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NEST PROVISIONING AND HOMING BEHAVIOR OF CERCERIS FUMIPENNIS (HYMENOPTERA, CRABRONIDAE): A USEFUL TOOL IN THE BIOSURVEILLANCE OF BUPRESTID BEETLES

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ABSTRACT

NEST PROVISIONING AND HOMING BEHAVIOR OF *CERCERIS FUMIPENNIS* (HYMENOPTERA, CRABRONIDAE): A USEFUL TOOL IN THE BIOSURVEILLANCE OF BUPRESTID BEETLES

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Cerceris fumipennis (Hymenoptera, Crabronidae) is a ground-dwelling wasp that provisions its nest with woodboring jewel beetles (Buprestidae), making it a useful tool in biosurveillance of forest pests. In particular, *C. fumipennis* aggregations have been used for monitoring the invasive Emerald Ash Borer (*Agrilus planipennis* (Coleoptera, Buprestidae)) and by using this biosurveillance technique researchers have tracked the spread of this pest into new states and provinces. However, despite its success as a biosurveillance tool, information about much of the biology of *Cerceris fumipennis* is lacking. This study is focused on the biology and life history of *C. fumipennis* to better understand its use in long-term pest monitoring, in particular the nest provisioning and homing behaviors of this species are examined.

The nests of *Cerceris fumipennis* were observed through the summers of 2016 and 2017. Nest creation and abandonment was recorded and analyzed, plus nests were also excavated and the contents of each nest was examined to determine the maternal investment and prey constancy of each wasp. Maternal investment was divided into three categories: number of young, the number of beetles brought back to the nest, and the biomass of these beetles. Each of these three categories was determined for each cell and for each nest. Nests that had regular human

interference and nests that had been left undisturbed throughout the summer had similar rates of maternal investment in each of the three categories. The constancy of prey species selection by each wasp was examined by looking at the species composition of the prey from each nest.

The homing range of the wasps was studied by releasing *Cerceris fumipennis* from three distances from their nests: 0.2 km, 0.4 km, and 1 km. The wasps returned from all three distances, with a significant difference in the average time that it took for wasps to return from 0.2 km and 1 km. This suggests that 1 km is well within the homing range of *C. fumipennis*. Determination of the homing range of the wasp is an initial step in determination of their hunting range, and produce information on their flight behavior.

Chapter 1: Introduction

Background

Cerceris fumipennis Say is a solitary ground-dwelling wasp of the family Crabronidae; subfamily Philanthinae. It is native to North America, ranging east of the Rocky Mountains and north of the Mexican border into the southern Canadian provinces of Ontario and Quebec (Map 1) (Scullen, 1965; Evans, 1971). It nests in disturbed areas with low amounts of vegetation surrounded by trees or bushes. The wasp prefers hard-packed sandy soils where it will form a nest consisting of one opening that leads down to a variable number of cells (Evans, 1971; Kurczewski and Miller, 1984).

Map 1. A *map* of the range of *C. fumipennis.* The dark grey area indicates known captures of the wasp, while the dashed lines represent the suspected range. (Careless, 2009)

Cerceris fumipennis females average 15 mm in length, and males are about 10 mm, and have distinctive markings that can be used for species identification (Figure 1). Both sexes are mostly black with a single broad yellow band on the second tergite of the abdomen, and have three yellow markings on the thorax. Female wasps have three yellow square markings on the frons between the eyes and above the clypeus. The male has only two yellow markings on the frons between the eyes and above the clypeus (Scullen, 1965). The species also has dark blue/black wings that have led to its common name, the “Smokey-Winged Beetle Bandit” (Careless, 2008).

Figure 1: On the left is a female *C. fumipennis* with identifying facial marks. The image on the right shows the yellow body marking and dark wings of *C. fumipennis*.

The seasonality of *C. fumipennis* changes throughout its range. In the Northeast region the wasp emerges as an adult in late June or early July. Males and females mate for one or two weeks, after which the males die (Mueller et al., 1992; Careless, 2009). The females will then spend the rest of the summer provisioning their nests and laying eggs. The adult female wasps die from late August to early September.

Cerceris fumipennis specializes in hunting beetles of the family Buprestidae. The female wasp finds and then paralyzes the beetle. She then carries it back to her nest where she will place it in a cell by itself or with other beetles (Scullen & Wold, 1969). She lays one egg on top of the mesosternum of one of the paralyzed beetles. The egg hatches and the larva feeds on the buprestids. The larva later becomes a prepupa in the cell and overwinters buried beneath the soil. It pupates in the spring, and remains there until it emerges as an adult in late June or early July (Kurczewski and Miller 1984; Careless, 2009; Rutledge et al., 2015).

Nesting

While *Cerceris fumipennis* will nest in a variety of substrates, it prefers hard-packed sandy soils in flat areas with low amounts of vegetation that are surrounded by trees or bushes which provide a source of buprestids (Evans, 1971; Kurczewski and Miller, 1984). Consequently, it often nests in areas of anthropogenic disturbance where vegetation has been removed and sand has been added, for example in baseball diamonds or sandy parking lots (Nalepa et al., 2012).

The nest entrance is surrounded by a small tumulus 2-6 cm in circumference. Often there are discarded buprestid beetles lying nearby that researchers have termed "drops" (Careless, 2009; Grossbeck, 1912). The nest consists of cells 10cm to 20cm deep, which the female wasp fills with varying number of beetles (Evans, 1971; Kurczewski and Miller, 1984) (Figure 2).

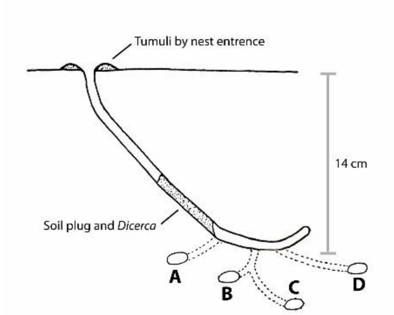


Figure 2. Drawing of a *C. fumipennis* nest. (Careless, 2009)

The wasps often aggregate in an ideal nesting habitat. Clusters of up to 500 nests can be formed in an area for a few years before the area is abandoned (Careless, 2009). Despite living in large clusters, *C. fumipennis* is a solitary wasp, though females have been observed to share nests. However the only observations of this behavior were during the first two weeks of adult activity, and nest sharing lasted only 24 hours (Kurczewski and Miller, 1984; Mueller et al., 1992).

During the first two weeks after emergence, nest abandonment and nest usurpation are common. Larger females will usurp nests from smaller females while the latter are out hunting (Mueller et al., 1992). After the first two weeks during which the wasps mate and establish nests, nest fidelity increases. Factors that increase nest fidelity include higher soil temperature and higher moisture levels (Careless, 2008, 2009).

After the mating period, *C. fumipennis* females will begin provisioning their nests. Female wasps returning with a buprestid beetle circle the nest to deter parasites and kleptoparasites that are often following (Mueller et al., 1992). After entering the nest the wasp will lay an egg on the mesosternum of the one of the beetles and backfill the cell. Keeping the nest backfilled is another protective measure against invasion by parasites while the female is out hunting (Kurczewski and Miller, 1984; Mueller et al., 1992).

Hunting

The hunting season for *C. fumipennis* varies based on their geographic location, and generally starts after a week of mating during the summer (Mueller et al., 1992). The wasps emerge in late morning around 9:00-11:00 am and fly into nearby wooded areas where they may forage for 45 min to 4 hours. During this time it will both hunt for buprestid beetles and feed on nectar from flowers. After locating a beetle, the wasp will paralyze the beetle by inserting its ovipositor into the metacoxal joint. Paralyzed prey are then carried back to the nest by clamping the beetles with its mandibles and holding it with its forelegs and midlegs (Careless, 2009). Often, smaller species of buprestid beetles do not get paralyzed, such as with members of the genus *Agrilus*. These smaller species are carried utilizing a special abdominal clamp, in addition to clasping the beetle with the mandibles and legs of the wasp. The clamp is located on the fifth metasomal sternite of the abdomen, and is termed the “buprestid clamp” (Nalepa, 2015). Carrying the beetle in this way often gives the wasps a J-shape in flights (Nalepa, 2015).

Cerceris fumipennis is restricted in its prey preferences by two factors, chemical cues and the size of the beetle. It has been shown in laboratory experiments that the wasps react positively to a class of chemical cuticular hydrocarbons (CHCs) produced by buprestids. CHCs are a group of chemicals with five different classes, and are common to wood and leaf boring beetles (Rutledge, 2014). The buprestid CHCs trigger stinging and consequent paralyzation of the buprestids. Buprestids that have been washed with a solvent to remove CHCs did not elicit this behavior. This could explain why even though *C. fumipennis* hunts mainly buprestids, it occasionally brings back species of other leaf-feeding or wood-boring beetles in New England, such as *Neochalmisus bebbianae* Brown (family Chrysomelidae) (Rutledge et al., 2011), that are presumed to share the same CHCs.

Body size of *C. fumipennis* also dictates its prey preference. The species can carry a range of beetles from 4.1-18.9 mm in length, with the wasps preferring larger species of buprestids. However, smaller wasps are limited to carrying smaller beetles, while larger individuals consistently carry larger beetles (Hellman and Fierke, 2014).

When *C. fumipennis* hunts it can be gone from the nest for 45 minutes to a few hours before it comes back with a beetle (Careless et al., 2013). However, relatively little is known about its activity during this time. Questions about *C. fumipennis* during this period focus on its flight and foraging behavior, 1) how far do they go to forage, 2) how is the flight path chosen, and 3) how do they locate buprestids in trees. Proposed limits of the foraging range of the wasp vary in the distances suggested. In one catch-and-release study by Careless (2008), two out of eight wasps returned to their nest from 2 km, while all returned from 1 km. Careless (2008) hypothesized that 2 km was the maximum foraging range for this species, and this result has been restated in a number of papers, though not retested (Swink et al., 2013; Nalepa et al., 2013, Careless, et al., 2014). A more conservative study calculated foraging range by estimating how far the wasp needed to fly from the nest to find a tree infested with the buprestids that the wasp then brought back (Nalepa et al., 2013). This study suggests that while wasps can fly further, most hunting occurs within 200 m of the nesting area (Nalepa et al., 2013).

The Value in Studying This Species

Biosurveillance is a term recently coined by ecologists to describe the method of using animals as tracking measures for a variable in the environment (Careless, 2009). Biosurveillance can be used to measure both biotic and abiotic variables. *Cerceris fumipennis* has been used over the past ten years as a biosurveillance tool for monitoring for the presence and abundance of buprestid species, including finding pest species of beetles newly introduced to North American forests, and in assessing the overall species composition of buprestids in forest areas near nesting sites.

Cerceris fumipennis is an ideal species for use in biosurveillance of buprestids for several reasons. First, *C. fumipennis* hunts buprestid beetles almost exclusively. Second, its provisioning behavior and nest fidelity easily allows researchers and volunteers to collect the buprestids. Third, since nests often occur in areas of anthropogenic disturbance, they are easy to find and access. Fourth, it is an easy wasp to identify. Its pattern of yellow and black markings distinguishes it from other ground nesting wasp species, so volunteers can quickly learn to recognize the species. Finally, this species does not sting humans (Careless et al., 2014).

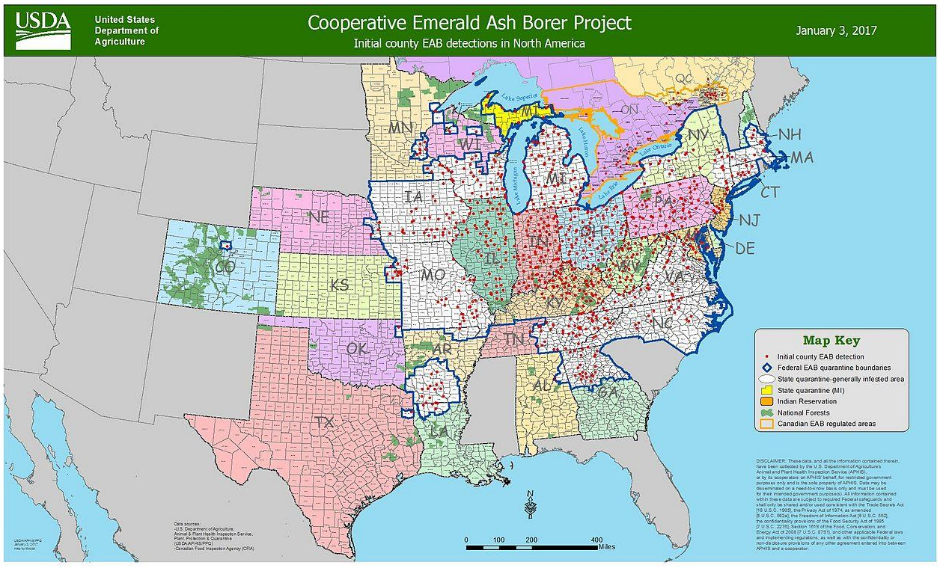
The first use of *C. fumipennis* as a biosurveillance tool was documented by Marshall et al. (2005). By monitoring nests they found three buprestid species new to eastern Ontario (Marshall et al., 2005). Subsequent studies have confirmed the effectiveness of collecting beetles from the wasps to assess beetle composition in the surrounding forest. When compared to museum records of buprestid species for a given area, *C. fumipennis* aggregations brought back a similar species diversity and were more effective at returning with buprestid beetles characterized by small population sizes (Hellman and Fierke, 2014). Species in the family Buprestidae are hard to find and collect for all but the most experienced entomologists. Traditional methods of collecting include sweeping or beating vegetation, the use of sticky purple or green prism traps, and inspection of trees. Surveillance at *C. fumipennis* nests has been shown to find a higher diversity of buprestid species and a larger number of buprestids than by way of these traditional methods during the hunting season of these wasps (Nalepa and Swink, 2015), and with a minimal investment of effort. It has therefore been suggested that *C.*

fumipennis be used to monitor the spread of buprestid pest species, in particular for the invasive Emerald Ash Borer (*Agrilus planipennis* Fairmaire) (Marshall et al., 2005; Careless et al., 2013).

Emerald Ash Borer (*Agrilus planipennis*)

The Emerald Ash Borer is native to eastern Asia (China, Japan, Taiwan, and Russia). In its original range, the Emerald Ash Borer is considered a minor pest to the native ash trees (*Fraxinus chinensis* var *chinensis* Roxburgh, *F. chinesis* var *rhynchopylla* (Hane) Murray, *F. mandshurica* Ruprecht, *F. mandshurica* var *japonica* Maxium). Emerald Ash Borer was first discovered in the United States in Michigan in 2002 (Bauer et al., 2003; Haack, 2002). It is believed that Emerald Ash Borer came to the United States 10 years earlier in wooden crates; however, a population was not established until 2001, and trees did not start dying from their attacks until 2003 (Siegert et al., 2007; McCullough et al., 2009). In the US Emerald Ash Borer attacks native White Ash (*F. americana* Linnaeus), Green Ash (*F. pennsylvanica* Marshall), and Black Ash (*F. nigra* Marshall) (Haack, 2002). However, there is some fear that it could attack species in other tree genera. Outside of it native range it has become a major forest pest in North America, and has spread to 29 states and two Canadian provinces since its introduction (USDA, 2017), killing millions of ash trees (Map 2) (Herms and McCullough, 2014).

The life cycle of Emerald Ash Borer starts when the female lays an egg on the bark of an ash tree. After hatching the larva tunnels into the tree to the cambial region where it feeds, eventually girdling the tree. There are four distinct larva instars. The last instar will move from the cambial region to below the outer bark of the tree where it will create a pupation chamber. In the pupation chamber it overwinters as a prepupa in diapause. It becomes a pupa in late spring and ecloses in early summer. Adults chew through the tree bark to emerge, leaving distinct D-shaped holes, and live for two to three weeks (Haack, 2002; Bauer et al., 2003). As adults the beetles feed on ash leaves and mate near the trees, and later the females lay 68-90 eggs (Haack, 2002; Bauer et al., 2003).



Map 2. A map of the current distribution of Emerald Ash Borer in the United State and Canada by county. (USDA, 2017)

The Emerald Ash Borer causes ecological, economic, and cultural damage. The forest ecosystems in many states and provinces have been severely impacted by ash tree death caused by Emerald Ash Borer, as ash is one of the most widespread tree genera in North America. Their death affects the nutrient cycle and topography of forests leaving physical gaps for invasive species (Herms and McCullough, 2014). Ash deaths also affect the many arthropods that feed on them, including 43 native species of arthropods, all of which are specialists that are at risk of extinction if ash trees are extirpated (Gandhi and Herms, 2010).

Much of the economic burden generated by Emerald Ash Borer comes from the removal and replacement of trees (Gandhi and Herms, 2010). Ash is one of the most popular trees planted in the Midwest, and removal and replacement of ash trees is estimated to cost between $10.7 and $12.5 billion by 2020 (Kovacs et. al, 2009). There is additional economic loss from the death of nursery and commercial trees (Herms and McCullough, 2014).

Human residents of areas with high ash tree mortality rates also suffer. There is a correlation between higher respiratory and cardiovascular disease rates and tree loss in urban areas (Donovan et al., 2013). The ash tree is also culturally significant among some Native American tribes that use black ash in basket weaving, which is both a valued cultural practice and source of income, particularly among the Abenaki Nations of New England and eastern Canada (Herms and McCullough, 2014).

Due to the widespread damage caused by Emerald Ash Borer, it is important to track spread of the infestation. It is especially important to look for the beetle in areas where it has not been previously recorded so that quarantine measures may be initiated, because damage to the trees is not visible for at least three years (Bauer et al., 2003). Quarantines slow spread of the species by stopping movement of infested wood into new areas, and may include tree removal, and chemical control for protection of locally important trees. The two standard methods of monitoring for appearance of Emerald Ash Borer in new areas are: 1) inspection of bait trees, and 2) use of traps. Bait trees can be used to increase the chances of finding Emerald Ash Borer outside of known infestation areas. Bait trees are girdled, which produces damage that attracts female beetles looking for oviposition sites. When the bark is stripping away from the dying trees later in the year it makes the search for larvae much easier (Francese et al., 2008; Herms and McCullough, 2014). Trap monitoring of Emerald Ash Borer has mainly used differently colored prism traps. These traps have three large panes that have a chemical lure in a container hung on the inside. The panes are covered with a sticky substance to which the beetles adhere upon contact (Francese et al., 2010). The most effective prism traps are purple on the top half and green on the bottom half, and work best when hung in the forest canopy (Poland et. al, 2011). However, these methods have been shown to be less efficient at early detection than is biosurveillance using *C. fumipennis* (Nalepa and Swink, 2015).

Because *Cerceris fumipennis* can find buprestid beetles even at low abundances, it was first used to monitor Emerald Ash Borer in 2005 (Marshall et al., 2005). Since then many states have started Wasp Watcher Programs that use volunteers and researchers to monitor *C. fumipennis* aggregations for the appearance of Emerald Ash Borer. These programs have had success in tracking the spread of the beetles; Emerald Ash Borer was first detected in Connecticut using this wasp (Rutledge et al., 2013). The expansion of biosurveillance programs and further understanding of *C. fumipennis* behavior will lead to effective early detection of the appearance of Emerald Ash Borer.

Objectives

The objectives of this project are to lead to a better understanding of *Cerceris fumipennis* behavior that will increase its usefulness as a biosurveillance tool.

Objective 1: Determine the pattern of nest building and abandonment during a season.

Objective 2: Determine the maternal investment of an individual wasp through the summer.

Objective 3: Determine the constancy of beetle prey species for an individual wasp.

Objective 4: Determine the homing range of this species.

Objectives 1-3 are treated in Chapter 2. Objective 4 is covered in Chapter 3.

Chapter 2: Nest Provisioning of *Cerceris fumipennis*

Introduction

Cerceris fumipennis nest structure has been studied by several authors using nest excavations and observations. These studies show that *C. fumipennis* nests in sandy, hard-packed soil in disturbed areas with low amounts of vegetation, often surrounded by trees or bushes (Grossbeck, 1912; Rau, 1922; Evans, 1971; Evans and Rubink, 1978; Kurczewski and Miller, 1984; Hook and Evans, 1991; Marshall et al., 2005). The wasps are solitary nesters, though there have been some observations of two wasps in a single nest for short periods of time (Kurczewski and Miller, 1984; Mueller et al., 1992). The nesting areas are often in areas of anthropogenic disturbance, which is useful for biosurveillance efforts as these areas are easy to find and access (Nalepa et al., 2012; Careless et al., 2013).

The entrance to the nest of *C. fumipennis* is surrounded by a tumulus that is 2-6 cm in circumference and 1-2 cm high. The entrance hole varies from 0.4-0.7 cm wide, based on the body size of the wasp (Careless, 2009), though openings of nearly twice this size (1.2 cm) are commonly observed in New Hampshire late in the season. The wasp uses its mandibles and foretibiae to dig a tunnel that ranges from 10-20 cm deep (Evans, 1971; Kurczewski and Miller, 1984; Careless, 2009). Researchers hypothesize that new nests are built from the emergence tunnel produced by the wasp after eclosion from the pupal stage (Field, 1992; Careless, 2009). The female wasps excavate new cells one cell at a time. She will fill the cell with paralyzed buprestid beetles, and once the cell is full she will lay one egg on the mesosternum of one of the beetles in the cell. Once a cell is filled with beetles, it is backfilled with dirt, and the wasp will then start digging a new cell. Previous studies have found a range of 5-24 cells per nest (Evans, 1971; Evans and Rubink, 1978; Kurczewski and Miller, 1984; Hook and Evans, 1991; Mueller et al., 1992).

Kleptoparasites are common around *C. fumipennis* aggregations. Wasps in the family Mutillidae (velvet ants), and many species of Diptera in the family Sarcophagidae (miltogrammine flies), will try to lay eggs on beetles collected by wasps by entering the nests and trying to invade the cells (Evans, 1971; Spofford et al., 1988; Spofford and Kurczewski, 1992; Careless et al., 2013). By backfilling the cells, a wasp can try to keep these parasites out while she is away and cannot guard the nest. The kleptoparasites sometimes succeed and their larvae are often found in some cells of the wasp (Hook and Evans, 1991; Careless, 2009; Careless et al., 2013).

Within the nest the larvae emerge from the egg and feed on the paralyzed beetles. The larvae eat the internal tissues of the beetles and leave the hard exoskeleton behind (Evans, 1963; Careless, 2009). The undigested exoskeletons of the buprestids are helpful in determination of the contents of each cell upon excavating a nest. The elytra and pronota of the beetles can often be pieced together to determine the beetle species found within the nest (Evans, 1971; Lund, 2015). The last larval instar becomes a prepupa, and the individual diapauses in this stage over the winter, pupating when it gets warmer in early spring (Careless, 2009). It emerges as an adult in New Hampshire in late June-early July, when there is a soil degree-day accumulation of 696.2 ± 16.8 degrees C (Rutledge et al., 2015).

In areas that are good sites for nesting, the wasps will cluster in groups that can range from 5-500 nests (Careless, 2009), with the nests often being very close together. Evans (1971) described the nests situated in a cluster as being between 4-15 cm apart. It has been hypothesized that aggregations with tight clusters are healthy colonies because nests that are closer together have a higher degree of relatedness, or philopatry. It is thought that tightly clustered holes are a result of sister wasps creating nests near the nest of the original mother (Careless, 2009), but no studies have been developed to confirm this.

Studying *C. fumipennis* nesting behavior can be difficult because of these many behaviors. The proximity of the nests and the fact that they backfill cells as they are filled with prey makes it hard to determine the true size of the nest unless the excavated nest is a significant distance away from its neighbors, so that it is not geographically interdigitated with other nests. In this study technique was developed to determine the maximum size range of nests, which was critical in choosing nests that did not overlap when excavated.

Objectives 1-3 for this portion of the study can be proposed as three main goals surrounding nesting provisioning: 1) determination of the above-ground activity of the wasps in terms of nest creation and abandonment; 2) determination of the maternal investment of a single wasp throughout the summer; along with determination if a summer-long monitoring program, involving high human interaction with the wasp adversely affects wasp maternal investment; 3) determination of the prey constancy of individuals.

<u>Above ground activity of the nest</u>

Nests may be created throughout the summer; however they are usually formed shortly after emergence within two weeks of the being of the summer during the nest-founding phase (Mueller et al., 1992). In warmer states the first nest entrance is thought to be the same tunnel as that was dug by the mother, whereas in colder states the mother's tunnel is destroyed and the new wasps will dig a new hole to the surface (Mueller et al., 1992; Careless, 2009). The creation and abandonment of nests examined after the first two weeks of emergence. During this time there are high rates of nest abandonment, nest creation, and nest usurpation. Activity after the nest-founding phase is thought to slow down, and wasps remain with a single nest longer

(Mueller et al., 1992). The length of time a wasp remains in a nest is considered critical in establishing nest-fidelity. Careless (2008) studied nest-fidelity in Ontario and found that a wasp stayed faithful to a nest anywhere from 1-48 days. This study examines nest founding and abandonment longitudinally throughout the reproductive season to quantify rates of each, and their correlation with seasonality.

Maternal investment

Few studies examine maternal investment of individuals *C. fumipennis*. Most studies are focused on the usefulness in biosurveillance of the total aggregation. Usually it is in the context of the total number of buprestid species an aggregation will bring back in a hunting summer (Careless et al., 2013; Hellman and Fierke, 2014; Napela, 2015). This maternal investment is determined and analyzed as three distinct groups: 1) the number of wasp young, 2) number of beetles taken into the nest, and 3) the amount of beetle biomass in the nest. This study examined at both beetle numbers and biomass as two dependent variables in producing young because *C. fumipennis* brings back buprestid beetles that range in size from 4-19 mm (Hellman and Fierke, 2014). The final goal of understanding maternal investment was to determine how many individual beetles were in the nests, and how much beetle biomass was in each nest or cell. These data were analyzed at independently due to the size variation exhibited by the different buprestid species.

Comparison of monitored to undisturbed nests

There are two methods of biosurveillance for Emerald Ash Borer using *C. fumipennis* aggregations. The first is active monitoring, which involves interference with the wasps by taking their beetles from them when they return to their nest (Careless, 2009). This may be done in different ways, including catching them with nets (Careless, 2009), or placing some sort of block over their nest (Careless, 2009). Currently there are two types of blocks that researchers and foresters use; a clear cup with a screen over the nest, or an excluder made from a piece of paper with a hole cut in it that is large enough for the wasp to pass through as it exits, but prevents entry of both wasp and beetle, with the wasp dropping the beetle when entrance is denied (Careless, 2009). The cup method is preferred by researchers who want to know when the wasp is exiting or entering the nest. If using the cup method there must be a vent in the cup or the temperature in the plastic cup can rise rapidly and kill the wasp (Careless, 2009). The second method is a passive approach in which beetles are located by looking for paralyzed "drops", beetles. For example, *C. fumipennis* often drop their beetle on the ground when they are attacked by kleptoparasitic flies (Careless, 2009). In observational studies, human interaction with the wasps has not changed their behavior in terms of increasing nest abandonment or prey diversity (Evans and Hook, 1982; Hook, 1987; McCorquodale, 1989; Alexander and Asis, 1997). However, since there has not been a study to examine the maternal investment of a wasp, there has not been a study to determine if human interaction affects the wasp's hunting behavior and reproductive output. This study aims to compare the maternal investment of nests, with some having been actively monitored with consequent high levels of direct human interaction throughout the summer, in contrast to nests that have been left undisturbed through the adult period of activity.

Determination of the constancy of prey beetle species

Previous studies investigating prey composition of *C. fumipennis* have focused on the diversity of prey species brought back by a whole aggregation of wasps. These studies have found that the greatest diversity in species is obtained when both active (taking beetles from wasps coming back to the nest) and passive methods (looking for drops) are utilized (Careless, 2009; Careless et al., 2014). The wasps bring back a high diversity of Buprestidae that match museum records from surrounding areas, and with a similar diversity to collections produced by purple prism traps, which are often used to monitor Buprestidae (Careless et al., 2014; Hellman and Fierke, 2014; Nalepa, 2015). Hellman and Fierke (2014) documented that *C. fumipennis* has a preference for large species of buprestids sized 5.0-5.8 mm, but this factor co-varied with prey choice and hunting success. The goal of this portion of the study is to determine there is constancy in prey selection. Do the wasps as a species or individual have a preference in the species they hunt? If so, they should be constant in their prey choice.

Methods

Field Site Characterization

Field studies were conducted in the summers of 2015 and 2016 in the towns of Epsom and Boscawen, NH. These locations were chosen because they held two of the largest aggregations of *Cerceris fumipennis* known in New Hampshire, and are the sites used for earlier studies of the wasp. Both sites are in Merrimack County, which is the county where Emerald Ash Borer was first recorded in the state.

The Epsom site is the parking lot of American Legion Post # 112 along Short Falls Road; (43.20226, -71.38483). The flat parking lot is on the west side of the American Legion building and forms a 575 m^2 rectangle. The surface of the lot is compacted coarse sand. Besides the open east side of the lot, which faces the American Legion Hall, the other margins of the lot are lined with trees consisting primarily of Red Pine (*Pinus resinosa)* on the west and south sides. In 2014, red pines on the west row side were removed by the city of Epsom from the American Legion parking lot to a pavilion owned by the state (Tibbets, 2014). This decreased the number of dead/dying tree resources near the nesting site the year before this project was initiated.

However, despite this loss of trees there is still a dense forest the south edge. On the north edge, there is a line of trees between the parking lot and Short Falls Road with the forest continuing north on the other side of the road. The forest itself is a mixture of deciduous and coniferous tree species, with the dominant trees being 34% maple species (Dube and Chandler, 2017). At this site the majority of the wasp nests are clustered at the north edge of the lot and extend south halfway across the parking area (Figure 3).

The Boscawen wasp site is along the northern perimeter of the New Hampshire State Forest Nursery, situated at 405 Daniel Webster Hwy; (43.37196, -71.65719). The nursery is a 103,656 m^2 field, with over 50 species of planted trees. The trees are 1-4 years old, and are planted in sandy and compact soil, with this e large field surrounded by a forest of coniferous and deciduous composition. The two dominant trees are Eastern Hemlock (39%) and American Beech (22%) (Dube and Chandler, 2017). The Daniel Webster Hwy is 600 m to the east of the field. *Cerceris fumipennis* originally colonized the north edge of the field, where there is a compacted road that is not plowed for planting (Figure 4). While this remains the largest grouping of *C. fumipennis* at the site, in 2016 wasps were seen at other areas of the nursery that were not regularly plowed. During weeks of peak activity there were 167 nests across the entire field. Only ground nests near the north edge were studied, which formed the densest part of the aggregation.

<u>Nest above ground activity</u>

The nesting surface area was measured on June 6th, 2016 before the wasps emerged for the summer. A meter grid map was created for each of the sites. For Epsom, this area was a total of 113 m^2, and for Boscawen it was total of 195 m^2. The total number of square-meter plots was entered into a random number generator, and 10 numbers were chosen for each site to represent the 10 randomly chosen meters^2 within the nest area (Figure 5). These were the areas that were monitored throughout the summer for nest activity.

The 10 meter-square plots chosen for the study were marked at each of their corners with an orange golf tee, so that the perimeter of the square could be easily seen throughout the summer. At each northeast corner a golf tee with piece of flagging was marked with the number of the square.

The squares were observed for nest activity once the wasps emerged. At Epsom the first nest activity was observed on June 24, 2016. At Boscawen the wasps emerged a week later, and the first observations of activity was on July 1, 2016. Starting with the initial emergences the squares were checked for nest activity once a week until the wasps ceased hunting season, and the majority of the wasps in the colony were either dead or inactive.

For each observation period the number of new *Cerceris fumipennis* nests were counted. New nests were marked with a white golf tee, so they could be recognized as "old" nests for future weeks. The number of abandoned nests was also recorded during each observation period. Abandoned nests were recognized by being flattened and not opened by 1 PM, filled with debris, or infiltrated by other species of insects, usually ants.

Summer-long monitoring *C. fumipennis* hunting behavior

Monitoring the wasps started two weeks after emergence, which corresponded with the end of the nest-founding phase, and continued twice per week for five weeks until hunting had ceased. In 2015, ten nests at each site were monitored. Due to the low numbers of returns with beetles in 2015, the number of nests being monitored was increased to 20 nests per site in 2016. The nests were chosen on the first day of monitoring, and were selected from a large cluster so that they could all be easily monitored at the same time. Cups were placed over more than 20 nests, and the first 20 wasps to emerge were monitored for the summer. Nests were given a number and marked with a golf tee and short piece of colored flagging tape. On the first day of monitoring when the wasps from these nests emerged, they were painted with a unique color combination of dots with Craft Smart© Outdoor Acrylic Paint and released.

Each site was monitored twice a week for five weeks on days without rain, with the goal of monitoring being the capture of all beetles brought back to specific nest during the day. Monitoring started before the wasps emerged around 9:00 AM. A clear plastic cup with a pair of mesh windows was put over a nest. When a wasp emerged to leave its nest, it would fly around within the cup and was promptly let out of the cup, and the time it left was recorded. The nest was then covered with a cup again, and when the wasp returned it was let back into the nest if it had no beetle, and the time was recorded. If the returning wasp had a beetle, it was caught in a net and the beetle was taken, the time recorded, and the wasp being then released back into its nest area. Monitoring ended at 5:00 PM when most of the wasps had returned to their nests (Virgilio, 2012).

The beetles collected were air dried for one week. Dry mass was recorded and body lengths measured, followed by rehydration and pinning. Beetles were identified using the keys and figures from Bright (1987) and Paiero et al. (2012), and confirmed by comparison with specimens in the UNH Insect and Arthropod Collection.

Nest excavations

Nest excavation began when the hunting season for the wasps concluded during the third week of August in both 2015 and 2016. Excavations continued until all chosen nests had been uncovered, ending about mid-September for both years. Both monitored and undisturbed nests that were not monitored were excavated. In 2015, seven holes were excavated: four were undisturbed nests and three had been monitored. The preliminary data from 2015 was used to determine how wide and deep the nest excavations should be in 2016. In 2016, 18 of the 20 selected, nests were excavated. At Boscawen five were monitored and five were undisturbed, while at Epsom four nests were monitored and four were undisturbed. Nest entrances were chosen that were at least 40 cm away from any other nest entrances. Any nest within 150 cm of the studied nest entrance were mapped for direction and distance. Nests that had been monitored were marked with an orange tee and a number written on the flagging. Consequently, all monitored nests are named after their flagging: *e.g.* “monitored nest #orange.” Some of the undisturbed nests had golf tees near them with a letter that allowed tracking of the entrance hole, while others were numbered. There was no other specific organization to the naming system.

Size determination of nests

In 2015, nest excavation was initiated by outlining a circle with a 60 cm radius based on the nest entrance, with this circle then etched into the ground. The perimeter of this circle was excavated 30 cm into the ground, creating a ring around the nest entrance (Figure 6). This area was large enough to include any cell made by the nest inhabitant based on Evans (1971). Once the trench was dug around the nest, the interior sides were slowly scraped away while looking for cells containing beetle parts, larvae, or prepupae. When a cell was found, the horizontal distance (cm) from the nest entrance, the depth from the surface (cm), and the orientation from North (using a 360° compass system) were recorded and kept with contents from that cell. The excavation was complete once the center had been reached and examined.

To create an image that showed the full extent of the nest without any connecting tunnels, 3D images of the nests based on coordinates of the cells were developed using Sketch Up© (Figure 7).

To determine the size of each nest and remove outlier cells the horizontal distances and orientation where used. The depth of the cell was not used in this statistic as the average depth did not appear to vary significantly and there was a normal distribution of average cell depths (Figure 8). Horizontal distance and orientation were treated as x and y coordinates, with these coordinates placed in a scatter chart that represented a bird's eye view of the nest looking directly vertical to the ground (Figure 9). Mahalanobis outliers for each nest were located by using the stats program JMP© (Version 12.1.0, SAS Institute Inc., Cary, NC, 2015), that then removed from the analysis.

Once the outliers were removed, the average and maximum size of each nest was determined (Table 1, Appendix). Based on the results from the 2015 survey, the maximum depth for a nest was 25 cm. In 2016, the holes were dug to only 30 cm deep, and based on the determination that the maximum distance from the nest entrance was 37 cm the excavations began using were dug with a radius of 40 cm.

Identification of insects from nest excavations

Specimens from the cells were identified in the lab. The beetles consisted of broken parts with the soft tissues removed, with the elytra and pronotum remaining while being disarticulated. These parts could be successfully used for identification to species or genus by was successful using Paiero et al. (2012). Larvae were identified to see if they were *Cerceris fumipennis* using the key in Evans (1957). If they were not *C. fumipennis*, they were identified only to order. All specimens and fragments from a single cell were kept together in vials of alcohol to preserve the larval or pupal stages with the beetle fragments.

<u>Determination of maternal investment</u>

The maternal investment of the nest was determined by looking at multiple variables: the number of prepupae or larvae, the number of beetles the wasp brought back, the average biomass of beetle species per nest, and the average amount of beetle biomass that produced a prepupa in single cells. The number of *Cerceris fumipennis* prepupae found in each nest is used to represent the reproductive success of the nest. The prepupal stages were not raised to adulthood, since development of a protocol to break winter diapause was beyond the scope of the project. It is assumed that all prepupae and larvae found intact were viable, not parasitized, and would have become adults the following summer. The number of beetles per cell in the nest were determined by connecting the major parts left behind, such as the elytra and pronota of the beetles.

Biomass could not be determined directly from the beetle parts in the nests, so whole voucher specimens were used to obtain an average mass for each species. Voucher specimens collected from the wasps during the 2015 and 2016 field seasons were used for the measurements. These beetles were dried for one week and then weighed on an analytical balance (Sartorius Research R200D©) and their lengths measured with calipers. Any buprestid species that did not have enough individual beetles brought back to the sites by the wasps was supplemented with museum specimens. Museum specimens were rehydrated so that they could be carefully removed from their pin or point mount without breakage, dried for a week, and then weighed and measured. The mass of 10 specimens was used to calculate an average mass, except for uncommon species that had less than 10 specimens present in the University of New Hampshire Insect Collection. There were also a few species that had fewer than five available voucher specimens, for which an average biomass was not calculated. Cells with any of these species are used for the citation of all species, but the species were not included in their accumulative average of biomass. There were also a number of cells with beetles that did not have enough parts to place them to species that were usually of the genera *Chrysobrthis* and *Agrilus*. Cells with beetles only placed to the generic level were also left out of biomass calculations. Since these totals are on averages, they do not reflect the size variation found between individuals of the beetle species.

The relationship between these measures of maternal investment were analyzed using JMP© (Version 12.1.0, SAS Institute Inc., Cary, NC, 2015). A multivariate correlation and a restricted maximum likelihood (REML) test was used to compare the length of the prepupa with the amount of biomass in a cell. Multivariate correlation and the REML tests were also used to analyze correlation between the number of prepupae in a nest and the amount of beetle biomass in a nest, as well as the number of prepupae in a nest to the number of beetles in a nest. A final multivariate correlation and a REML test was used to analyze the correlation between the amount of beetle biomass in a nest and the number of beetles in a nest.

Comparison of monitored nests to undisturbed nests

Monitored nests were compared with undisturbed nests to determine if the monitoring procedures were affecting wasp maternal investment. JMP© was used to analyze the three separate measures of maternal investment: the number of young wasps (prepupae), the number of beetles per nest, and the amount of beetle biomass per nest. Each measure of maternal investment was analyzed to determine if the distribution was normal using the Shapiro-Wilk test to analyze goodness of fit. If the distribution was normal, then a t-test was performed to determine variation between the monitored and the undisturbed nests. If the distribution was not found to be normal, a Mann-Whitney U test was performed to determine the degree of variation between the monitored and the undisturbed nests.

Determination of constancy of prey beetle species

The constancy in selection of prey beetle species by an individual wasp was determined by examination of the beetle remains found during each nest excavation, which allowed determination of species composition. The composition of a nest was analyzed by comparing the content of the pooled species found in each nest. Activity of the monitored nests was observed throughout the summer to be certain that there was only one active wasp maintaining the nest, while undisturbed nests were not checked. Whether a single wasp or more were active in provisioning the undisturbed nests during the summer is unknown.

Figure 3. An aerial view of the New Hampshire State Forest Nursery in Boscawen, NH. Nests of the wasps used in the homing study were on the north border of the field within the red oval.

Figure 4. An aerial view of the American Legion Parking Lot in Epsom, NH. Nests of the wasps used in the homing study were in the red oval.

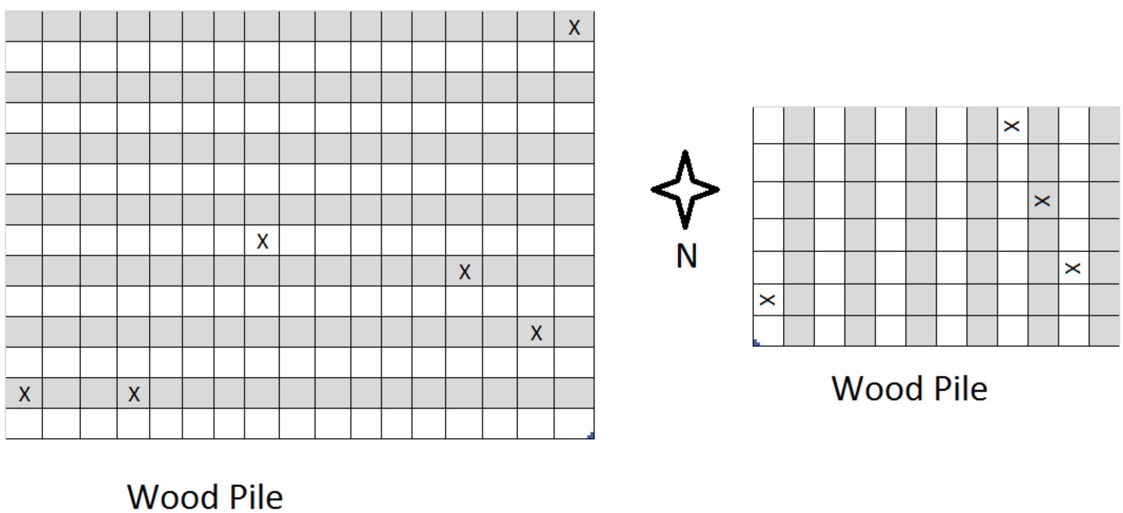


Figure 5. Example of the square meter grid. Each chart is a measured nesting area at Boscawen, NH. Each X is a randomly chosen meter square that was observed for nesting activity throughout the summer.

Figure 6. A photo of the circle drawn in the sand with a 40 cm radius centered on the nest entrance, which has been marked with a star. A photo of the 30 cm deep trench that had been dug around the nest.

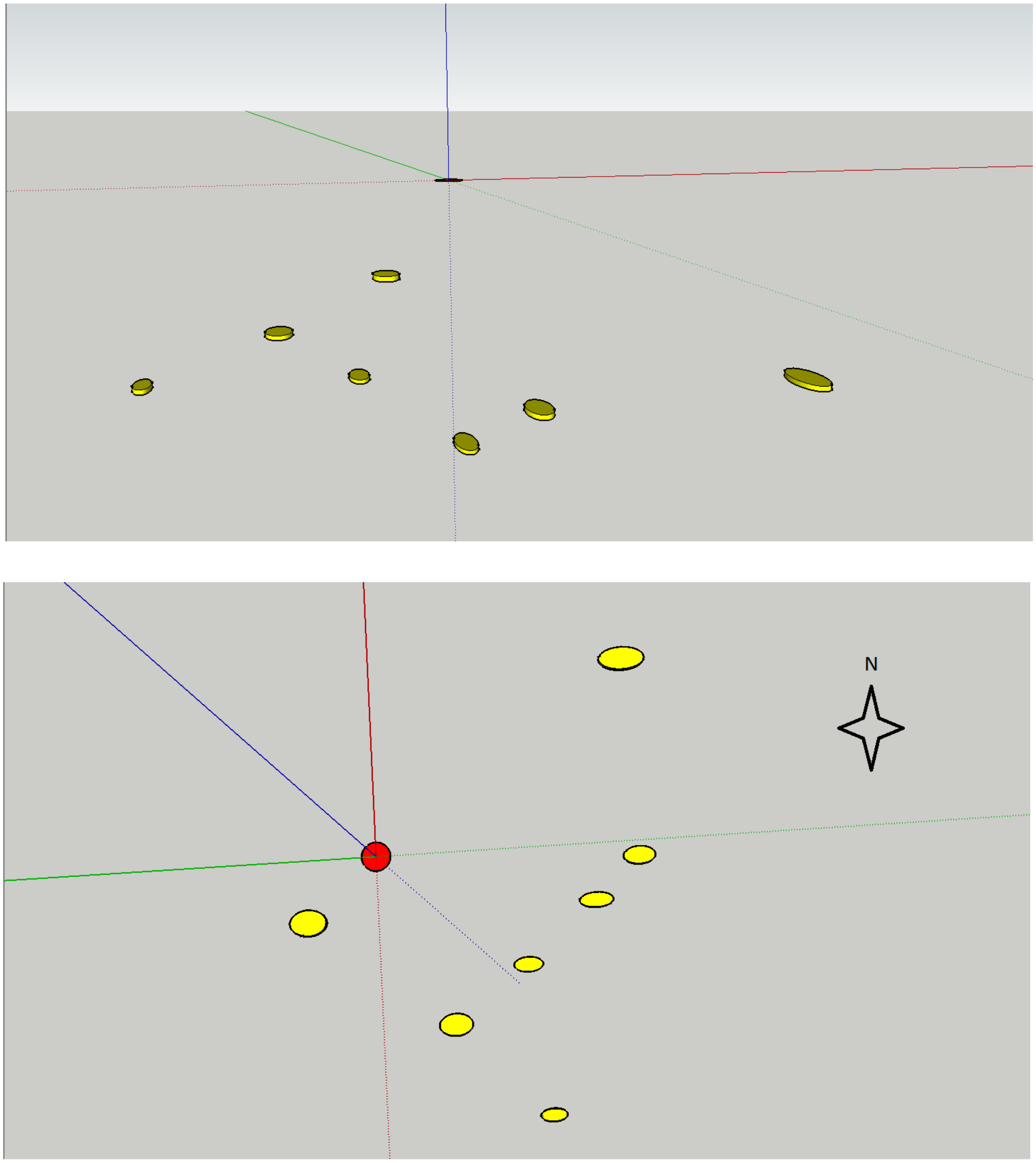


Figure 7. An example of a 3D model of a *Cerceris fumipennis* nest. Based on wasp “monitored 5” at Boscawen in 2015. The first view is a view of the nest in a lateral view side. The second is a bird’s eye view looking down on the nest.

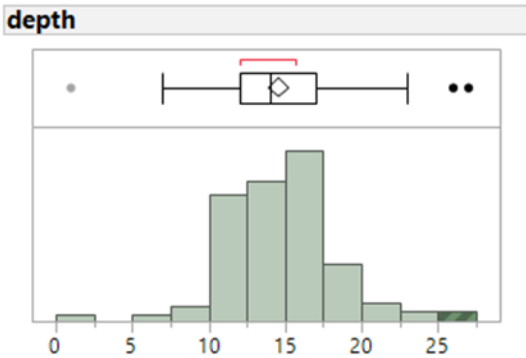


Figure 8. A distribution graph of the depth of the cells within *Cerceris fumipennis'* nest. It shows a normal distribution around the average depth of 14 cm (p=0.0001)

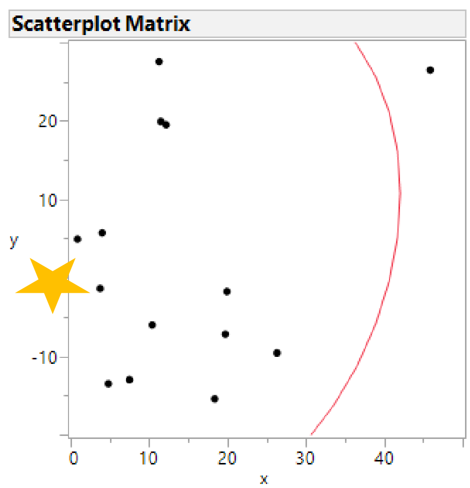


Figure 9. An example of how a scattered plot matrix was used to identify outliers. This is the nest of wasp "6 undisturbed" at Boscawen in 2015. The entrance of the nest is marked with a star at 0,0. Each point represents a cell. The cell outside the ellipse is a Mahalanobis outlier.

Results

Nest building

Of the 20-randomly selected meter^2 plots of wasp habitat that were studied in 2016, 14 of them had nesting activity: eight in Epsom and six in Boscawen. There was nest-building activity in the squares throughout the summer. When an individual square is monitored, such as square 17 at Boscawen, there were nests that were created and abandoned throughout the summer and not just at during the nest-founding period. On the first day of observation, July 1st, there was one nest. However, five days later (July 6), this nest was abandoned, and there was a new nest in the northwest corner of the plot. This nest entrance was abandoned after two weeks, and on July 30 a new nest appeared in a small grassy patch within the plot. This nest entrance was used for at least a week, but by August 11 had been abandoned. The activity of grid 17 is depicted in Figure 10. Despite some high abandonment, over a third of the nests, 14 of 37 remained active for more than two weeks. On July 14, the third week of observation, square 14 at Boscawen had its first nest entrance appear. This nest was maintained until the last observation period on August 11, and was an active nest for four weeks. This activity is graphed in Figure 11.

Looking at individual nest activity provides some interesting insights, but also the appearance of new nests and abandoned nests was analyzed. A scatter plot (Figure 12) portrays the number of new nests per week at both the Epsom and Boscawen sites. The peak number of new nests for both sites was within the first seven days of July. This was after the first week of emergence for the wasps at Epsom, and after the first week of activity at Boscawen. At Epsom there were nine new nests on July 5, and at Boscawen there were eight new holes on July 1. However, the appearance of new nests declined after these dates, but new nests were being dug throughout the entire period of aggregation activity. During the last week of observation there were no new nests at Epsom, while at Boscawen, an abandoned nest was reactivated.

The number of nests abandoned per week is depicted in Figure 13. One week after the initial emergence at Epsom there were no abandoned nests. However, after the peak of new nests appearing on July 6, there were nests that were abandoned at both sites. The wasps occupying these nests had not been marked, so it could not be determined if the occupants abandoned the nests or had died.

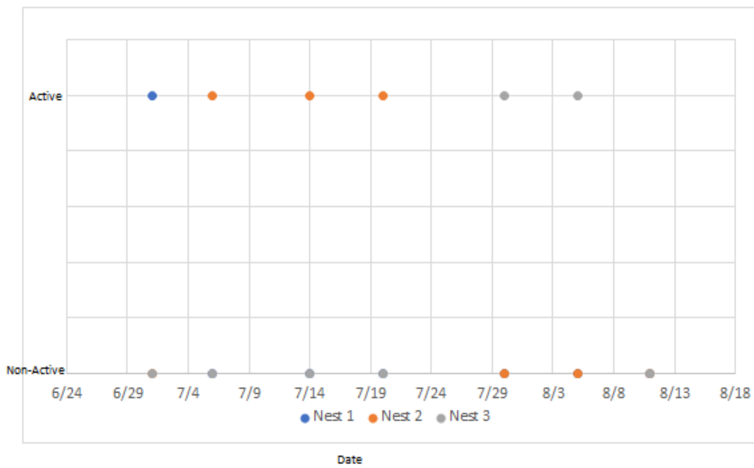


Figure 10. The nest activity of grid 17 throughout the weeks of monitoring, showing when each nest was active.

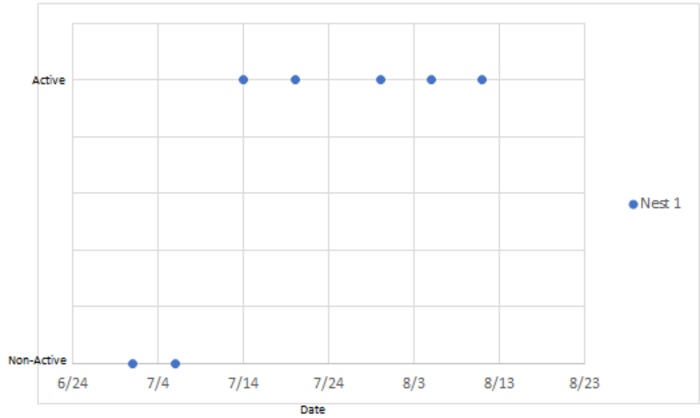


Figure 11. The nest activity of grid 17 throughout the weeks of monitoring, showing when each nest was active.

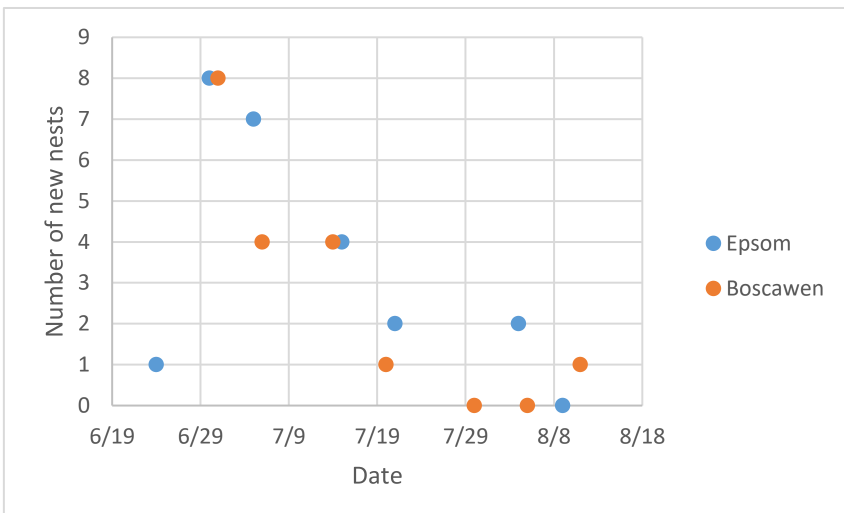


Figure 12. Number of new nests within the monitored plots each week for Epsom and Boscawen.

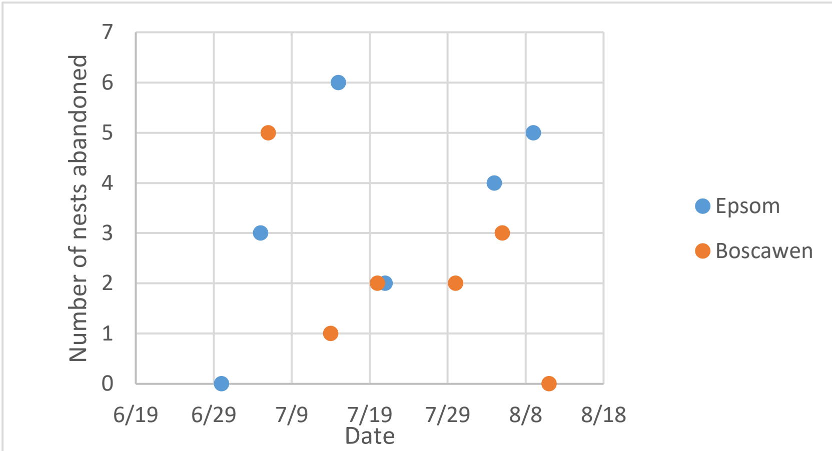


Figure 13. Number of abandoned nests within the monitored plots each week for Epsom and Boscawen.

Maternal investment

Maternal investment: production of young

The average number of prepupae per nest was 4.3 when the nests from both sites are pooled. There was a wide range in the number of prepupae that developed in each nest, ranging from 0 to 11. The nest with the highest number of prepupae was "nest 1 Orange" from 2015. There were two nests that failed to produce any viable pupa, "unmarked nest 3" from 2015 and "monitored nest 15 Orange" from 2016. In "unmarked nest 3" a muscoid dipteran pupa was present in one of the cells. "Monitored nest 15 orange" produced one cell that lacked a larva or prepupa.

There was also a variation in the number of prepupae between years. In 2015, the average number of prepupae per nest was 5.66, while in 2016 it dropped to 3.88 prepupae per nest. A table of all nests and their contents can be found in the Appendix (Tables 3a and 3b).

The length of the prepupae was recorded in 2016. The smallest prepupa was 10.69 mm and the longest was 24.99 mm. The distribution of prepupal lengths is shown in Figure 15, with an average of 17.5 mm. There is a large cluster around the average, and a second small cluster at around 20.7 mm. The correlation coefficient between the pupal length and the amount of biomass in cell was found to be (r= 0.4821, p= 0.0001) (Figure 16).

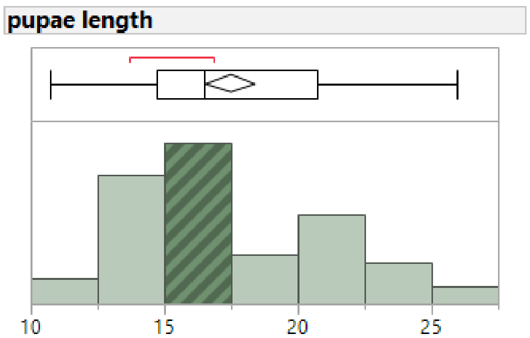


Figure 14. A distribution plot of the length (mm) of the prepupa of the *Cerceris fumipennis*. The bars indicate abundance. The box and whisker above the graph marks the mean, the 75% quartile, the 25% quartile, the maximum, and the minimum. The average is 17.5 mm which is near a large cluster. There is also a small cluster around 20.7 mm.

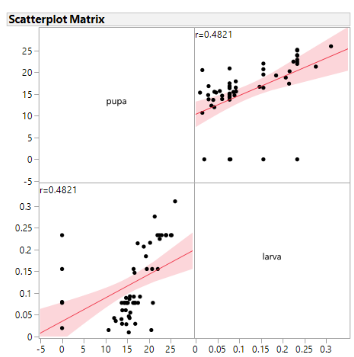


Figure 15. A multivariate correlation between the amount of biomass in a cell and the length of the pre-pupa found in the cell (r= 0.4821). This is a significant correlation (REML, p= 0.0001).

Maternal investment: prey beetle

The average number of beetles brought back was 18 beetles per nest. There was a wide variation in number of beetles found in a single nest, from 1-64 beetles. The nest with the highest number of beetles was “unmarked nest 6” from 2015 at Boscawen. This nest was very active and had a high number of cells (14), but the high beetle count may be explained in part by the species of beetles brought back by the wasp. In this nest 81% of the buprestids present were a smaller class of Buprestid (Table 2). The most common species found in this nest was *Chrysobothris sexsignata,* with 29 specimens in the nest. The nest with the fewest beetles was “unmarked nest F4” from 2016 at Epsom. This nest only had one cell with one beetle in it, a *Dicerca divaricata,* which was also had a prepupa within the cell. *Dicerca divaricata* is a large beetle and provided enough resources for the larva to develop into a prepupa. There is between-year variation in the average number of beetles found in the nests. In 2015, the average number of beetles per nests was 30.5, and in 2016 the average number of beetles per nest was 14.3.

The number of beetles/cell is related to the size of beetle species being caught. The cell with the most beetles was from “unmarked nest 6”, which had 17 beetles. In this cell, there were 10 *C. sexsignata,* 1 *Chrysobothris azurea,* 2 *Chrysobothris rotundicollis,* and 4 *Agrilus anxius*. All of these beetles besides *C. rotundicollis* are in the small size class of buprestid. Of the 206 total cells, 16.5% (34) had only one beetle in it. Most of the cells, 30 out of 34 (88%), had a beetle that fell into the large class of buprestid, such as *D. divaricata* or *D. caudata*. The average number was 3.3 beetles per cell.

Maternal investment: prey beetle biomass

The results of the average beetle biomass for each species can be found in Table 2. Using the average mass each buprestid species was grouped into a small, medium, or large size class

(Table 2). The beetles that are left out of this table are *Brachys aeruginosus* Gory, *Phaenops aeneola* Melsheimer, *Anthaxia quercata* Fabricius, and *Chrysobrthis azurea* LeConte. These are all rare and physically small species. The largest buprestid species the wasps regularly returned with was *D. divaricata*, with an average biomass of 0.078 g. The smallest buprestid species that the wasps returned with was *Brachys ovatus* with an average biomass of 0.004 g.

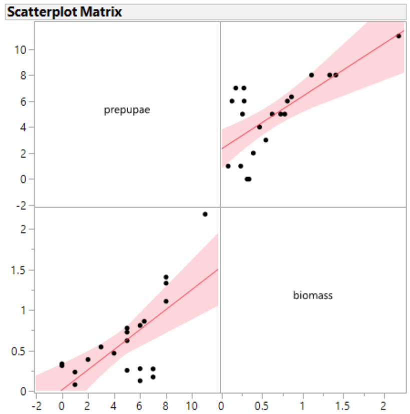
The average biomass per nest was 0.621 g (SD= 0.533g), the range being 2.129-0.078 g. The nest with the most biomass was “monitored nest 1 orange” from 2015 at Epsom with a total biomass of 2.129 g. While this nest did have a high number of beetles in it (30), it was not the nest where the most beetles were found, with “unmarked nest 6” having 61 beetles. The nest with the smallest biomass and a *C. fumipennis* larva in it was “monitored nest 13 orange” present at Boscawen in 2016 with a biomass of 0.0758 g. This nest had only one cell with two beetles in it, which were two specimens *D. punctulata*, a medium-sized *Dicerca* species. Following a similar pattern as the number of beetles, there was between-year variation in the amount of biomass in each nest. In 2015, the average amount of biomass/nest was 0.975 g, and in 2016 the amount of biomass/nest was 0.488 g.

The average biomass per individual cell was 0.123 g. However, once the cells that did not have a viable prepupa were removed from the analysis, the average mass/cell increased to 0.13 g. The cells that lacked a prepupa had an average biomass of 0.09 g. There is a positive correlation between the biomass per cell and whether a viable prepupa was present (REML, r= 0.223, p= 0.0133). The cell with the most biomass was “monitored nest 1 orange” from 2015 at Epsom with a biomass of 0.388 g. This cell had 5 *D. divaricata* and a *C. fumipennis* prepupa. The cell that had the smallest amount of biomass (0.03 g) that still supported a prepupa was “undisturbed nest M" from 2016 at Boscawen. This cell had three beetles of the same species, *C. sexsignata*, a medium-sized species, plus the prepupa.

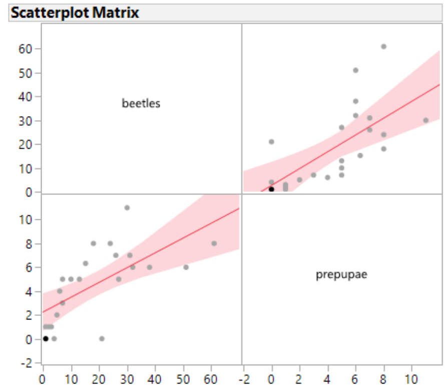
When looking at the accumulated biomass for the whole nest there was a significant correlation between the amount of biomass in the nest and the number of prepupae produced (REML, $r= 0.661$, $p= 0.0008$) (Figure 16a). There was also a significant correlation between the number of beetles per nest and the number of prepupae produced (REML, $r= 0.692$, $p= 0.0003$) (Figure 14b). However, the number of beetles per nest does not have a significant correlation to biomass (REML, $r= 0.343$, $p= 0.118$) (Figure 17).

Table 2. A list of the dried masses of the beetles found in nests. Masses are from an average of ten beetles unless it has an asterisk after the mass. *Brachys ovatus* was averaged from the mass of 6 dried individuals; *B. consularis* was averaged from the mass of 9 individuals; *D. pugionata* was averaged from the mass of 5 dried individuals. The species were placed in size classes based on their mass: small (s) (0.3 mg ≥ 1.5 mg, length 4.25 mm < 9.69 mm), medium (m) (1.5 mg ≥ 5.0 mg, 12.75 mm < 19.03 mm), and large (l) (5.0 mg- 7.77 mg, length 10.4 mm < 11.69 mm).

Species	Average dried mass (mg)	Size Class
Agrilus anxius	1.07	s
Agrilus psedocoryli	0.56	s
Brachys ovatus	0.36*	s
Buprestis consularis	6.33*	l
Buprestis striata	6.89	l
Chrysobothris femorata	2.37	m
Chrysobothris harrisi	1.34	s
Chrysobothris rotundicollis	1.51	m
Chrysobothris sexsignata	0.98	s
Dicera divaricata	7.77	l
Dicerca caudata	7.96	l
Dicerca puctulata	3.79	m
Dicerca pugionata	2.68*	m
Neochlamisus bebbinae	0.85	s
Phaenops fulvoguttata	1.75	m



16a.



16b.

Figure 16a. Multivariate correlation plot between the amount of biomass in a nest and the number of prepupae (r= 0.6608). The correlation is significant (REML, p= 0.0002). 16b. Multivariate correlation plot between the number of beetles in the nest and the number of prepupae (r= 0.6924). The correlation is significant (REML, p= 0.0003).

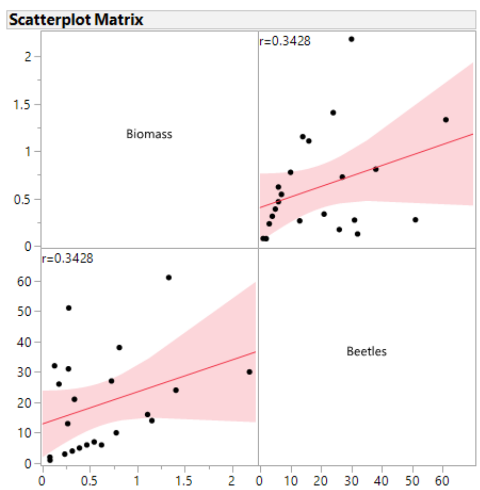
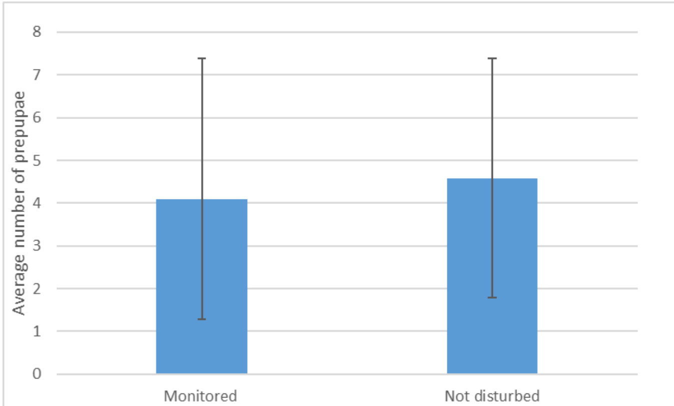


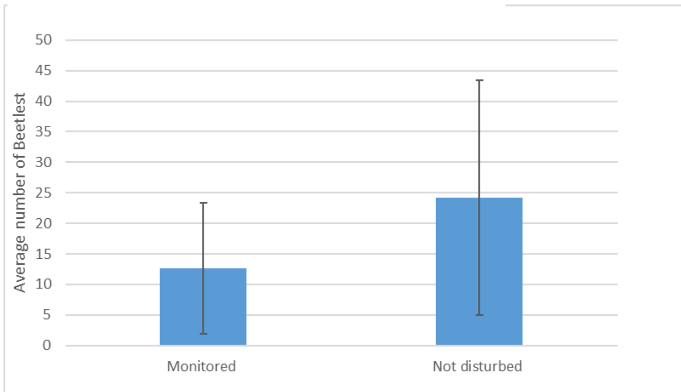
Figure 17. A multivariate correlation between biomass and number of beetles (r= 0.3428). This is not a significant correlation (REML, p= 0.1184).

<u>Comparison of monitored nests to undisturbed nests</u>

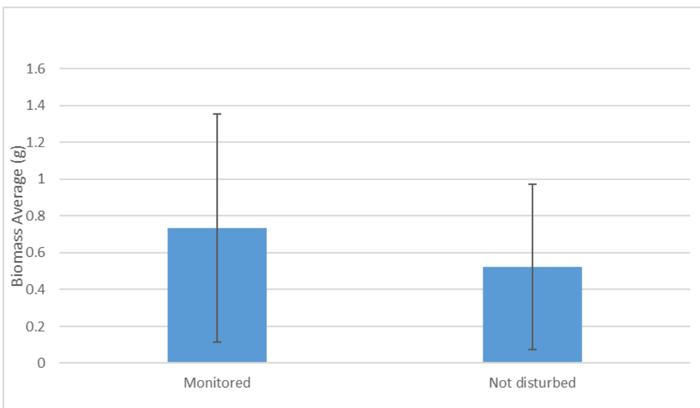
Maternal investment was not significantly different between nests that were monitored or left undisturbed. The number of prepupae per nest followed a normal distribution (Shapiro-Wilk, p= 0.2957). The average number of prepupae in nests that were monitored was 4.09 (SD= 3.33), and for the undisturbed nests it was 4.58 (SD= 2.84) (T-test, t= 0.3797, p= 0.7082). The number of beetles per nest did not follow a normal distribution (Shapiro-Wilk, p= 0.0181). The average number of beetles per monitored nest was 12.63 (SD= 10.69), and for the undisturbed nests it was 24.16 (SD= 2.84) (Mann-Whitney U, U= 109.5, p= 0.1755). The amount of biomass in a nest did not follow a normal distribution (Shapiro-Wilk, p= 0.0047) The average amount of biomass for monitored nests was 0.736 g (SD= 0.62), and for undisturbed nests it was 0.524 g (SD= 0.45) (Mann-Whitney U, U= 126, p= 0.4483) (Figure 18).



18a.



18b.



18c.

Figure 18a. Histogram of the average number of prepupae in each nest between monitored and undisturbed nests. 18b. Histogram of the average number of beetles in each nest between monitored nests. 18c. Histogram of the average amount of biomass in a nest between monitored and undisturbed nests. The error bars are standard deviation. There is no significant difference exhibited by any of these comparisons.

<u>Constancy of prey species</u>

There were 20 different species of beetles recovered for a total of 422 beetles (Table 3) from the 22 excavated nests. Three species, the buprestids, *Brachys aeruginosa* and *Anthaxia quercata*, plus a scarab beetle, *Dichelonyx albicollis*, comprised the first records of these taxa ever taken by *Cerceris fumipennis*. The most common species brought back to the nest was *Dicerca divaricata*. Seventeen of the 23 nests (73.9%) brought back at least one *D. divaricata* (124). Of the total beetles brought back, *D. divaricata* made up 29% of the individuals. The two next most common species brought back were *Agrilus anxius* (16.5% of individuals) and *Chrysobrothis sexsignata* (16.5% of the individuals). Figure 19 breaks down the composition of prey species found from all the excavated nests.

The beetle species were organized into size categorizes: small, medium, and large (Table 2). Fifty percent of the beetle species fell into the small size category (mass 0.3 mg ≥ 1.5 mg, length 4.25 mm < 9.69 mm), 37% percent of the beetle species fell into the large size category (mass 5.0 mg- 7.77 mg, length 10.4 mm < 11.69 mm), and 13% percent of the beetles were placed in the medium category (mass 1.5 mg ≥ 5.0 mg, length 12.75 mm < 19.03 mm).

Eight of the 23 excavated nests had only one buprestid species present (34%). Of these eight nests, seven of had only *D. divaricata,* while the other nest had only *D. punctulata*. Thirty-four percent of the nests had four or five beetle species (Figure 20). The most diverse prey assemblage was found in nest “monitored orange 6”, and had nine beetle species totaling 64 beetles in 14 cells: *Chrysobothris harrisi* (1), *C. azurea* (2), *Neochlamisus bebbianae* (5), *Dicerca divaricata* (10), *D. caudata* (1), *C. sexsignata* (29), *C. rotundicollis* (5), *Brachys ovatus* (1), and *A. anxius* (10) (Figure 21). A breakdown of the beetle composition for two nests is shown in Figure 22: one nest with one beetle species (*D. divaricata*, 18), and another nest whose wasp brought back three beetle species (*C. sexsignata* (10), *D. caudata* (2), *A. anxius* (1)).

Summer-long monitoring of individual wasps can be used to understand the constancy of individual wasps to prey species. However, in monitoring only one day a week, there were often only one or two specimens recovered from the wasps even after multiple weeks of monitoring. The wasps were not adequately active in a single day to determine if there is a pattern to the species being taking throughout the summer, but it does provide a glimpse of what is happening on the days the wasps were monitored and the days they brought back prey. The most active individual was wasp 17 from Epsom in 2016. On July 15 she brought back four beetles: three *D. caudata* and one *D. divaricata*, while on July 21 she brought back one *D. divaricata*. This wasp was active in its nest until August 8 (Figure 23). In another nest that was active until August 8, wasp 3 from Boscawen in 2016 brought back three *D. divaricata* on July 13, and on July 20 she brought back one *D. divaricata* (Figure 24).

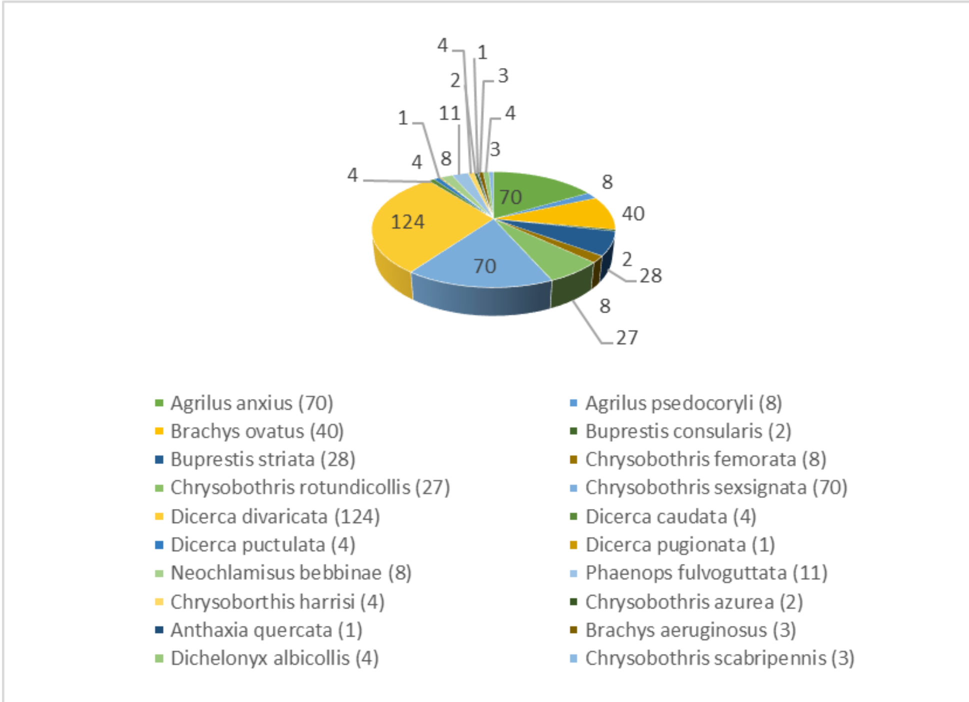


Figure 19. The total number individuals of the prey species from all excavated nests.

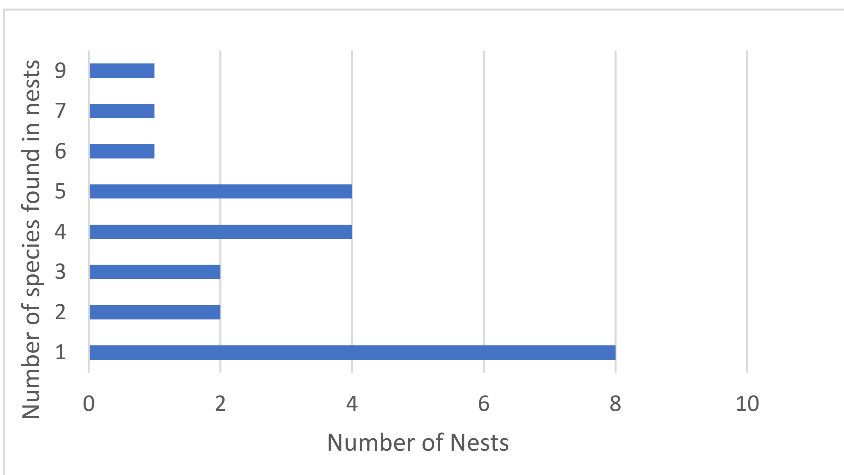


Figure 20. A histogram of the number of species found vs the number of nests.

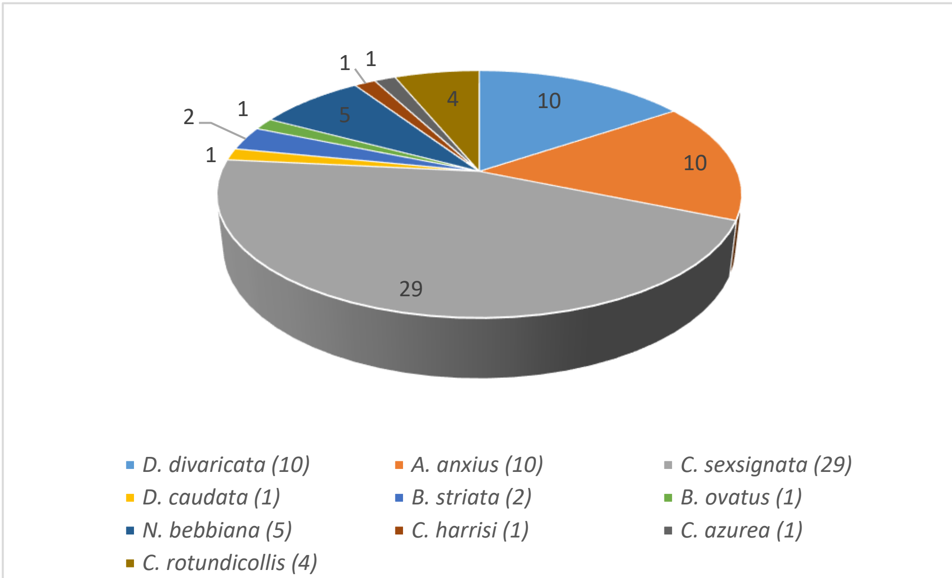
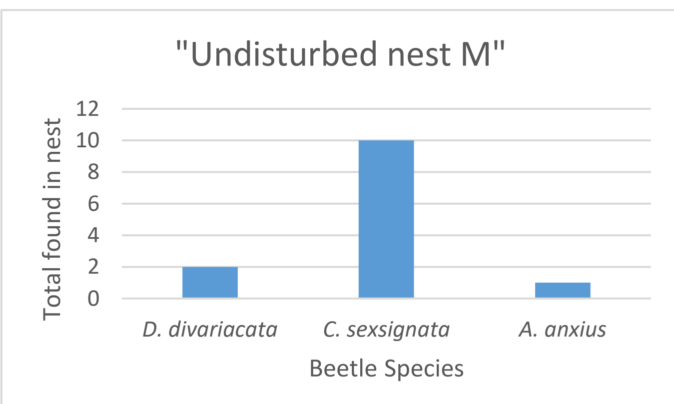
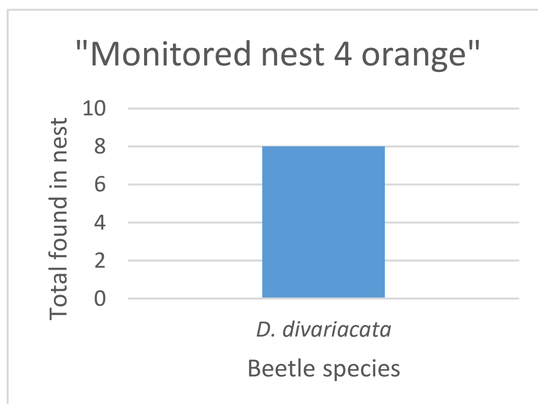


Figure 21. The species break-down of nest "6 monitored orange" with 64 beetles from 9 different species in 14 cells.





22 a. 22b.

Figure 22a. The prey composition for a nest whose wasp caught 3 species. The nest had 13 beetles; *C. sexsignata* (10), *D. caudata* (2), and *A. anxius* (1). Figure 22b. The prey composition for a nest whose wasp caught only one species, producing 8 *D. divaricata*.

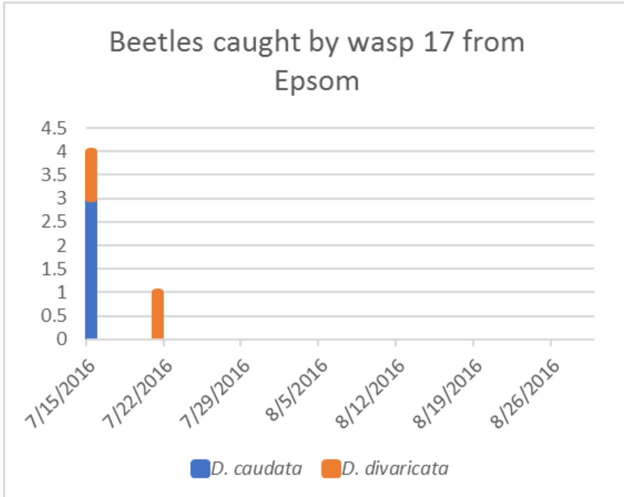


Figure 23. Beetles caught by wasp 17 from Epsom in 2016, after 7 weeks of monitoring. The beetles caught were within the first two weeks of monitoring. This wasp brought back two species *D. caudata* and *D. divaricata*.

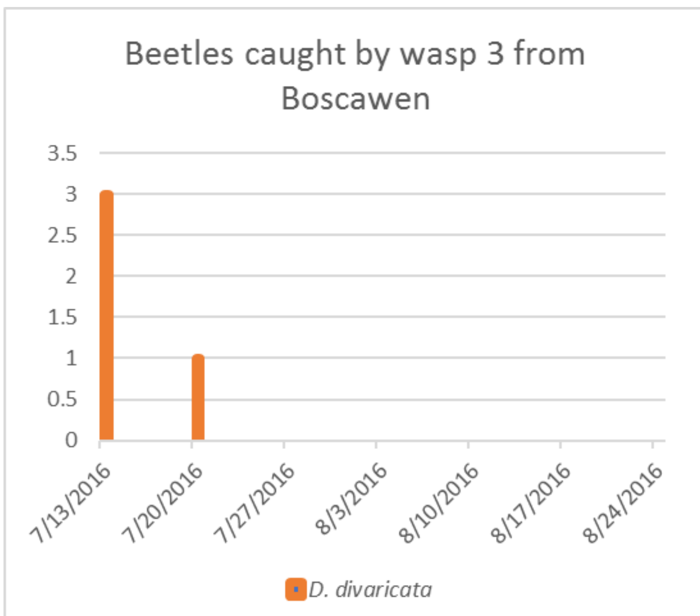


Figure 24. Beetles caught by wasp 3 from Boscawen in 2016, after 7 weeks of monitoring. The beetles were caught within the first 2 weeks of monitoring. This wasp only brought back one species of beetle *D. divaricata*.

Discussion

Nest Building

The data from the above-ground nest grids showed that *Cerceris fumipennis* creates new nests throughout the entire summer. The peak of nest creation was during the first week of July, which was during the second week of post-emergence. Nest creation did drop for both sites after the peak in creation, but new nests were seen until the end of the study in late August. Studies have found that there is a high rate of nest abandonment and creation during the first two weeks post-emergence, but these studies were not continued through the rest of the summer (Mueller et al., 1992; Careless, 2009).

After the first week of emergence nests were being abandoned at an irregular rate (Figure 12). For this portion of the study none of the wasps were marked, so it is unknown what happened to the wasps that abandoned these nests. They may have died or created new nests. Both of the sites, Epsom and Boscawen, experienced periodic disturbances from drive-throughs by cars and trucks, and Epsom was also mowed once a month. Some of the nest abandonments could be due to the wasps returning from hunting, and spending more time trying to find their original nest, but with their nest entrance flattened and the landscape slightly altered they may have simply started a new nest. Square number 16 at Boscawen was half tilled, and the holes in the tilled portion of the square were abandoned. This could be due to death of any wasps that had been resting inside the nests.

Another question is the strength of nest fidelity among *C. fumipennis*. It was thought that after the initial two-week active period of nest creation and abandonment, that the wasps would be loyal to their nests for the rest of the summer. This idea was based mostly on the observations of Careless (2008). He found that moist soil and cool temperatures would increase nest fidelity in mobile colonies (Careless, 2009). For an individual grid like square number 17 at Boscawen, the longest a nest remained active was 2 weeks, suggesting that there is low nest fidelity. The low nest fidelity may be due to a high disturbance rate, such as cars and tractors driving and destroying the nest entrances. However, these higher rates of nest abandonment and creation could be from wasps dying and new wasps were building the nests nearby. There were also nests such as Square 14 in Boscawen, which were dug during the third week, but remained active for 4 weeks. We cannot assume that it was the same wasp in the nest for the entire time. However, if it was, remaining in a nest for four weeks suggests that wasps have long-term nest fidelity.

When looking at these data, we can see that *C. fumipennis* individuals are creating and abandoning nests throughout the summer. However, since the individual wasps were not tagged it is hard to determine why this was occurring. In the future this study should be repeated with all of the wasps marked in an aggregation so it could be determined if newly emerged wasps were creating the nests, or wasps were abandoning and creating new nests, or simply moving from one nest to another nest. Strength of nest fidelity could then be determined.

<u>Maternal investment</u>

<u>Maternal investment: production of young</u>

Though nests have been previously excavated, the reproductive success of *C. fumipennis* has not been examined (Evans, 1971; Kurczewski and Miller 1984; Careless, 2009; Lund, 2015). In this study, the number of prepupae of a single nest was used to define reproductive success. While there was only single prepupa per cell, there was a range of numbers of cells with prepupae in each nest, with some cells lacking a prepupa or larva. The highest number of young that a wasp was able to produce during this study was 11. This came from a nest that had been monitored throughout the summer for 5 weeks, and the wasp did not abandon the nest. Therefore, it can be concluded that all 11 prepupae were produced by one wasp. The lowest number of young per nest was 0, which occurred in two nests that were possibly abandoned. One of the nests contained a dipteran mucoid pupa in it, and the other nest had only one cell with beetles, but no larvae or prepupae. The nest may have been abandoned because of kleptoparasites or other unknown reasons, but this wasp could have made a new nest in a different area. So, it cannot be concluded that her reproductive success was zero.

The average number of young in the nests was 4.33. This could be considered the average reproductive success of an individual wasp. This average could be used to determine female fitness in an area; if the average is significantly smaller, it could be due to changes in the abundance of buprestid populations or long-term adverse weather conditions, as well as random individual differences in the wasps studied. *Cerceris fumipennis* aggregations are known to decline and eventually abandon an area (Careless, 2008), and by monitoring the number of young produced by individuals the decline can be tracked. It can also provide useful information on the longevity of large aggregations.

This study showed a positive correlation between the amount of biomass in an individual cell and the length of the prepupa in it. In some ways this is intuitive, as the more food a larva has the larger it will become. However, in *C. fumipennis* the female is about a third larger than the male (Scullen, 1965), which would suggest that much smaller prepupae will become males and larger ones will become females. In excavation studies where the prepupae where reared, it was discovered that the male to female ratio in *C. fumipennis* 1:1. The males are haploid and receive less food than females, and are smaller (Evans, 1971; Kurczewski and Miller 1984). Female adults do vary in size, and this influences which prey they hunt, or more accurately which prey they bring back to their nests (Hellman & Fierke, 2014). To determine the effects of prey size or biomass an experiment could be devised where larvae in a lab are fed artificial diets of different amounts and then raised to adulthood to see if they were male or female. Then it would be possible see what effect diet and the amount of biomass in the cell had on male to female ratios, and the size of adult wasps.

Maternal Investment: prey beetles

The number of beetles excavated from nests ranged widely from 1 to 64. "Unmarked nest 6" had the largest number of beetles. This was an undisturbed nest, which means that no beetles had been taken from the wasp during the summer. Two wasps could have been living in this nest, which has been observed on occasion (Evans, 1971; Kurczewski and Miller, 1984), or the nest may have been taken over by another wasp when the first wasp died and the nest was abandoned. Taking that into consideration, there is evidence to suggest that this nest was occupied by a small wasp that was focused on smaller prey beetles and therefore needed more beetles per nest than do larger wasps that specialize on large beetles to provide enough biomass in its cells. Eighty-one percent of the beetles in this nest were in the smallest size-class of beetles. The largest number of beetles found in one cell for a single larva was 17. In the nests with fewer beetles, such as "unmarked nest F4," one beetle of the large-size class, a *D. divaricata*, supported a prepupa. This suggests that a smaller wasp can only carry smaller prey, or perhaps that it simply targets smaller prey (Hellman & Fierke, 2014), and must bring back more beetles to a single cell and forage more often than a wasp that is able bring back larger prey. Unfortunately, the holes were excavated when the wasps had died for the season, so there is no information on the size of the wasp associated with each nest. To support the idea that wasp size is influencing the hunting choices of individual wasps, the length of the wasps should be measured for nests that will be excavated at the end of the season.

The average number of beetles per nest could be used to estimate how many beetles the members of a colony will bring back in a summer based on the number of nests. This has been done with other species within the genus *Cerceris* (Polidori et al., 2007). There is variation between years on the average number of beetle caught, which affects the efficacy of the estimate. At the Boscawen site there were 167 nests, and with an average of 18 beetles present per nest, the aggregation could bring back an estimated 3006 Buprestidae during the summer from the surrounding area. Even aggregations at smaller sites could bring back a considerable number of beetles. For example, a site with only 25 nests could collect 450 buprestids assuming the observation based 18 beetle/nest average.

Maternal Investment: prey beetle biomass

The large range in the number of beetles brought back to the nest reflects the large size ranges of the beetle, which can be seen in the biomass table (Table 2). With the wide range of size variability for Buprestidae, using biomass as an indicator of maternal investment is more accurate than use of the number of beetles.

The biomass present in individual cells is a useful indication of how much food is needed for a prepupa to successfully develop. There was a positive and significant correlation between the total biomass in the cell and whether or not the cell contained a prepupa or larva (REML, $r = 0.2235$, $p = 0.0133$). This suggests that there may be a threshold of biomass that is required before a wasp will lay an egg.

Finally, there is positive and significant correlation between both the amount of biomass in the nest and the number of prepupae, as well as the number of beetles and the number of prepupae. However, since beetles have such a wide range of mass, there is not a significant correlation between the number of beetles in the nest and amount of biomass. These data suggests that a wasp can be successful by bring back a large amount of biomass, which may come from a small number of larger beetles, or a large number of small beetles.

Comparison of monitored and undisturbed nests

The data comparing production of monitored and undisturbed nests did not indicate loss in maternal investment due to the summer-long monitoring of the wasps. The number of young, number of beetles, nor biomass were significantly affected by monitoring. This information is beneficial to research and biosurveillance programs, as it indicates that human-interaction with wasps such as: marking them with paint, the weekly covering their nests with clear cups, and their occasional interception as they approach their nest with their beetles and having them taken from them, does not lead to nest-abandonment or lower maternal investment. Other studies have shown that *Cerceris* behavior for aggregations in disturbed areas, does not change with human interaction (Evans and Hook, 1982; Hook, 1987; McCorquodale, 1988; Alexander and Asis, 1997), but this is the first to show that their maternal investment does not change with repeated weekly interactions.

Constancy of prey species

Of the 422 beetles that were excavated from the nests, there were three species recorded that were brought back by *Cerceris fumipennis* for the first time: the buprestids *B. aeruginosa* and *A. quercata*, of which both have been caught in purple prism traps (Nalepa et al., 2015), and a member of the family Scarabaeidae, *Dichelonyx albicollis* Burmeister. There were three *D. albicollis* found in one cell, which was most likely an opportunistic feeding event. *Dichelonyx albicollis* adults feed on *Pinus strobus* Linnaeus (eastern white pine) (Kriska & Young, 2002), and most likely a large cluster was found on a white pine during a hunting flight for buprestids. This also suggests that when a wasp finds a spot with many beetles, they will return to that site for at least short periods of prey constancy.

The most common species found in nests was the buprestid *Dicerca divaricata*, which made up 29% of the beetles from the nests, and were found in 73.9% of the excavated nests. *Dicerca divaricata* is a common species in the study areas (Dube & Chandler, 2017). It may be more attractive to *C. fumipennis* than other common species because it is large, or that their search patterns have evolved to locate large beetles. The next two most frequently taken species were *A. anxius* (16.5% of beetles taken) and *C. sexsignata* (16.5% of the beetles taken), which are not large species. These are also common species that feed on many tree species, and their frequency in collections reflects their abundance in nature (Dube & Chandler, 2017). *Dicerca divaricata* is found on many genera of deciduous trees, which in New Hampshire include members of: *Acer*, *Betula*, *Fraxinus*, *Ostrya*, and *Quercus*. *Chrysobothris sexsignata* is found on both deciduous and coniferous tree and shrub species including: *Acer rubrum*, *A. saccharum*, *Amelanchier arborea*, *Betula alleghiensis*, *B. nigra*, *Carya ovata*, *Castanea dentata*, *Crataegus viridis*, *Fagus*, *Fraxinus americana*, *F. nigra*, *F. pennsylvanica*, *Gleditsia triacanthos*, *Juglans cinerea*, *J. nigra*, *Larix laricina*, *Picea mariana*, *Pinus rigida*, *Quercus alba*, *Q. bicolor*, *Q. macrocarpa*, *Q. stellata*, *Tsuga canadensis*, *Ulmus alata*, *U. rubra*, and *Vitis*. *Agrilus anxius* has been found on members of *Betula* and *Populus*, both deciduous groups of trees (Paiero et al., 2012).

When the beetles were divided into size classes, the smallest size class made up 50% of the beetles excavated from all nests. Hellman and Fierke (2014) documented that wasp size is correlated with the size of beetles that brought back to the nest. If the smaller wasps are indeed hunting smaller beetles, the fact that there are a high numbers of small beetles suggests that many of the wasps were small. From these findings, it is hypothesized that the smaller wasps provision their nests with more beetles rather than a few large beetles. This hypothesis would explain why there are more many small beetles than large beetles in the nests.

Eight of the 23 excavated nests exhibited constant prey choice and had only one species present. Seven of those wasps with high prey constancy were bringing back beetles in the largest beetle class, which were mostly *D. divaricata.* These data suggest that when the wasp is able to target a common large species, it will remain constant. Nests with a higher species diversity, including nests with four or five species, had a mixed composition of large and small beetles. The nest with the highest composition of beetle species had 9 different species. Of the 64 beetles in this nest, (75%) were in the small beetle category (Figure 19). These data suggest that small and medium wasps are catching the largest diversity of beetles. This could be due to the fact that they have to bring back more beetles to their nest to support the same number of young. There is also the fact that wasp have been known to occasionally if briefly share nests (Mueller et al., 1992; Careless, 2009). This makes analyzing the constancy of a wasp difficult. Unmonitored nests that produced multiple species of beetles could have been provisioned by two different wasps if early in the season, but the undisturbed nests were active through the season, and the beetles in the cells are most likely taken by a single individual.

Understanding if the smaller wasps primarily hunt smaller beetles and therefore collected a higher diversity of beetles, is important for biosurveillance efforts. The Emerald Ash Borer, for example, is a medium-sized beetle. Therefore, small or medium sized wasps are more likely to return with these beetles because they are not searching for the largest beetles. However, since the nests that were excavated have no size data for their corresponding wasps this hypothesis is yet to be truly tested, and in the future the wasps from any nests that will be excavated should be measured.

There were only two wasps that brought back more than two beetles during the entire 5-week monitoring period for both 2015 and 2016. These wasps were “wasp 17” from Epsom and “wasp 3” from Boscawen. For both wasps, the peak of their hunting activity was in the second week of July, when “wasp 17” from Epsom brought back four beetles in one day (Figure 23) and “wasp 3” brought back 3 beetles in one day (Figure 24). These two wasps also brought back a single beetle the following week. However, after the third week of July the wasps did not bring back anymore beetles during the monitoring days, though they were observed to be alive and actively leaving their nest for two more weeks. The other wasps observed during the monitoring periods brought back only one or two beetles throughout the five weeks of monitoring. The average number of beetle found in an excavated nest was 18 beetles. Assuming this average for all wasps, the wasps would have to successfully find and return with one to two beetles a day during the five week hunting season. Based on wasp 17 from Epsom and wasp 3 from Boscawen, it appeared that the height of hunting activity is earlier in the season, when they both brought back multiple beetles in one day. This could be natural with wasps more active in hunting beetles earlier in the season, or it could be that wasps are responding to the possible decline in abundances of buprestid population during the summer. The low numbers brought back by the monitored wasps suggest that to successfully monitor an aggregation for the appearance of pest species during a single day per week, it would need to be a large colony with multiple nests monitoried in order for the wasps to return with a high diversity of beetle preys.

Chapter 3: The homing range of *Cerceris fumipennis*

Introduction

A question that is of particular interest to biosurveillance programs is both: 1) how far can the wasps fly, and 2) what is their hunting radius? Once the hunting range of *C. fumipennis* is known, then researchers and foresters can establish a likely distance within which they can locate the trees that have produced Emerald Ash Borers. Based on the size and biology of the wasp, the current technologies for tracking insects, such as harmonic radar and radiotelemetry, cannot be used (Nalepa et al., 2013; Zhu et al., 2011). However, with catch and release experiments we can begin to estimate the homing range of *C. fumipennis*. This is the distance at which released wasps cease to return to their nests, which can be used to provide an estimate of their maximum flight range, which can be used to set the distance limits for searching for infested trees.

A previous study of the hunting ranges of other species of *Cerceris* described the maximum distance from which the displaced wasp returns is the foraging range (Fabré, 1915). This distance does not truly equal the foraging range, as it does not accurately reflect the distance a wasp would travel during its normal flight through the day. It does show that wasp can find its way back to its nest by navigating the landscape within a day. Careless (2009) used a method of establishing the foraging range that was first described by Wilmer (1985). A wasp was taken from its nest in Florida and released at a spot 1 km from the nest. The fastest time it took to return to its nest was recorded as the navigation speed. The navigation speed is not the same as top flight speed, since wasps do not fly in a straight line when navigating within a forest (Collett & Collett, 2002; Careless, 2009). The navigation speed was then used to estimate how far wasps could have flown based on how long they were away from the nest. This distance was given a maximum range of 2 km based on of catch and release tests done in 2008, when *C. fumipennis* returned from 2 km, but did not return from 3 km (Careless, 2008).

Nalepa (2012) used a different method to determine foraging range of *C. fumpennis*. In this study they captured beetles from the wasps, and then found the closest tree that the beetles could have come from, relying on specialization of the beetles' preferred host. A conservative range was then calculated to be 200 m for the average foraging range. This method does not consider that the beetles are strong fliers and could searching in the forest further than the distance established by the infected trees. Additionally, only some species feed on the leaves of the same trees used for oviposition while others feed on flowers and nectar of other species (Webster and DeMerchant, 2012).

None of the methods used by researchers can determine a definitive range. The most accurate way to determine the wasp's foraging range would be to attach a tracking device to the wasp. Unfortunately, due to the wasp's biology and limitations of the current technologies for tracking insects, the application is not feasible at this time. Harmonic radar can track insects through open areas, or through plants with a low water content. It is not strong enough to trace the wasp as it flies through forest, as the signal cannot penetrate through trees and their dense foliage (Zhu et al., 2011; Boiteau et al., 2011). Radiotelemetry was considered as a possibility because it can track for long distances through forests. However, the devices are too large for attaching to *C. fumipennis*, and it would interfere with the wasp's ability to leave and enter its nest (Careless, 2009; Nalepa et al., 2012). Therefore, experiments and observations are used to estimate the foraging range.

This portion of the study aimed to determine the homing distance of *C. fumipennis*, and to see what could be inferred about its flight distance and speed, based on our observations. It was hypothesized that 1 km was within their homing range, and if release points were set up at 0.2, 0.4, and 1.0 km then wasps would successfully return from these three distances. The amount of time it took each wasp to return was recorded. Using their return times, it was hypothesized that there would be high variation as the wasps would not all return directly to the nest, and that different patterns between the three distances would be evident.

Methods

Field tests were conducted at two locations in Merrimack County, New Hampshire in the towns of Epsom and Boscawen. These study sites had been chosen because of the large *Cerceris fumipennis* aggregations living here. In Boscawen there were approximately 167 nests during the peak hunting weeks. This site was spread out over the dirt roads and fields at the north edge of the New Hampshire State Forest Nursery. Nests were situated along the most northern road of the nursery (Figure 25). In Epsom there were approximately 247 nests during peak hunting weeks. This site is located on the northern portion of a sandy parking lot and the nests were more closely clustered than at Boscawen (Figure 26).

Release sites were at three distances from the nesting aggregations, at 0.2, 0.4, and 1 km. The release sites were chosen using Google Earth© (Figure 27). Release points were selected in open areas in a straight line to the south of the nesting site. Open areas were chosen so that the wasps could recognize landmarks when released if the site was within it flight range. The straight line to the south was chosen to reduce one of the variables in selecting a release point.

Each site was visited four times, with four wasps being released at each distance for each site per visit, producing n= 96 observations across all sites. The time was recorded for the duration the wasps needed to navigate back from their release distances to their nests.

Flight tests were started the last week of July and were conducted once a week until the third week of August. The last half of the season was chosen so that the wasps could be assumed to have a higher rate of nest fidelity, and a greater familiarity with the landscape. Arrival at the site was at 8:30 AM before wasps had emerged from their nests. Clear plastic cups with mesh windows were placed over nests that had previously never been used for flight tests or used for other observations. When a wasp emerged from her nest, and flew around in the cup, she and was taken from the cup and placed in a net. In the net, the thorax of wasp was painted with a unique color combination so that its identity could be confirmed when it returned to the nest. After being painted, it was placed into a clear jar with a mesh top and placed in the shade. Generally, at least two wasps were released at a time for each distance, and the wasps were kept in the jar for no more than 15 minutes. Wasps were transported to the release sites in cars with the jars placed on the dashboard, so that the sun would be clearly visible as they were moved.

The time was recorded at each distance when the wasps were released. The clear plastic cups remained over their nests, so the wasps could return and see their nests, but could not enter. The nests were watched and the time recorded whenever a wasp returned to its nest. The marking on each wasp was checked to make sure that the correct wasp was attempting to enter the nest. The study ended at sunset, and the cups were removed. Any wasps that had not returned at this point were considered lost or dead. The following day the nests of those individuals that had not returned during the release day were checked to see if they had returned, which was recorded.

The return time for the distance and the average number of wasps returning from each distance were analyzed as two RCB ANOVAs blocked by site using the program JMP©. The data was blocked by site to account for any possible differences between the two populations of wasps. Post-hoc analyses include a Tukey HSD test to examine the source of variation in return time.

Figure 25. Aerial view of the New Hampshire State Forest Nursery in Boscawen, NH. Nests of wasps used in the homing study were on the north border of the field within the red oval.

Figure 26. Aerial view of the American Legion Parking Lot in Epsom, NH. Nests of wasps used in in the homing study were in the red oval.

Figure 27. Release points to the south of the nesting site at 1 km, 0.4 km, and 0.2 km. Top image has Boscawen, NH release points. Bottom image has Epsom, NH release points.

Results

Of the 96 wasps released in the 2016 flight homing trails, 82 returned to their nests within the time constraints of the experiment. Twenty-nine out of 32 wasps returned from the 0.2 km distance; 28 wasps out of 32 returned from 0.4 km, and 25 wasps out of 32 returned from 1 km. There was no significance difference in the number of the returns between the distances (ANOVA, $F_{3,92}$= 0.4323, p= .7303) (Figure 28).

The wasps returned over a range of times. The fastest return was from the distance 0.2 km at Boscawen on August 8, with a return time of 3 minutes. Assuming the wasp flew directly back to the nest its speed was 1.11 m/s. The longest a wasp took to return to it nest was from the distance 0.4 km at Boscawen on July 27, with a return time of 9 hours and 8 minutes. From each distance there were a number of wasps that returned in both under an hour, and also wasps that took multiple hours to return to their nest. The fastest return time from each distance was 3 min from 0.2 km, 8 min from 0.4 km, and 21 min from 1 km. The speed was not calculated for most of the wasps, because the routes that the wasps flew back to the nest is not known. They may have hunted in the surrounding forest before returning, but none of the wasps returned to the nest with beetles.

A comparison of the average return times or for the three distances and 1 km, 0.2, 0.4, a yielded a significant difference (ANOVA, Tukey HSD, $F_{2,77}$= 4.5025, p= 0.0057) (Figure 29). The quickest average return time was 2.11 hours for wasps released at 0.2 km. The wasps released at 0.4 km had an average return time of 2.98 hours, and the wasps released at 1 km had an average return time of 3.90 hours. The average return time from 0.2 km was significantly different from the 1 km return times (Tukey HSD, p= 0.0043). However, the 0.4 km average return times were not significantly different from the 1 or 0.2 km.

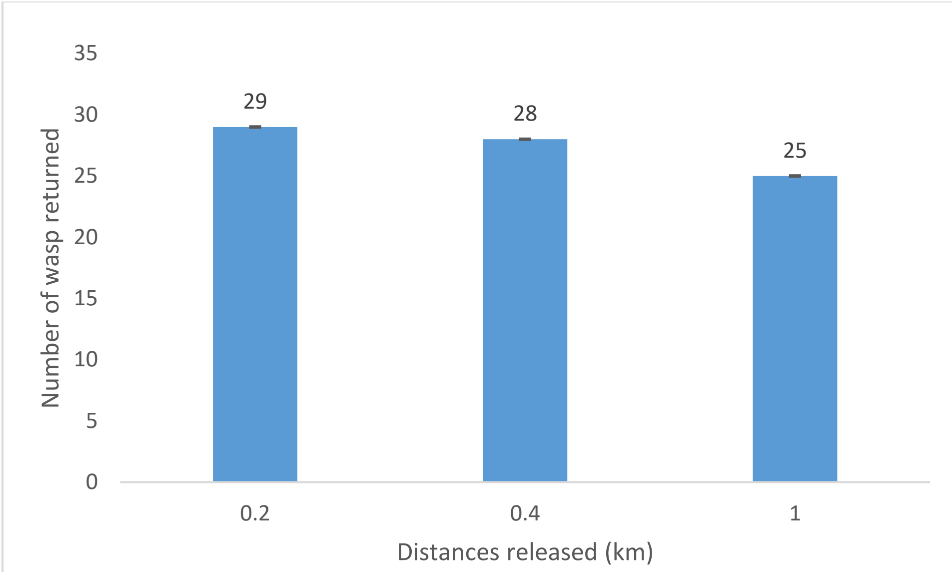


Figure 28. A histogram of the wasps that returned from each of distances, from which there were 32 wasps released. There was no significance difference in the number of wasps that returned between the distances (ANOVA, $F_{3,92}$= 0.4323, p= .7303).

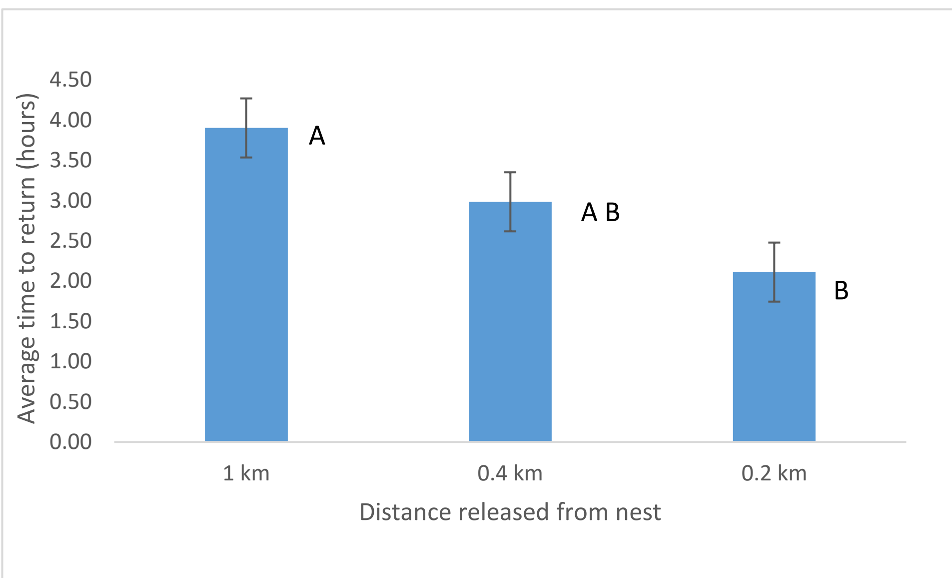


Figure 29. A histogram of the average return times from each of the distances released. There was a significant difference between average return time (ANOVA, $F_{2,77}$= 4.5025, p= 0.0057). Averages marked with the same letter are not significantly differently (p>0.05).

Discussion

This study has shown that 1 km is well within the homing range of *Cerceris fumipennis*. Of the 32 wasps released from each distance, a few less (25 of 32) returned from 1 km, but not to a significant degree. This shows that when taken from their nests and released in clear areas 1 km from their nest, they tend to return to their nests successfully. This homing range is supported by Careless (2008) who determine a maximum homing range of 2 km in Florida. To confirm the maximum homing range, the distances of released wasps were be increased in increments up to 3 km when this study was continues in 2017.

In this study, the fastest wasp flight time provided an estimated 1.11 m/s navigation speed. Careless (2008 & 2009) used the fastest return times of wasps from 1 km to estimate a maximum hunting range based on how long the wasps were gone. He used the "Careless flight speed" equation B*C/2=A, where A equals the radius of navigation, B equals duration of foraging flight, and C equals navigation speed. Navigation speed takes into account that the wasp flies in an indirect manner, zig-zagging and using landmarks to orient themselves during flight in their return to the nest (Collett and Collett, 2002). From these experiments, Careless (2009) estimated that the greatest maximum hunting range is 2 km. Using a speed of 0.79 m/s, he calculated that if a wasp was away from its nest for 57 minutes this would indicate the wasp had a hunting range of 1351 m (Careless, 2009). If this formula is used based on a top speed of 1.11 m/s, a wasp could theoretical fly 1998 m (nearly 2 km) in one hour. This is double the successful 1 km homing ranging established in this study, but it is within the 2 km range estimated by Careless (2009). However, there are several flaws with this equation, many which Careless (2009) has acknowledged. Primarily, this equation does not account for the hunting or handling time of the beetles, and it assumes the wasps are flying continually. It also does not consider whether the wasps might be stopping during flight to rest or feed on nectar. The assumption is that they are flying at top navigation speed for the duration of the flight. Another major problem is that there is often large variation in the flight duration of the wasps during foraging. If a wasp was gone for five hours, then if you apply the "Careless flight speed equation" it yields a radius of 9990 m. This is far outside the current estimated flight range.

There was a large degree of variation in return times of the wasps (Figure 27). Some wasps took many hours to return to their nests, while others took minutes to return from each distance. However, despite the range there was a significant difference in the return times between wasps released at 0.2 and 1 km from the nest ($F_{2,93}$= 3.15 p=0.0043). The significant difference in return time could be explained by the difference in distance: 0.2 km is only 20% of the distance of 1 km. The wasps need more time to fly the longer distance. However, it could also suggest that the wasps had to learn the landscape or use the orientation of the sun to fly in the correct direction until they recognize the landscape because the release point could be outside of their natural hunting range. To determine if 1 km is really within their hunting range or only within their homing range, tracking technology small enough to fit on the wasps without interfering with their biology, and strong enough to track them through a forest must be developed (Nalepa et al., 2013; Zhu et al., 2011; Careless, 2009).

The variation in return times from each distance strongly supports that many of the wasps were not traveling in a direct line from the release distance to the nests. It was observed that after being released many chose to go into the forest and to the North in the clear areas back to their nests. The variation in return times could be attributed to searching for beetles, however, none of them returned with beetles. Many of the wasps were gone for multiple hours, the longest being nine hours after its release. Since many of the catch-and-release wasps were gone for hours at a time, they most likely were not flying continually, and stopped to feed on nectar to replenish water and sugars. This is supported by the wasp's life history that while hunting it will often feed on nectar (Evans, 1963; Careless, 1963). Wasps that returned quickly were most likely flying directly back to the nest.

Conclusion

This experiment shows that 1 km is clearly within the homing range of *C. fumipennis*. To determine the extent of the homing range for distances beyond 1 km future studies were planned 2017. The wasp with the fastest estimated speed flew at 1.11 m/s. When this is used in "The Careless Equation" to calculate the potential buprestid capture radius, or foraging radius was found to be a distance similar to that found in his analysis. However, because all of the potential activities and time investments while wasps are away from their nests, this is an unreliable estimate. The significant differences in average return times between the distances could be explained by the differences between the distances themselves, or by the fact that wasps need to learn to recognize new landmarks at the furthest distance of 1 km. Alternatively, the variation could be attributed to the wasps exploring the forest and flying in a zig-zag pattern, orienting to scattered landmarks, stopping for nectar, or searching for buprestids (Evans, 1969; Collett & Collett, 2002; Careless 2008). To understand how far *C. fumipennis* flies to find buprestids, new technological tracking systems need to be developed. Transmitters are needed that can be attached to the wasps so that they can be traced for long distances though the forest without interfering with the biology of the wasp (Nalepa et al., 2013; Zhu et al., 2011.; Careless, 2009).

Chapter 4: General Conclusions

Previous studies of *Cerceris fumipennis* have shown that the wasp can be a useful tool for the biosurveillance of buprestid forest pests, in particular for detecting spread of the invasive Emerald Ash Borer. In fact, *C. fumipennis* is currently being used in many successful programs to monitor for the appearance of Emerald Ash Borer. Despite this, there are gaps in our knowledge concerning the life history of *C. fumipennis.* This study has filled some of those gaps in both the nest provisioning and potential flight range of the wasp. Both nest provisioning and flight behavior have important implications for biosurveillance planning.

Cerceris fummipennis nest maternal investment could be used as a sign of colony health. The average number of prepupae or the number beetles that an aggregation brings back per year could be tracked as a measure of colony health. This study also documented that biosurveillance did not affect prey accumulation on the basis of an individual nest, which means that when wasps vacate a site it is more likely due to a trend in decreasing quality of habitat or available prey, and is not due to human interaction. This is an important insight as it shows that the same site may be used for years to monitor for the appearance of Emerald Ash Borer and other pest buprestids without affecting the *C. fumipennis* population. When looking at constancy within an individual nest, these data suggest that individuals who hunt for large species of beetles, where 1 or 2 prey individuals are adequate for larval development, were constant in prey choice and brought back fewer, but large beetles. Nests that were supplied with smaller beetles had a more diverse range of prey species, and the cells had more beetles. This may have important consequence for monitoring, if a biological characteristic of the wasp could been shown to be correlated to prey choice diversity. Researchers engaging in active monitoring of pest could focus their monitoring effort on wasps bringing back more species beetles, especially since

Emerald Ash Borer falls into a smaller size category. The homing range of *C. fumipennis* was studied, and 1 km was found to be well within the homing range. Determination of the maximum homing range will let researchers know how far a wasp could possibly forage during the day and return to their nest with prey. These data can be used to suggest a maximum hunting range of the wasps for beetles, and a maximum range to search for an ash tree infected with Emerald Ash Borer.

This study documents some behaviors of *C. fumipennis*, however there is still more to learn. With the nest excavations there were patterns revealed that appeared to be based on the size of the beetles being brought back to the nest. The assumption is that the larger wasps were the ones continually bringing back larger beetles based on earlier literature. In future, this could be confirmed if prior to the nest excavations, the wasps were measured. If it is confirmed then this information could be applied to biosurveillance programs as the smaller wasps would bring back smaller beetles, and would therefore be the most likely to bring back a greater variety of species, and would be the best wasps to be individually monitored for Emerald Ash Borer and other invasive buprestids. The above-ground nest activity experiment confirmed that wasps were creating and abandoning nests throughout the summer. For future studies the wasps should be marked so that the wasps that made each nest could be followed. Finally, a maximum homing range for the wasps was not determined. The trials for this were to be expanded with distances starting at 1 km and increased to 3 km to find the distance where no wasps return, and by implication establish the maximum foraging distance for this species.

Appendix

Table 1: Excavated nests with the following measurements: deepest cell (cm); average cell depth (cm); furthest cell from the nest entrance (cm); and the average horizontal cell distance from the nest entrance (cm).

Site	Year	Nest	Maximum depth (cm)	Average depth (cm)	Maximum distance (cm)	Average distance (cm)
Boscawen	2015	5 Orange	19	14.5	33	22
Boscawen	2015	4 Unmarked	14.5	13.3	32	17.1
Boscawen	2015	3 Unmarked	11	7	34	12.7
Boscawen	2015	6 Unmarked	22.5	14.3	28	16.5
Epsom	2015	1 Orange	20	12.2	37	16.9
Epsom	2015	7 Umarked	18	15.3	14	6.25
Boscawen	2016	1 Unmarked	18	17.3	28	16.8
Boscawen	2016	10 Orange	19	16.6	38	24.3
Boscawen	2016	13 Orange	11	11	10	10
Boscawen	2016	14 Orange	19	15.1	36	26
Boscawen	2016	4 Orange	17	13.8	23	17.3
Boscawen	2016	B	18	16.5	21	15.8
Boscawen	2016	H	15	13.4	17	15
Boscawen	2016	I	12	11.1	19	10
Boscawen	2016	M	22	17.4	28	16
Epsom	2016	1 Unmarked	16	14	18	15.7
Epsom	2016	13 Orange	15	13.7	41	31
Epsom	2016	15 Orange	15	15	15	15
Epsom	2016	4 Orange	26	18	29	23.7
Epsom	2016	Grid 8	15	12.8	30	23.3
Epsom	2016	8 Orange	18	16.8	19	15.6
Epsom	2016	B4	22	22	27	27
Epsom	2016	F4	11	11	7	7

Table 3a: Excavated nests and the beetle species found.

Site	Year	Nest	*Agrilus anxius*	*Agrilus psedo-coryli*	*Brachys ovatus*	*Buprestis consul-aris*	*Buprestis striata*	*Chryso. femorata*	*Chryso. rotund-icollis*	*Chryso. sexsig-nata*	*Dicerca divari-cata*	*Dicerca caudata*	*Dicerca puctulata*	*Dicerca pugio-nata*	*Neochla-misus bebbinae*	*Phaen. fulvog-uttata*	*Chryso. harrisi*	*Chryso. azurea*	*Anthaxia quercata*	*Brachys aerugin-ousus*	*Diche-lonyx albicollis*	*Chryso. scabrin-pennis*	Total
Boscawen	2015	5 Orange	5	0	10	0	7	0	1	0	3	0	1	0	0	0	0	0	1	0	0	0	28
Boscawen	2015	4 Unmark	1	0	0	0	0	0	0	14	6	0	0	0	0	0	0	0	0	0	0	0	21
Boscawen	2015	3 Unmark	0	8	0	0	1	0	0	0	2	0	0	0	3	0	0	0	0	0	0	0	14
Boscawen	2015	6 Unmark	10	0	1	0	0	0	5	29	10	1	0	0	5	0	1	2	0	0	0	0	64
Epsom	2015	1 Orange	2	0	0	0	2	0	4	0	26	0	0	0	0	0	0	0	0	0	4	0	38
Epsom	2015	7 Umarke	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	6
Boscawen	2016	1 Unmark	4	0	0	0	11	1	0	0	7	0	1	0	0	0	0	0	0	0	0	0	24
Boscawen	2016	10 Orange	2	0	2	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	8
Boscawen	2016	13 Orange	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2
Boscawen	2016	14 Orange	3	0	0	0	1	0	1	0	13	0	0	1	0	0	0	0	0	0	0	0	19
Boscawen	2016	4 Orange	6	0	0	0	5	0	1	6	8	0	0	0	0	0	0	0	0	0	0	0	26
Boscawen	2016	B	24	0	27	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0	55
Boscawen	2016	H	11	0	0	2	0	4	0	9	3	0	0	0	0	0	3	0	0	0	0	0	32
Boscawen	2016	I	0	0	0	0	0	3	15	1	0	0	0	0	0	11	0	0	0	0	0	0	30
Boscawen	2016	M	1	0	0	0	0	0	0	10	0	2	0	0	0	0	0	0	0	0	0	0	13
Epsom	2016	1 Unmark	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	4
Epsom	2016	13 Orange	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	4
Epsom	2016	15 Orange	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4
Epsom	2016	4 Orange	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	8
Epsom	2016	Grid 8	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	10
Epsom	2016	8 Orange	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	7
Epsom	2016	B4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	4
Epsom	2016	F4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
		TOTALS	70	8	40	2	28	8	27	70	124	4	4	1	8	11	4	2	1	3	4	3	422

Table 3b: Excavated nests with the total number of beetles, the number of *Cerceris fumipennis* young, and the number of Diptera larvae found in the nest.

Site	Year	Nest	Beetles	*C. fumipennis* young	Diptera pupae
Boscawen	2015	5 Orange	28	5	0
Boscawen	2015	4 Unmark	21	6	0
Boscawen	2015	3 Unmark	14	0	1
Boscawen	2015	6 Unmark	64	8	0
Epsom	2015	1 Orange	38	11	0
Epsom	2015	7 Umarke	6	4	0
Boscawen	2016	1 Unmark	24	8	0
Boscawen	2016	10 Orange	8	7	0
Boscawen	2016	13 Orange	2	1	0
Boscawen	2016	14 Orange	19	3	0
Boscawen	2016	4 Orange	26	7	0
Boscawen	2016	B	55	6	0
Boscawen	2016	H	32	6	0
Boscawen	2016	iii	30	7	0
Boscawen	2016	M	13	5	0
Epsom	2016	1 Unmark	4	2	0
Epsom	2016	13 Orange	4	1	0
Epsom	2016	15 Orange	4	0	0
Epsom	2016	4 Orange	8	7	0
Epsom	2016	Grid 8	10	5	0
Epsom	2016	8 Orange	7	3	0
Epsom	2016	B4	4	1	0
Epsom	2016	F4	1	1	0

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