cells at the Skowhegan site contained more than one genera of beetle prey. Dedham which had the highest level of prey fidelity in nests still had nearly 15% of the cells containing more than one genera. Several nests had 3 or 4 different species with in a single cell. We would expect that the taxonomic composition and abundance levels of Buprestidae availability of specific species would be variable among sites. Tree species composition of the surrounding landscape would strongly influence the buprestid populations in an area. Nine of the species we identified in *C. fumipennis* nests are considered softwood borers (*Buprestis maculativentris, B. striata, B. consularis, Chrysobothris dentipes, C. harisi, C. neopusilla, Dicerca dumolini, D. tenebrosa, D. tuberculata*), while the remaining eleven species can be found most commonly in hardwoods (Paiero et al. 2012). We would also expect a significant variation in abundance levels of different prey to vary within seasons (time of year) at sites. The capacity of *C. fumipennis* to hunt and utilize more than one prey species could be an important factor underlying the presence of wasp populations along the East Coast and throughout the Midwest of North America.

Taxonomic variation between sites is not surprising but the variation between nests, suggested by the distribution of less common prey items, is surprising. These differences could be a learned component in prey choice or it simply reflects the local abundance of certain prey at the time of nesting. Unfortunately, we were unsuccessful in our objective to identify the background Buprestidae population found in the landscape surrounding *C. fumipennis* colonies. We collected only four species across all the trap designs, tree species and sites. This is further evidence that we are not nearly as successful in our techniques for collecting member of the family Buprestidae as *C. fumipennis* female wasps are.

In females, a larger adult body size has been shown to result in more space for storing eggs, larger fat bodies for producing more eggs, greater food carrying capability in flight, and the ability to subdue larger prey (O'Neil 2001). We observed an interesting relationship between the relative size of different dominant genera of beetles excavated from cells at each site and the mean weight of the wasps at the corresponding sites. Those sites where the largest beetles make up the highest proportion of our sampled provisioned beetles yielded the largest sized wasps. The genera *Dicerca* contains the largest members of the family Buprestidae (Paiero et al. 2012) and we found a significantly higher proportion of *Dicerca* identified in cells at China and Dedham than at the other two sites (comprising approximately 90% and 80% respectively). Dedham and China also had the largest mean weights of wasps collected at their sites. Beetles excavated at the Skowhegan and Veazie sites consisted of up to 30% *Dicerca* and 70% other smaller genera and had smaller sized wasps.

As previously reported, female wasps provision their young with the same mean number of beetles across all sites sampled, but the weight of beetles that they provision in their cells is variable (Table 3.12, 3.13). There is also a positive relationship between the weight of provisioned prey per cell and the resulting size of pre-pupae (Figure 3.15). At the Dedham and China sites we found that the mean weight provisioned per cell was higher than at the other sites, the resulting pre-pupae were larger, and in this chapter we found that the adult wasps were also largest at these sites.

Polidori et al. (2005, 2010) studied several species of *Cerceris* predatory wasps and generally found that smaller individuals carry smaller prey, lowering the overall diversity of prey available to them. Our findings seem to contradict this. Out of the 20 species of beetles we collected in 2013 and 2014, the highest number of species collected at any site was seventeen species at Skowhegan. Skowhegan also had the smallest sized adults. However, these findings may be suspect because we did not have the same sampling effort at all the sites, and sampling effort influences the diversity of species recovered (Figure 4.01).

Many studies have shown a positive correlation between female size and their prey size in species of solitary wasps (Byers 1978, Kurczewski and Elliot 1978, Laing 1979, Cowen 1981, Gwynne and Dodson 1983). This has interesting implications for the *C. fumipennis* when considering the spread of EAB throughout North America. *Agrilus planipennis* is a relatively small member of the family Buprestidae. In EAB infested areas in Ontario, EAB has been found to make up to 86% of a wasp colony's daily capture (Careless 2009). The question then becomes, will the spread of EAB result in pushing *C. fumipennis* populations to become smaller sized over time?

The ability for *C. fumipennis* to utilize several different hosts as prey items is essential for the continued use of it as a biosurveillance organism. Other researchers have noted that *C. fumipennis* is able to detect populations of EAB at low densities, most recently finding EAB infestations in Connecticut, Illinois, and New York, while forest managers and nearby traps failed to detect the beetles (Rutledge et al. 2013). Besides EAB, there are several other potentially harmful buprestids that we can use *C. fumipennis* to detect. We identified two pest buprestids in the excavated nests of *C. fumipennis*. The Flat-headed appletree borer (*Chrysobothis femorata*) a pest of apple (*Malus* spp.) and stone fruit (*Prunus* spp.) and bronze birch borer (*Agrilus anxius*) is a pests of birches (*Betula* spp.) and poplars (*Populus* spp.) (Paiero et al. 2012).

It has been estimated that at least 10 exotic species of *Agrilus* (Coleoptera: Buprestidae) are established in the United States (Haack 2010). *Agrilus subrobustus* and *A. sulcicollis* are two species of concern. *Agrilus subrobustus* was first collected in Georgia in 2006 feeding on Mimosa trees, *Albizia julibrissin* (Hoebeke and Wheeler 2011). The European oak borer (*A. sulcicollis*) was first confirmed in Ontario in 2008 and Michigan and New York in 2009 and has been found infesting stressed oak trees in those areas (Haack et al. 2009). In both cases, little tree mortality has been attributed to these two *Agrilus* species, but the potential for future problems exists (Haack et al. 2009, Hoebeke Wheeler 2011).

Tracking native species that have broken out of their range is also a potential area of use for biosurveillance. The gold spotted oak borer, *Agrilus coxalis*, was recently discovered in California and is causing damage to oaks (Coleman and Seybold 2008a, 2008b). Native to Arizona and New Mexico, its introduction to other parts of the US outside its native range has been problematic. Finally, some buprestid species have been intentionally introduced, as in the case of, the St John's wort root borer, *A. hyperici*. *Agrilus hyperici* was introduced as a biological control agent of St. John's wort (*Hypericum perforatum*) and has had some success controlling the invasive weed (Nechols 1995). *Cerceris fumipennis* could potentially be used to monitor the spread of biological control introductions.

Cerceris fumipennis's ability to find and to utilize several different hosts as prey items and its ease of adaptation to alternate hosts makes this insect a good candidate for its use as a biosurveillance organism for many different areas of monitoring and research. Understanding the relationship between *C. fumipennis* and its native prey can aid in our overall understanding of how the introduction of invasive beetles in the family Buprestidae (EAB) could potentially affect populations of *C. fumipennis*.

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Jennifer Lund was born in White Plains, NY on September 13, 1978. She was raised in Brewster, NY and graduated from Brewster High School in 1996. She received a Bachelor of Science in Environmental and Forest Biology from the State University of New York College of Environmental Science and Forestry (SUNY-ESF) in 1999. She started a Master of Science program in 2000 at SUNY-ESF but never completed it. She worked as a quarantine technician at Cornell University from 2003 – 2004, where she maintained an Asian longhorned beetle (*Anoplophora glabripennis*) colony at a USDA quarantine facility. From 2004-2005 she worked on fungal pathogens infecting the Asian longhorned beetle as a research technician at Cornell University. In 2005, Jen started as a research assistant at the University of Arkansas where she examined novel management practices on tarnish plant bug (*Lygus lineolaris)* damage in cotton. In 2007, Jen moved to Maine and started a position with the Department of Inland Fish and Wildlife in Bangor sampling and identifying the invertebrate community of the salt marshes. She also spent time sampling vernal pools in southern Maine for marbled salamander populations and rare dragonflies. In 2008, she accepted a laboratory technician position at the University of Maine where she conducted research on the ecology and management of European fire ant (*Myrmica rubra)* and examined honeybee health. Jennifer entered the School of Biology and Ecology Master of Science program at the University of Maine in 2012 where her research focused on the ecology and development of the predatory wasp *Cerceris fumipennis*. Jennifer is a candidate for the Master of Science degree in Entomology from the University of Maine in December 2015.