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Biology and prey of the predatory wasp Cerceris fumipennis (Hymenoptera: Crabronidae) and its use for bio-surveillance of the emerald ash borer

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BIOLOGY AND PREY OF THE PREDATORY WASP *CERCERIS FUMIPENNIS* (HYMENOPTERA: CRABRONIDAE) AND ITS USE FOR BIO-SURVEILLANCE OF THE EMERALD ASH BORER.

BY

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THESIS

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by

Morgan C. Dube

University of New Hampshire, September, 2018

Cerceris fumipennis is a colonial wasp that preferentially preys on native and non-native members of the family Buprestidae including the emerald ash borer (EAB), *Agrilus planipennis*, which is a major threat to North American ash (*Fraxinus* spp.). *Cerceris fumipennis* has been used for bio-surveillance of this destructive pest because it catches, stings, and paralyzes buprestids that are then easily intercepted at their nests and documented.

Two large aggregations of *C. fumipennis* in Merrimack County, NH, USA were monitored during the summer of 2013 and 2014 to determine regional baseline information on aggregation activity, seasonality, paralyzation rate, and prey preference in different forest types for New Hampshire to aid in determining the efficacy of *C. fumipennis* as a bio-surveillance tool. The 2013 field season determined that emergence of wasps appears to be synchronous, with 200 females emerging over a 15 day span. There were 890 individual buprestids collected from females returning to their nests, and their prey species consisted of 33 buprestid species and one chrysomelid. In 2014, individuals of eleven of these species were brought to the nest without being successfully paralyzed. This non-paralyzation occurred in 11% of the total collected prey. These data showed little correlation between percent coniferous and deciduous trees and the

collected prey's preferred feeding hosts. Factors such as lack of host tree specificity in the family Buprestidae, age of forests, diseases, and other environmental conditions could have led to this lack of correlation.

Research should continue to assure and guide government and non-governmental agencies that use of *Cerceris fumipennis* in bio-surveillance of this destructive invasive species and other non-native threats is an effective monitoring tool and can assist in documenting species that are difficult to survey as well as local buprestid diversity.

CHAPTER 1

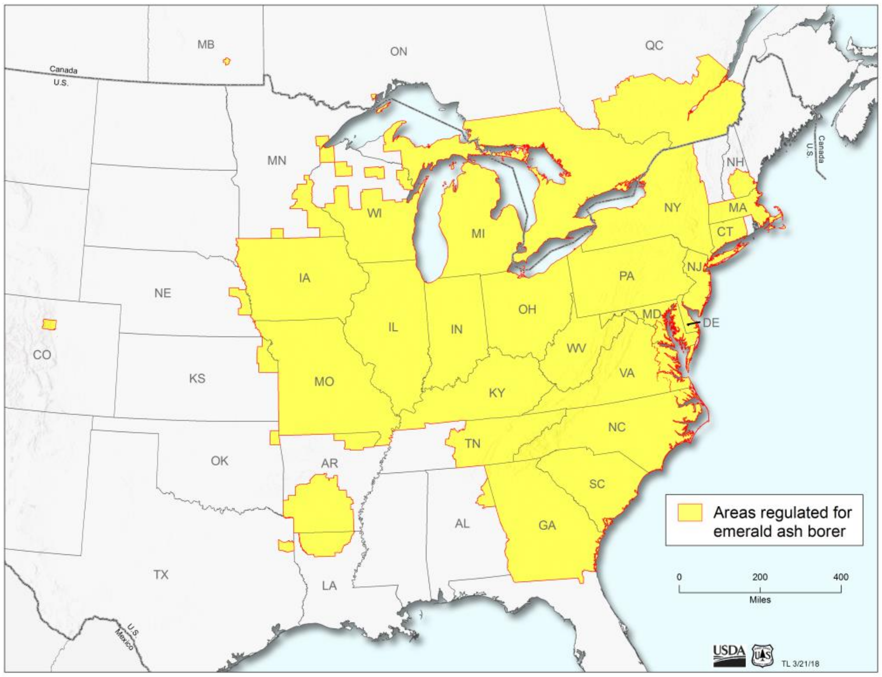
Emerald Ash Borer, *Agrilus planipennis* Fairmaire and Ash trees, *Fraxinus* spp, in New Hampshire

1.1 Emerald Ash Borer (EAB) History in North America

International commerce and trade make introductions of new forest insects and pathogens inevitable. History has documented the importance of imported nursery stock and solid wood packing material as major sources of potentially invasive forest pests (Niemela & Mattson, 1996; NRC, 2002; USDA APHIS-FS, 2000). In addition to EAB, at least 10 nonindigenous forest insects associated with solid wood packing material have been discovered in the United States or Canada since 1990 (Haack, 2005).

Emerald ash borer, *Agrilus planipennis*, was first discovered in North America in southeastern Michigan in 2002 and shortly thereafter in Windsor, Ontario (Haack *et al*., 2002). This wood-boring beetle, which aggressively attacks and kills all species of North American ash (Cappaert *et al*., 2005; Anulewicz *et al*., 2008), was associated with large numbers of dead and dying ash trees, and at the time of initial detection, the infestations at these locations were extensive. Initially, there was very little information on EAB beyond taxonomic descriptions (Jendek, 1994) and a few paragraphs published in Chinese reference books (Chinese Academy of Science, 1986; Yu, 1992). This species originated from the Russian Far East and northeastern China where it is occasionally a minor pest of native ash species. Two native Asian ash species, *Fraxinus mandshurica* Ruprecht and *F. chinensis* Roxburgh, appear to be more resistant to EAB than do the North American species (Rebek *et al*., 2008). Limited reports from Asia suggest that EAB is a secondary pest that is present in low densities and attacks only stressed or declining trees in its native range (Akiyama & Ohmomo, 2000; Schaefer, 2005; Williams *et al*., 2005, 2006). The story in North America is quite different.

Once the implications and severity of this new pest introduction was determined, a quarantine was quickly initiated to prevent movement of the insect through the commercial and recreational transport of ash trees and firewood (Haack *et al*., 2002). A dendrochronological analysis indicated that this beetle was most likely introduced in the early to late 1990's (Cappaert *et al*., 2005; Siegert *et al*., 2014). By 2004 Emerald ash borer had spread to Indiana and Ohio and has since spread throughout southeastern Canada and the eastern United States as far west as Colorado (Map 1). While EAB is capable of short range dispersal via flight (Bauer *et al*., 2004) this rapid range expansion is believed to result from long-distance human-assisted movement of firewood (Jacobi *et al*., 2009) or nursery stock (Sargent *et al*., 2009).



Map 1: Map of known emerald ash borer infested counties and territories as of February 2018. Image credit: N. Siegert.

1.2 Emerald Ash Borer Biology

Emerald ash borer is an inconspicuous insect whose biology and life cycle continue to challenge state and federal regulatory agencies. Challenges include early detection of low density populations, permitting continued movement of wood products while preventing the movement of the pest to new areas, and preserving forested ash through use of biocontrol. Adults emerge from the ash trees during New Hampshire’s summer months, extending from mid-June through early August (Discua, 2013) leaving distinct D-shaped exit holes (2-3 mm in diameter) in the trunk and branches of the trees (Figure 1). Adults live three to six weeks, usually needing about one week of feeding on ash leaves before mating. Damage to trees from adult foliage feeding is minimal (Figure 2).

Figure 1: D-shaped exit hole in an ash tree caused by emerald ash borer. Photo credit: M. Dube.

Figure 2: Emerald ash borer adult feeding damage. Photo credit: N. Siegert.

On average, females are able to produce between 40-70 eggs, with the occasional long-lived individual producing more than 200 eggs (Rutledge & Keena, 2012; Wei *et al*., 2004). These small (1-1.4 mm) eggs are laid within bark cracks and crevices. When freshly laid, eggs are ivory-white to jade-green and slowly turn reddish-yellow to brown after a few days, and hatch in about two weeks (Wang *et al*., 2010). Upon emerging, the larvae bore directly through the outer bark and feed voraciously on phloem, creating serpentine (S-shaped) galleries that eventually girdle the tree, preventing transport of water and nutrients (Figure 3) by the fluid transport systems.

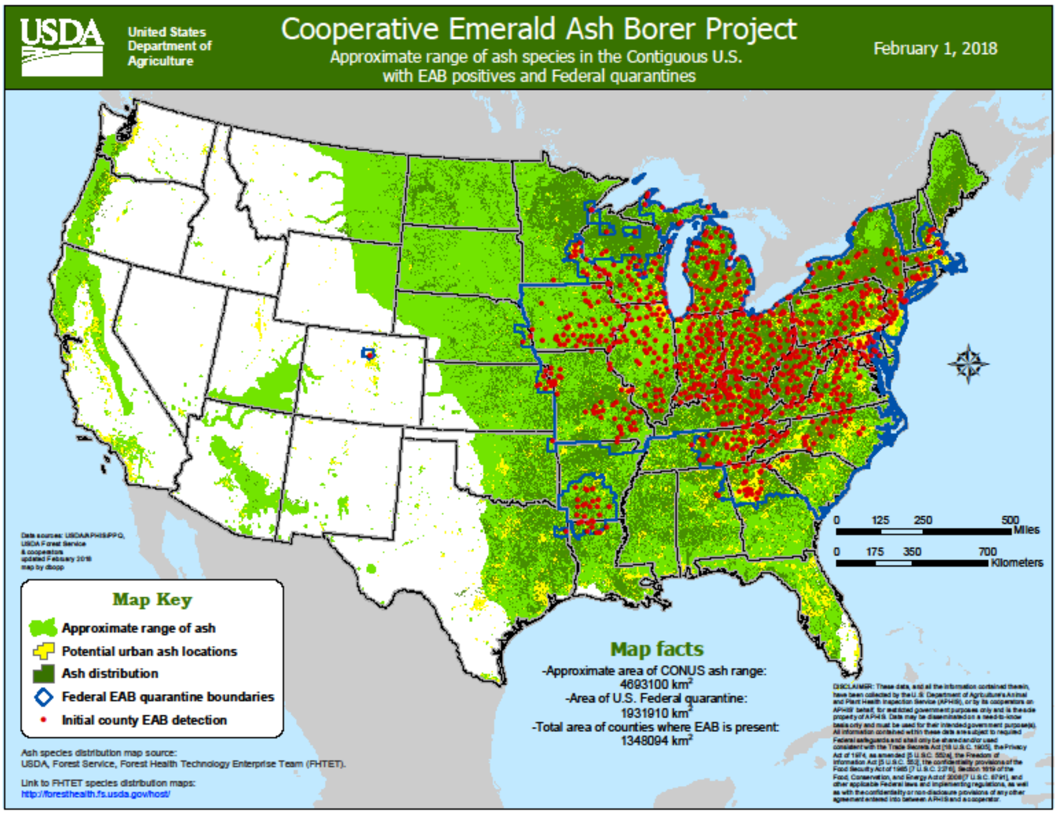
Figure 3: Serpentine gallery created by emerald ash borer larvae in an ash tree. Photo credit: N. Siegert.

Upper portions of the ash canopy are typically infested before the main trunk, therefore increasing the difficulty of early detection. Larvae typically feed from mid-summer into fall, completing four instars (Figure 4). They overwinter as prepupal fourth instars in small chambers in the outer bark or the outer 1-2 cm of sapwood. Most individuals complete their life cycle in 1 yr; however, in early infestations, when trees are healthier, individuals may require 2 years to complete development (Cappaert *et al*., 2005; Siegert *et al*., 2010; Tluczek *et al*., 2011). Pupation normally occurs in middle to late spring and adults emerge shortly thereafter.

Figure 4: Early and late instar emerald ash borer larvae. Photo credit: N. Siegert.

1.3 Ash, *Fraxinus* spp. (Family Oleaceae)

There are sixteen species of *Fraxinus* in North America that are threatened by EAB, with three of these comprising about 6-7% of New Hampshire's forests: white ash, *F. americana* Linnaeus; black ash, *F. nigra* Marshall; and green ash, *F. pennsylvanica* Marshall. Ash trees are a prominent feature throughout much of North America's hardwood forests and associated riparian ecosystems (Poland & McCullough, 2006) (Map 2) and are important in maintenance of the biodiversity of these landscapes. The destruction of large populations of *Fraxinus* species will have severe ecosystem-level consequences (Lovett *et al*., 2006).



Map 2: Map showing ash, *Fraxinus* sp. distribution in the continental United States. Known emerald ash borer infested counties (as of February 2018) are also shown with the initial find in each county marked with a red dot.

For example, *F. americana*, a common species throughout Central and Eastern North America, produces soil organic matter with a low carbon-to-nitrogen ratio and high nitrification rates (Finzi *et al*., 1998; Venterea *et al*., 2003). The loss of this species may have dramatic effects on the carbon and nitrogen cycles within the affected areas. In some forests, near the initial EAB detection in Michigan, more than 99% of ash trees greater than 2.5cm in diameter have been killed (Klooster *et al*., 2014). EAB-caused ash mortality tends to occur synchronously over just a few years (Klooster *et al*., 2014), leading to gaps in tree canopies. Drastic changes in canopies can also have cascading direct and indirect effects on forest ecosystems (i.e. altered understory environments and successional trajectories, spread of invasive plants and increased woody debris (Klooster *et al*., 2014). Ash is also important for approximately 282 arthropod species, of which 43 are monophagous natives in North America and could be at risk of coextirpation as ash declines (Gandhi & Herms, 2010).

Ash is also prominent in North America's urban landscapes (Herms & McCullough, 2014) and when urban canopies begin to decline it can result in the costly removal of hazardous trees. Soon after the arrival of EAB, ash trees in the urban and rural forests of the United States of America were valued at $282 billion (USDA APHIS-FS, 2000). This value has surely changed since that time.

Although there have been studies that show resistance in Asian ash species, such as *F. mandshurica* and *F. chinensis*, these are uncommonly planted in North America. There is hope that if the mechanism of resistance for these Asian species can be identified, it would prove useful in breeding EAB-resistant ash trees (Rebek *et al*., 2008).

Currently, the greatest threat to the continued existence of *Fraxinus* spp. in North America comes from the extensive and pathological infestations of *Agrilus planipennis*, the Emerald ash borer (EAB).

1.4 Current Methods of Control

Current methods of controlling EAB have focused on development of surveillance and detection techniques throughout the range of the beetle hosts to quickly quarantine areas where

EAB is found. Effective protocols are needed for detection of EAB at low density infestations and for monitoring the spread of populations. Visual inspections are widely used during the months of February and March when woodpecker feeding is high and their damage, called "blonding", is more noticeable (Figure 5). Unfortunately, when ashes are fully leafed-out blonding is very difficult to detect since the initial oviposition sites are in the upper canopy of trees (Cappaert *et al*., 2005). Not only are visual inspections limited by time of year, they are also very time-intensive.

Figure 5: Blonding from woodpeckers feeding on emerald ash borer under the bark of an ash tree. Photo credit: N. Siegert.

Various trapping techniques have been explored using attractive colors and odors (Francese *et al*., 2008; Francese *et al*., 2010). Unfortunately, EAB does not appear to use sex or aggregation pheromones but rather responds to olfactory cues such as kairomones, ash tree volatiles, and visual stimulants such as color (Poland *et al*., 2004, 2005; Francese *et al*., 2005). The USDA APHIS Plant Pest Quarantine (PPQ) surveys currently use purple panel traps (prism traps) for the National EAB survey in high risk areas where EAB has not yet been discovered. In 2017 USDA APHIS PPQ hung 18,000 purple panel traps baited with (Z)-3-hexanol nationwide of which 253 were in NH (USDA APHIS PPQ, 2018). Since 2013 the New Hampshire Department of Natural and Cultural Resources (NH DNCR) has conducted its own surveys using green panel traps baited with hexanol or a combination of hexanol and lactone. The New Hampshire Department of Agriculture, Markets & Food (NH DAMF) has used purple panel traps as well as green and black funnel traps baited with hexanol, manuka oil, or a combination of the two for monitoring EAB infestation levels in known populations. Though the large colorful traps have been useful in creating awareness of this invasive pest, they have been outperformed by almost every other trapping or detection method (Careless, 2009; Nalepa & Swink, 2015).

Human girdling of ash trees has been investigated for attractiveness by creating a "trap" that naturally produces odors of a stressed ash tree (Cappaert *et al*., 2005; McCullough *et al*., 2009a). Girdling an ash tree consists of removing a lateral band of bark and phloem, usually with a draw knife, causing stress volatiles to be released (Figure 6).

Figure 6: Ash tree girdling by removing the bark and outer cambium layer where EAB larvae feed with a draw knife. Photo credit: N. Siegert.

McCullough *et al*. (2009b) showed that this method generally captured more adult EAB, and had higher larval densities when compared to methods such as vertical wounding and herbicide treatment as well as outperforming the aforementioned manuka oil lure. Girdling has been shown to work at lower density EAB sites where there are no other visible symptoms of EAB activity. Ash tree girdling is also widely used to attract adult EAB to specific trees that are then destroyed, resulting in large scale EAB population reductions (Mercader *et al*., 2011). This method is effective for slowing the rate of infestation but, unfortunately, is labor intensive and should only implemented by trained sawyers.

1.5 Surveillance Using *Cerceris fumipennis*

Cerceris fumipennis, a solitary wasp that is a colonial nester in hard-packed sand, is recognized as an extremely effective and simple technique for sampling a local buprestid fauna that may include EAB (Marshall *et al*., 2005). *Cerceris fumipennis* outperforms the purple prism traps in detecting presence of buprestid species when the beetles are in small and diffuse populations (Marshall *et al*., 2005). *Cerceris fumipennis* specializes in catching, stinging, and paralyzing beetle species of the family Buprestidae, the metallic wood-boring beetles (Figure 7). Prey are then brought back to the nests to become a living source of food for the developing wasp larvae (Evans, 1971). The ease of intercepting wasps returning with prey allows us to document the presence of buprestid species that are typically elusive and difficult to survey.

Figure 7: A *Cerceris fumipennis* female with a *Dicerca* sp prey in its grasp. Photo credit: P. Careless.

Intercepting wasps not only aids in documenting diversity of the local buprestid fauna, but also has great potential for being a useful tool for detection of EAB and other potentially destructive buprestids. There has been a significant increase in recent research on *C. fumipennis*, particularly because of its affinity for members of the family Buprestidae, including several non-native species, such as EAB, that are potential threats to forest health (Poland & McCullough, 2006), *Agrilus auroguttatus* Shaeffer (Lopez & Hoddle, 2011), *A. sulcicollis* Lacordaire (Jendek & Grebennikov, 2009), *A. prionurus* Chevrolat (Wellso & Jackman, 2006) as well as several species of interest due to their potential for introduction from Eurasia such as *A. biguttatus* Fabricius (Kimoto & Duthie-Holt, 2006), *A. viridis* Linnaeus (Corte *et al*., 2009), *Phaenops cyanea* Fabricius (Wermelinger *et al*., 2008), *Melanophila picta* Pallas, and *Poecilonota variolosa* Paykull (Kezheng, 1996). Monitoring aggregations of *C. fumipennis* has allowed researchers to gain a better understanding of local biodiversity and seasonality within the family Buprestidae, information that would have been challenging to obtain until recently. *Cerceris fumipennis* aggregations have been used in all New England states as well as DE, FL, GA, IA, IL, KS, KY, LA, MD, MI, MN, MO, NC, ND, NJ, NY, OH, PA, TN, TX, VA, WI, and WV for bio-surveillance of emerald ash borer, other invasive forest pests, or for sampling of local buprestid faunas.

1.6 Lack of Knowledge

Of the eight New World buprestid-hunting species of *Cerceris* in North America, *C. fumipennis* is the only species of this genus east of the Rocky Mountains. To efficiently utilize *C. fumipennis* as a monitoring and collection tool for buprestids, regional baseline information on aggregation activity, seasonality, paralyzation rate, and prey preference across different forest types is needed for New Hampshire.

This study focused on documenting the basic biology of *C. fumipennis* which has not yet been documented for New Hampshire and is needed to better use long-term pest monitoring.

CHAPTER 2

Cerceris fumipennis

Cerceris fumipennis is a solitary ground nesting wasp that provisions its nest with both native and non-native species of Buprestidae (Scullen & Wold, 1969; Marshall *et al*., 2005). It has been known in the United States for over 170 years and is found throughout the continental United States east of the Rocky Mountains, from Texas to Florida and north to Maine and Wyoming. It is also known in southern Quebec and Ontario, Canada. Currently, *C. fumipennis* is the most studied member of its genus dating back to work published by J. A. Grossbeck (1912), and is the only known species of buprestid-hunting Crabronidae in the eastern United States.

2.1 Biology

Female *C. fumipennis* are “mass provisioning” Crabronid wasps that only provision one brood cell at a time (Careless 2009). Once an adult female provisions a cell with a sufficient number of beetles she will immediately lay a single white, sausage-shaped egg on the mesosternum of a beetle (Figure 8).

Figure 8: An excavated *Dicerca* sp. with a Cerceris fumipennis egg layed along the mesosternum. Photo credit: P. Careless.

The larva emerges from the egg after two to three days, and will consume all of its provisioned prey within five to ten days (Careless, 2009). Portions of the highly sclerotized exoskeleton are left behind and indicate cell locations during nest excavations. A common threat to successful egg and larval development is parasitism by various kleptoparasitic miltogrammine flies (Sarcophagidae) and velvet ants (Mutillidae) (Kurczewski & Miller, 1984; Hook & Evans, 1991). The final instar occurs between seven and thirteen days after oviposition, and a cocoon is formed (Evans, 1963). The larva then remains in a quiescent prepupal phase within the cocoon for up to nine months (Evans & O'Neil, 2007). There is a three to four week period in the pupal stage, from late May to late June, even extending to early August. Adults then will begin to emerge by excavating their own exit tunnel to the soil surface (Careless, 2009). Emergence dates can vary within the aggregations, between aggregations, and throughout North America, but emergence in New Hampshire is most likely with a soil degree-day accumulation of 696.2 ±16.8° C (base temperature of 10° C) (Rutledge *et al*., 2015).

Throughout most of the northeastern United States *C. fumipennis* is univoltine, having only one generation per year, but research has demonstrated that Florida's warm climate facilitates emergence in early April and late May, with the lengthy period of warm temperature there allowing the species to be bivoltine (Evans, 1963; Mueller *et al*., 1992). Males of *C. fumipennis* tend to emerge before females and never reenter their exit hole. Adult males will spend the following months near the aggregation visiting flowers for nectar and attempting to intercept and mate with passing females (Evans, 1971; Alcock *et al*., 1978).

2.2 Nest Structure and Nesting Behavior

Each adult female *C. fumipennis* builds a nest by digging a tunnel in the ground perpendicular to the soil surface. Most nests have a small circular mound of earth 1 – 2 cm high, a tumulus, surrounding the entrance. The entrance, usually 2 – 6 cm in circumference, is left open while the female is provisioning. Short lateral tunnels are then constructed at varying distances from the opening and end in a smooth oval cell that serves as a depository for the beetle prey and as a growth chamber for the larva (a brood cell). The adult females then locate, catch, and paralyze the desired beetle individuals that are then deposited within the cell (Scullen & Wold, 1969).

Studies show that typically each burrow can have 5-13 cells, but as many as 24 cells has been reported (Mueller *et al*., 1992; Hook & Evans, 1991; Kurczewski & Miller, 1984; Evans & Rubink, 1978; Evans, 1971). Time spent in the egg, larval, and pupal stages is approximately 10 months (Careless, 2009), all spent within the confines of these cells, located 10 to 20 cm below the surface (Evans, 1971; Kurczewski & Miller, 1984). Once a cell is provisioned with 2-18 prey items (depending on prey size), the female lays an egg on the ventral surface of one of the paralyzed beetle prey (Figure 8). Cell entrances are back-filled with 6-15 cm of soil before the wasp moves on to construct other cells from the main burrow (Careless, 2009). Late in the summer females stop provisioning their nests, and backfill the nest entrance until they die of exhaustion or exposure to the elements (Careless, 2009). Dead females are sometimes found on the ground near other nests or in a slight indentation in the substrate where there may have been a nest entrance at one time. At other times, especially late in the season, nests are abandoned and have evidence of webbing, possibly from opportunistic Araneae.

2.3 Nest and Aggregation Habitat and New Hampshire Distribution

There is much variation in the composition of the substrate in which *C. fumipennis* will nest, ranging from fine-grained, friable sand to hard-packed sandy clay, or occasionally gravel. These nests are usually made in a location attractive to other individuals of this species, forming aggregations or loose "colonies" of 2 to more than 200 nests per site. These aggregations are usually located where there is a considerable expanse of desirable soil type surrounded by trees or bushes where buprestid beetles are plentiful. Since the family Buprestidae consists of species that as a group feed on 31 different genera of trees or woody herbaceous plants (Paiero *et al*., 2012), these wasp aggregations could be successful near many different forest types with the proper soil substrate.

Often there are a variety of other hymenopteran groups sharing the nest sites us by *C. fumipennis*. Members of Mutillidae, Formicidae, Sphecidae, and Apoidea were seen at both study sites during this project. Two species, *Dasymutilla snoworum* Cockerell and *D. scaevola*

Blake are known parasites of *C. fumipennis* (Evans & Rubink, 1978; Hook & Evans, 1991; Careless, 2009). *D. scaevola* occurs in New Hampshire but was not seen at the study sites. Females of a closely related species of *Dasymutilla* were frequently seen entering, exiting, and digging near *C. fumipennis* nests, and males were observed in flight close to the ground or resting on low-level vegetation. These were most likely *D. nigripes* Fabricius or *D. vesta* Cresson based on comparison with identified specimens in the UNH Insect Collection. Tiger beetles (Carabidae: Cicindelinae) were also found nesting in the *C. fumipennis* aggregations.

New Hampshire has more than 100 known *C. fumipennis* aggregations ranging in size from two to greater than 200 nests (NH DNCR & DAMF, unpublished data). These data were collected over a nine-year period (2008-2016) by the NH DNCR and NH DAMF. These New Hampshire aggregations have been used for the past nine years to aid in the discovery of low density emerald ash borer infestations. Monitoring of the aggregations commenced in July and continued through August, based on seasonality data originally collected in Canada and Florida. There has been limited research regarding emergence and seasonality of *C. fumipennis* in New Hampshire. Known New Hampshire *Cerceris* aggregations range from latitudes 42.73314˚N to 44.05166˚N and longitudes 70.7522˚W to 72.4319˚W. The elevation range of the aggregations in New Hampshire range from 4 m to 551 m.

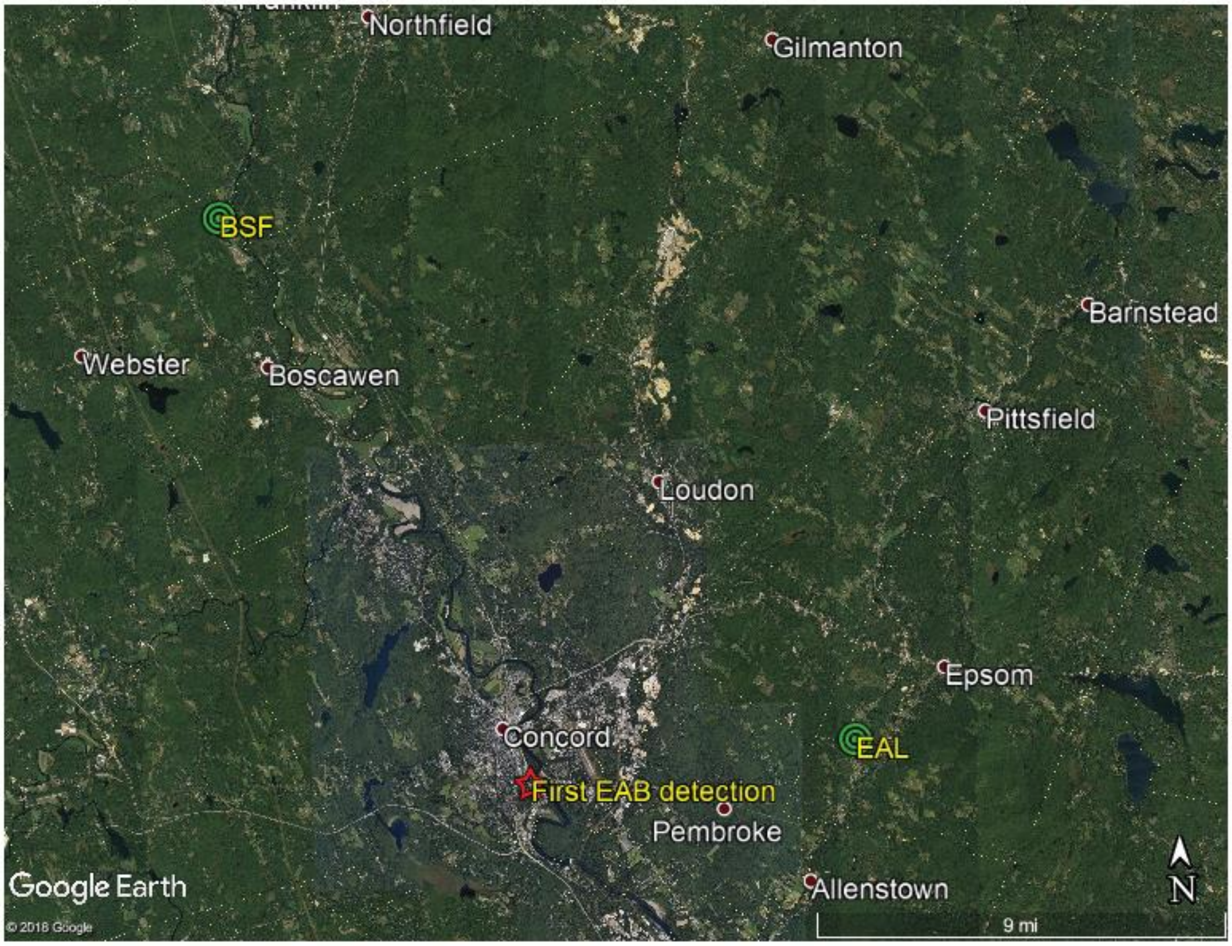
Two aggregations in New Hampshire, consisting of 200 or more *C. fumipennis* nests, were located during previous studies funded by the US Forest Service. These sites served as ideal locations for the objectives of this project. My goal was to determine if *C. fumipennis* is a productive and useful tool for monitoring native and non-native species of buprestid beetles. My objectives were to 1) increase our knowledge of the biodiversity and seasonality of New Hampshire's buprestid fauna, 2) provide a better understanding of the seasonality and prey selection of *C. fumipennis*, 3) determine if forest composition is accurately reflected by the prey brought back to the aggregation by *C. fumipennis*, 4) determine *C. fumipennis'* rate of paralyzation of the available prey species, and 5) monitor for the invasive emerald ash borer, previously not known to occur in New Hampshire.

CHAPTER 3

Methods & Materials

3.1 *Cerceris fumipennis* Aggregation Site Descriptions

The Epsom American Legion parking lot (EAL) and the upper fields of Boscawen State Forest Nursery (BSF) were chosen due to the large size of their aggregations (greater than 200 nests) and their proximity to the recently discovered New Hampshire EAB infestation in Concord (43.190655°N, 71.525646°W) (Map 3). Both sites had been monitored previously by NH Division of Forest and Lands during *C. fumipennis* surveys for EAB. Collection data are on file at the NH Division of Forest and Lands office located at Fox State Forest Nursery in Hillsborough, NH.



Map 3: Initial emerald ash borer detection and both research sites, Boscawen State Forest (BSF) and Epsom American Legion (EAL).

Epsom American Legion parking lot (EAL): 43.202307°N, 71.384829°W (Map 4)

The EAL site had been previously monitored by NH DNCR in 2012 and 2013. The Natural Resource Conservation Service (NRCS, 2018) soil survey provided the following information. The *Cerceris* monitoring site consists of mostly loamy sand with 3-15% slopes. The loose sandy glaciofluvial deposits are from granite, and schist or gneiss and are well drained. This site is 102.4 m above sea level and is approximately 550 m^2 , with a rectangular perimeter of approximately 100 m. The mean annual precipitation is 91-180 cm and the site remains frost-free for 140-240 days of the year (NRCS, 2017). The nearest body of water is the Suncook River 193 m to the east.

Map 4: Epsom American Legion research site.

Boscawen State Forest Nursery upper fields (BSF): 43.371979°N, 71.656961°W (Map 5)

The BSF aggregation had been previously monitored by NH DNCR in 2012 and 2013. The NRCS classifies this area as fine sandy loam with 15-60% slopes. This sandy outwash is derived mainly from granite, gneiss and schist and is well-drained. This site is 115 m above sea level and is approximately 2,000 m^2, with a perimeter of approximately 250 m. The mean annual precipitation is 100-127 cm and the site remains frost free for 90-135 days (NRCS, 2017). The nearest body of water is the Merrimack River 395 m to the east.

Map 5: Boscawen State Forest research site

Determination of forest composition

Forest composition was documented by walking three 0.5 km transects away from both the BSF and EAL sites. The Boscawen SF transects ran north, east, and west. A southern transect was not conducted due to the 0.3 x 0.4 km area of fields abutting the aggregation to the south. The EAL transects ran east, south, and west. A northern transect was not surveyed due to a 0.25 x 0.25 km housing development to the north containing very few trees. The method used for tree sampling was a modified point-centered quarter density method (Cottam & Curtis, 1956). There were 6-7 tree sampling points taken along each transect depending on terrain. At each sampling point along the transect 1 tree was identified from each quadrant surrounding the surveyor (Figure 9).

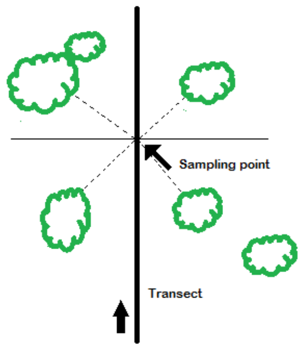


Figure 9: Diagram of point-centered quarter method for determining forest composition showing transect that was walked, and the point where the observer stopped and identified the closest tree species in each quadrant. Method modified from the point-centered quarter density method for determining forest density (Cottam and Curtis 1956).

The majority of *C. fumipennis* buprestid prey are generalist feeders on either coniferous or deciduous trees. There are few buprestids that are specific to a single host, so that although tree identifications were made to genus, comparisons of prey between the two surrounding forests were only made by placing coniferous or deciduous tree species into separate pools, and percentage was based on proportions of tree abundances at this level.

3.2 2013 Field Season

Set up

Monitoring of the EAL and BSF *C. fumipennis* sites began the third week of June, the earliest time known for *C. fumipennis* to emerge in the northeastern United States (Careless *et al.*, 2013). Both sites were monitored twice a week for 6-8 hours during each visit from July through August. Monitoring began between 9am and 11am and ended between 3pm and 6pm. Weather (temperature, wind speed and direction, and precipitation), both forecasted and current, and wasp activity were documented as well as daily monitoring duration with start and finish times.

Foraging times

For this project, foraging is considered to be the time a female spends away from her nest before returning with a prey item. This time is most likely spent collecting prey items to provision nest cells, but mating or feeding also could have occurred during this time. The time allotted to each of these activities cannot be determined so here it is treated as foraging as long as the female returned with prey. To record foraging time 9-14 nests were chosen at random at the start of each monitoring period. Each nest had a transparent 0.5 L numbered plastic cup placed over the entrance to capture or delay female *C. fumipennis* as they left from and returned to their nests. This protocol is based on the work of Careless (2009) in Ontario, Canada. The cups were vented on four sides by evenly spaced windows approximately 2.5 cm wide by 7.5 cm tall (Figure 10). These windows were screened with a 14-by-2.5 (14 squares per 2.5cm) cloth mesh so that air could flow through the cup and the females would not become overheated before the time was recorded and the wasp released to forage.

Figure 10: Clear plastic vented cup placed over *C. fumipennis* nest with tumulus.

Once the cups were placed over the randomly selected nests females were allowed to enter or leave their nest only with assistance. When a female was observed attempting to leave the nest by flying up into the cup it was flipped over and the female flew away. Wasp number and time was then recorded (i.e. #12 out @ 2:10pm). Time, wasp number, and presence/absence of prey were recorded each time a female tried to reenter the nest (i.e. #12 in @ 2:43pm, with prey).

After the wasp indicated preference for a nest, i.e., landing on a cup or circling a specific cup more than twice, the cup was slowly removed or tipped over gently to allow the wasp to enter.

Too much movement during this time could scare a female away but she would usually return a few minutes later. If a female returned to the nest with prey in her grasp she was either intercepted with an aerial net or, depending on the size of her prey burden, encouraged to drop the beetle by gently pressing on the dorsal surface of her thorax and abdomen once she had landed (Figure 7). The prey was then collected and placed in a snack-sized re-closable plastic bag along with a label documenting cup number, time and date.

Additionally, 3 hours of each site visit were used to intercept prey brought back by other foraging females in the aggregation to better document *C. fumipennis* prey species richness, abundance, and seasonality in NH. Interceptions were conducted at distances greater than 2 m from the cupped nests to prevent disruption in documentation of foraging times for the monitored individuals. Careless (2009) determined that a minimum of 50 prey individuals for each week is adequate for detecting changes in prey seasonality over the course of a season.

At the beginning, and occasionally throughout the monitoring period, “drops” were collected at the sites. *Cerceris fumipennis* females have been known to drop their prey near their nests if they are startled, or if the prey has been compromised in some way by an nearby awaiting parasite. Each site was visually searched in its entirety for these drops from a height of less than 1 meter at least once during the monitoring day, and drops were also randomly collected as they were found during normal monitoring procedures. These specimens were also placed in snack-sized re-closable plastic bags with a label documenting collection method (i.e. drop or catch), time and date. All collected specimens were placed in a cooler until they could be properly preserved. Specimens were frozen at the end of every collection day pending identification. Prey species were identified using keys (Bright, 1987; Wellso & Manley, 2007;

Paiero *et al*., 2012), or by comparison with specimens in the UNH collection or from a voucher collection of *C. fumipennis* prey items taken during previous studies.

Sampling days were only considered productive if more than 15 individual prey were collected as well as a minimum of three different species. Days that were not productive were usually due to high winds (>10 mph) or cooler temperatures (<70˚F). Data from these non-productive days were still used to help estimate foraging times and total species richness and abundances for each site but only data from the productive sampling days were used in documenting seasonality of the wasps and beetles. Buprestid seasonality was determined for the following most common species (> 10 individuals collected throughout the season) collected at the sites; *Agrilus anxius* Gory, *A. arcuatus* Say, *A bilineatus* Weber, *Buprestis striata* Fabricius, *Chrysobothris femorata* Olivier, *C. rotundicollis* Gory and Laporte, *C. sexsignata* Say, *Dicerca divericata* Say, *Eupristocerus cogitans* Weber, and *Neochlamisus bebbianae* Brown (Coleoptera: Chrysomelidae). Weekly averages were calculated for these species to show how their abundances changed over a seven-week period. Heavy rain and strong winds during week 3 (collection dates July 24 and 25, 2013) caused abnormally low abundances during that period, therefore week three data were not used for these calculations.

3.3 Field Season 2014

Prey rates of paralyzation and prey preference

The EAL aggregation was monitored for a shorter period in 2014 than in 2013 due to more concentrated effort on observations of prey paralyzation and preferences rather than *C. fumipennis* and buprestid seasonality. The site was monitored from July 13 to August 17, 2014, and was visited one to two days a week or five times a month. Sites were monitored 4-6 hours on each visit. Only the EAL aggregation was monitored for this project during the 2014field season. Twenty-five wasps were successfully marked with identifying dot color patterns and nests were numbered to correspond to each dot patterned female. No other wasps were used during this field season. The vented 0.5 L transparent plastic cups were placed over the numbered nests and prey was collected from an incoming wasp once it had displayed preference for a nest, i.e. landing on a cup or circling a specific cup more than twice. If a female returned to the nest with prey in her grasp she was either intercepted with an aerial net or was encouraged to drop the beetle by gently pressing on the dorsal surface of the wasp's thorax and abdomen once she had landed. Prey was then collected and placed in a snack-sized re-closable plastic bag with a label documenting cup number, time, and date. Before the prey was placed in a cooler for storage, each specimen was observed in its plastic bag for one hour after collection to determine if it was successfully paralyzed. Prey were considered unparalyzed if they displayed wing movement or crawling in the bag. Specimens were frozen at the end of each collection day to await identification.

The seven buprestid species with some unparalyzed individuals were: *A. anxius*, *A. arcuatus*, *A. bilineatus, C. femorata*, *C. sexsignata*, *D. divaricata*, *E. cogitans*, plus *N. bebbianae*. Ten specimens of each of these species were weighed using a 0.001 g resolution analytical scale to calculate an average weight for these species that were most frequently unparalyzed. Average weights were then graphed against percent paralyzation to determine a correlation coefficient. Rates of prey paralyzation were determined only for these seven species.

Changes in prey preference were analyzed for the 13 individual female wasps that returned to the nests with prey more than once per season. There were twelve wasps that returned to the nest with only one prey item throughout this monitoring season, and thus these wasps were not used in data analysis for changes in prey preference. Prey diversity index was calculated for the 13 female wasps. A prey diversity index of 1 means there was no variation in the species collected and an index of 0 means there was great diversity among the species of prey collected by one wasp.

CHAPTER 4

Results

4.1 Prey Species & Abundances

In 2013 853 individual beetles consisting of 35 different species were collected from July 11 to August 21, 2013 (Table 1). At BSF *C. fumipennis* females brought in 396 individuals and 26 different species on nine non-consecutive sampling days. At EAL the wasps brought in 457 individuals of 31 different species taken during 11 sampling days. The chrysomelid *N. bebbianae* was the only non-member of the family Buprestidae collected by *C. fumipennis* during this field season. There were 81 individuals of *N. bebbianae* collected throughout the sampling period, 50 from BSF and 31 from EAL.

Table 1: List of *Cerceris fumipennis* prey species and abundances at Boscawen State Forest and Epsom, NH site from July through August 2013.

Species	**Boscawen total**	**Epsom total**	**total**
Buprestidae			
Dicerca divaricata	188	236	424
Agrilus anxius	43	53	96
*Chrysobothris rotundicollis**	9	17	26
Chrysobothris sexsignata	13	10	23
Buprestis striata	11	10	21
Agrilus arcuatus	11	9	20
*Dicerca tuberculata**	7	7	14
Chrysobothris femorata	9	5	14
Dicerca caudata	1	12	13
Chrysobothris verdigripennis†*	7	5	12

*Agrilus pseudocoryli**	8	4	12
*Eupristocerus cogitans**	0	11	11
*Agrilus carpini**†	4	7	11
Agrilus bilineatus	6	4	10
*Phaenops fulvoguttata**	6	4	10
*Dicerca punctulata**	1	8	9
Agrilus politus	5	3	8
*Buprestis consularis**	0	6	6
Dicerca tenebrica	3	2	5
Poecilonata cyanipes	4	1	5
*Chrysobothris harrisi**	2	1	3
Chrysobothris scabripennis	2	1	3
Brachys ovatus	0	2	2
*Dicerca pugionata**	0	2	2
Chrysobothris dentipes	1	1	2
*Dicerca asperata**	2	0	2
*Actenodes acornis**†	0	1	1
*Agrilus corylicola**	0	1	1
Agrilus ruficollis	0	1	1
Brachys aerosus	0	1	1
*Chrysobothris adelpha**	0	1	1
Buprestis maculativentris	1	0	1
*Chrysobothris neopusilla**	1	0	1
*Phaenops aeneola**	1	0	1
Chrysomelidae			
Neochlamisus bebbianae	50	31	81
Total Number of Individuals	396	457	853
Total Number of Species	26	31	35

* uncommon, rare, or infrequently collected; † new NH state record

Prey abundances by date are shown in Table 2. Peak species richness of prey was observed on July 17 (19 species) at EAL and July 18 (15 species) at BSF.

Table 2: Prey abundances and species richness at EAL and BSF by week and date.

			Abundances		
Week	**Date**	**Julian Day**	**BSF**	**EAL**	**Species Richness**
1	7/11/2013	192		21	6
2	7/16/2013	197	67		13

2	7/17/2013	198		89	19
2	7/18/2013	199	117		15
4	7/28/2013	209		70	14
4	7/29/2013	210	42		7
4	7/30/2013	211		45	9
4	7/31/2013	212		83	13
4	8/1/2013	213	21		6
5	8/6/2013	218		45	11
5	8/7/2013	219	41		9
6	8/12/2013	224		47	8
6	8/15/2013	227		15	4
7	8/19/2013	231	58		5
7	8/20/2013	232		42	5
7	8/21/2013	233	50		6
	Total		396	457	
			total EAL species richness		31
			total BSF species richness		26

4.2 Prey Preferences in Different Forest Types

The surrounding forest at BSF was a well-mixed forest consisting of 51% deciduous tree species and 49% coniferous tree species (Figure 11). This forest contained 39% *Tsuga canadensis* and 22% *Fagus grandifolia* with the occasional *Acer*, *Pinus*, or *Quercus* spp. (all <10%) present at the sampling points along the transects. Boscawen SF also had more beetle individuals that are known to feed on *Tsuga canadensis* than EAL (50:39 individuals), while species richness for *T. canadensis* feeding species was not greater at BSF than at EAL (5:6 species).

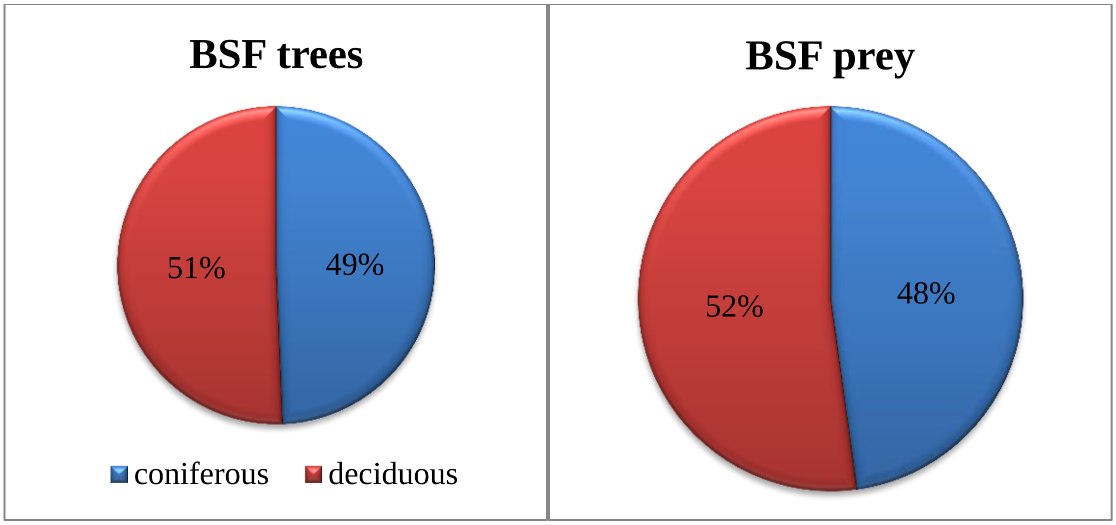


Figure 11: Pie charts showing the percent of coniferous and deciduous trees at BSF and percent of prey with coniferous and deciduous hosts.

The surrounding forest at EAL mostly consisted of *Acer* (34%) and *Pinus* (30%), but was primarily a deciduous forest (66%) (Figure 12). There was a greater abundance of beetle prey known to feed on *Acer* spp. tree species collected at EAL than at BSF (275:226 individuals). Species richness for *Acer* spp. feeding beetles was very similar between BSF and EAL (3:4 species). Species richness for species that feed on *Pinus* and *Quercus* was similar at BSF and EAL (*Pinus* = 9:10 species, *Quercus* = 4:5 species).

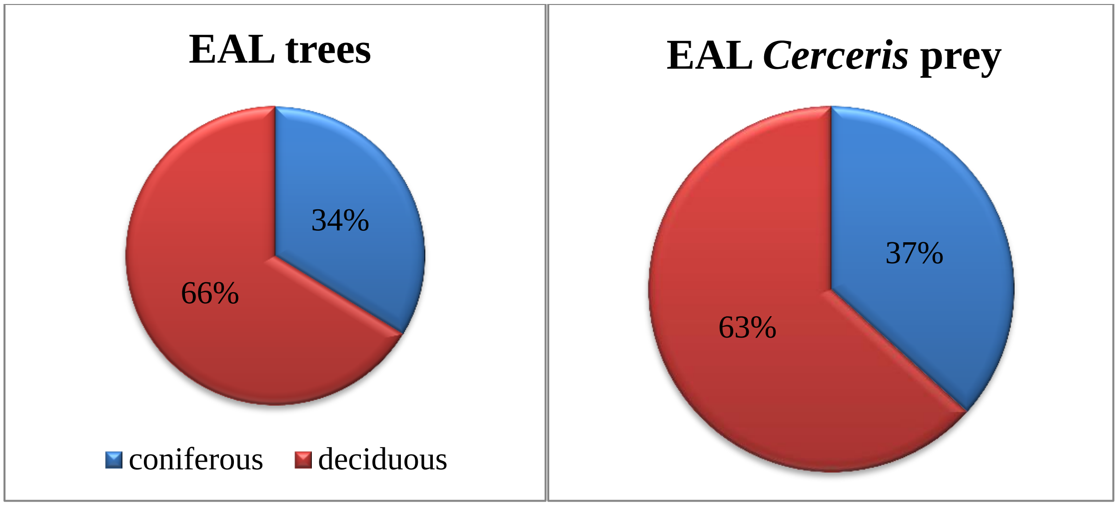


Figure 12: Pie charts showing the percent of coniferous and deciduous forest composition at EAL and percent of prey with coniferous and deciduous hosts.

4.3 Seasonality of *C. fumipennis* and its Buprestid Prey

Seasonality of C. fumipennis

A single male *C. fumipennis* was observed on June 26, 2013 with no other individuals seen that day. During the next two weeks, up to July 9, females emerged and were active, but none were observed returning to their nests with prey. Mating was observed in the tree canopies along the margin of the aggregations, probably not far from where they can find nectar or pollen on which to feed. On July 9 there were approximately 150 nests open at both BSF and EAL. Most female wasps were either hovering approximately 0.5 m above the ground or were visible at the entrance of their nests. New nest construction and emergences continued to occur during these two weeks.

On July 11 there were more than 200 nests at both BSF and EAL, and females were observed returning to their nests with prey at both sites. Wasp interceptions at EAL started on this date, with 21 individual beetle specimens collected representing six different species. Both aggregations were active after sampling ended on August 22. On August 31, there were 62 open nests remaining at BSF and 41 open nests remaining at EAL. Three *C. fumipennis* females were observed dead just inside or just outside their nests at BSF, and one dead adult was seen at EAL. Although females were observed in flight, none brought prey back to their nests.

Seasonality of buprestid prey

Buprestid seasonality was determined for the ten most commonly collected beetles, as well as *Phaenops aeneola* Melsheimer and *P. fulvoguttata* Harris because they were only documented during week 2. These collection data are separated into two figures with abundances from both study sites pooled, and shown with their corresponding Julian date to simplify the figures. Figure 13 displays seasonality for the five most frequently collected species and Figure 14 for the next five most frequently collected species. *Dicerca divaricata* was the most abundant of all the beetle species collected (424 individuals of 853) (Table 1). This species had two peaks during the sampling season, one on Julian day 198 during week 2 (mid-July), and another on day 233 during week 7 (mid-August), (Figure 13, x = 47 individuals/day and x = 23 ind/day respectively, See Table 2 for weeks and corresponding dates). *Agrilus anxius* was collected 110 times and was most abundant from Julian days 198 to 219, weeks 2-5 (9-14 ind/day). By Julian day 224 in week 6 there was only one *A. anxius* collected in a day and none by Julian day 233, week 7. *Buprestis striata* was also prevalent early, with a peak spanning Julian days 191 to 198, week 1-2 (x = 6, x = 5), without any individuals observed for the rest of the season. *Neochlamisus bebbianae* was not collected until week four (one individual) but abundance peaked around Julian day 233, during week 7 with a mean of 19 individuals per day. *Agrilus* *arcuatus* had two peaks, one on Julian day 212 during week 5 and the other on day 233 in week 7 (x = 6, x = 5 ind/day, Figure 13).

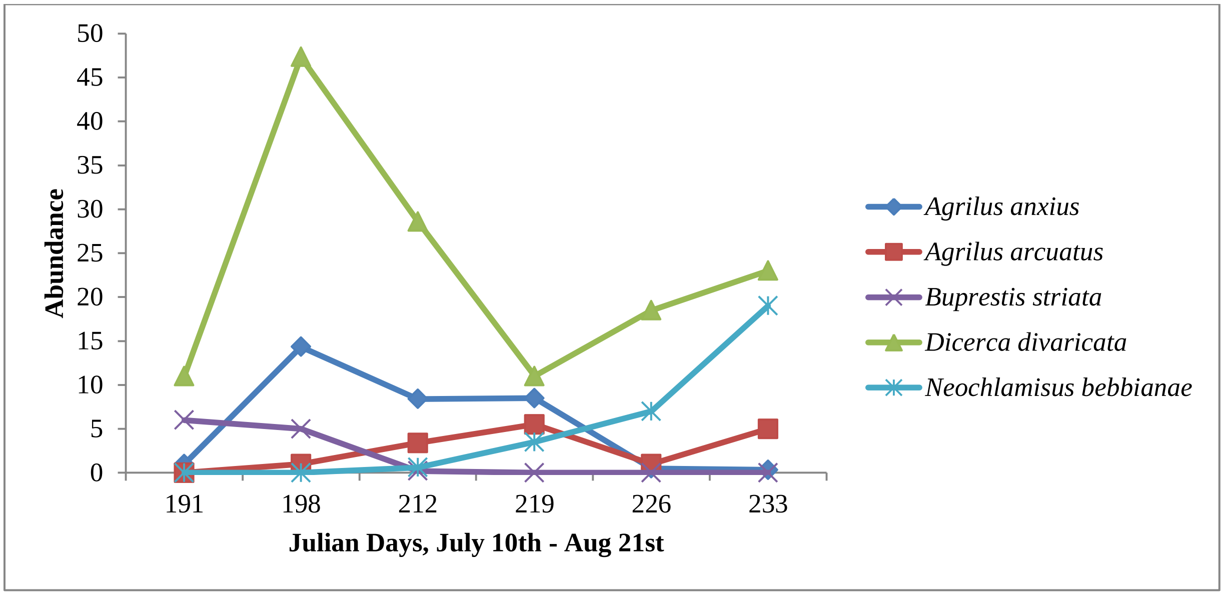


Figure 13: Graph showing weekly average prey abundances for the 5 most frequently collected prey species.

C. rotundicollis was not observed until Julian day 198, week 2 (Figure 14) with a mean of 4 individuals a day, but did not appear again until after Julian day 219 in week 5. *Agrilus bilineatus* was not collected until day 209 in week 4 (one individual), and peaked on day 219 in week 5 (x = 3 ind/day). *Chrysobothris sexsignata* also had two peaks on days 198 and 219, week 2 and week 5 (x = 5, x = 6 ind/day), and none were observed by day 233, week 7. *Chrysobothris femorata* peaked during week 2 (x = 3 ind/day) as well, but declined to 1 ind/day for the consecutive weeks and was absent by week 7 (Figure 14).

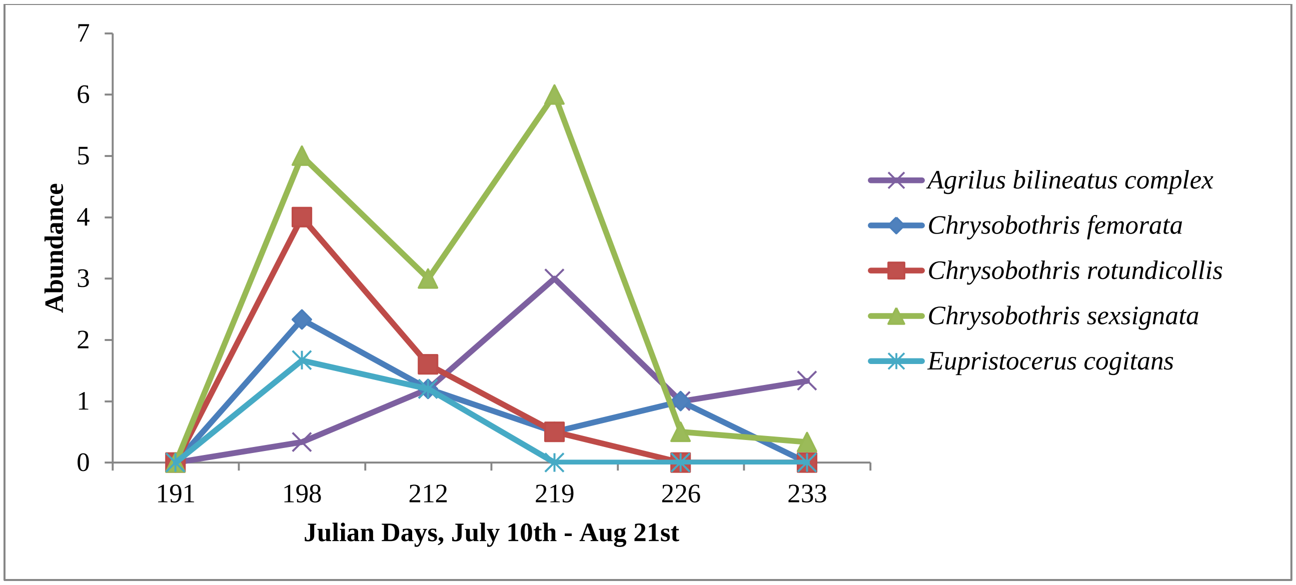


Figure 14: Weekly average prey abundances for the next 5 most frequently collected prey species.

Phaenops aeneola and *P. fulvoguttata* were observed only during week 2 with abundances of 1 and 2, respectively (Table 1). *Eupristocerus cogitans* was only observed between weeks 2 and 4 (2 and 1, respectively), and was not observed thereafter during the field season (Table 1).

4.4 Provisioning Rate & Foraging Times

There was an average of 1.9 prey collected by an individual wasp per monitoring period. Five females came back with prey five times in one monitoring day, seven females returned with prey four times in one day, 13 females returned with prey three times, and 24 females returned with prey two times during a day. There were 50 females that returned to their nest with prey once during an entire day (Figure 15).

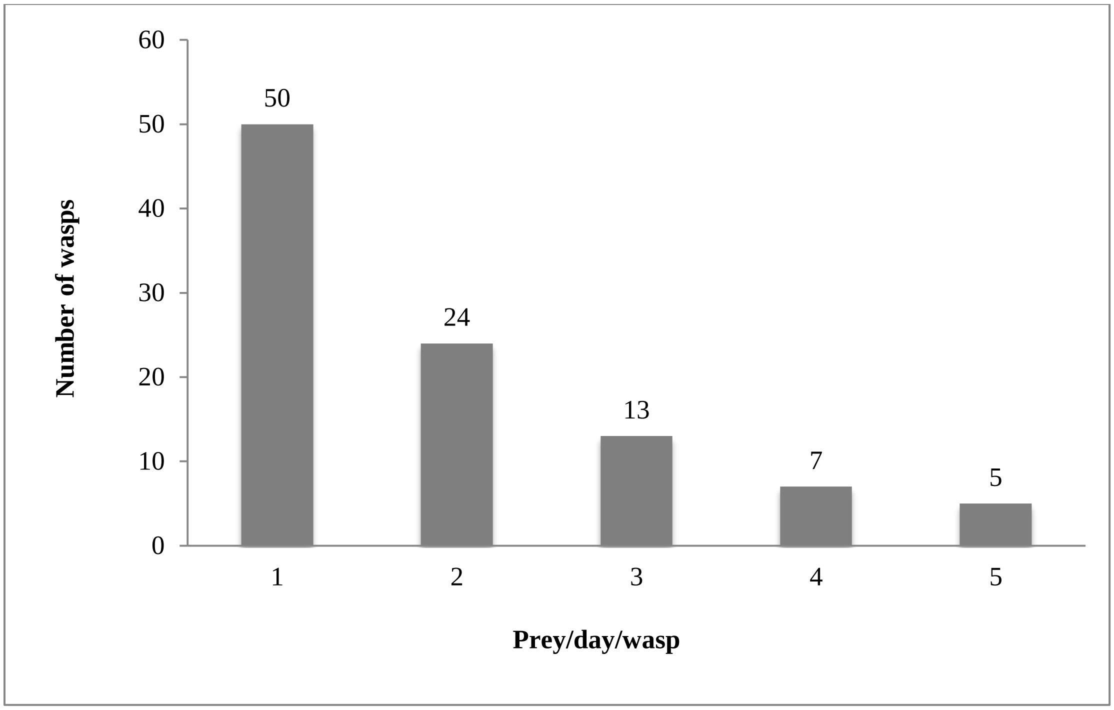


Figure 15: Prey per day collected from foraging *C. fumipennis* females. Mean prey per day was 1.9.

Wasp foraging times ranged from 6 to 217 minutes (Figure 16). The average foraging time for a female *C. fumipennis* that returned with a prey beetle was 47 minutes. Fifty-two females, 40%, returned to their nests with prey within 30 minutes. Ninety-seven females, ~75%, returned to their nest within one hour.

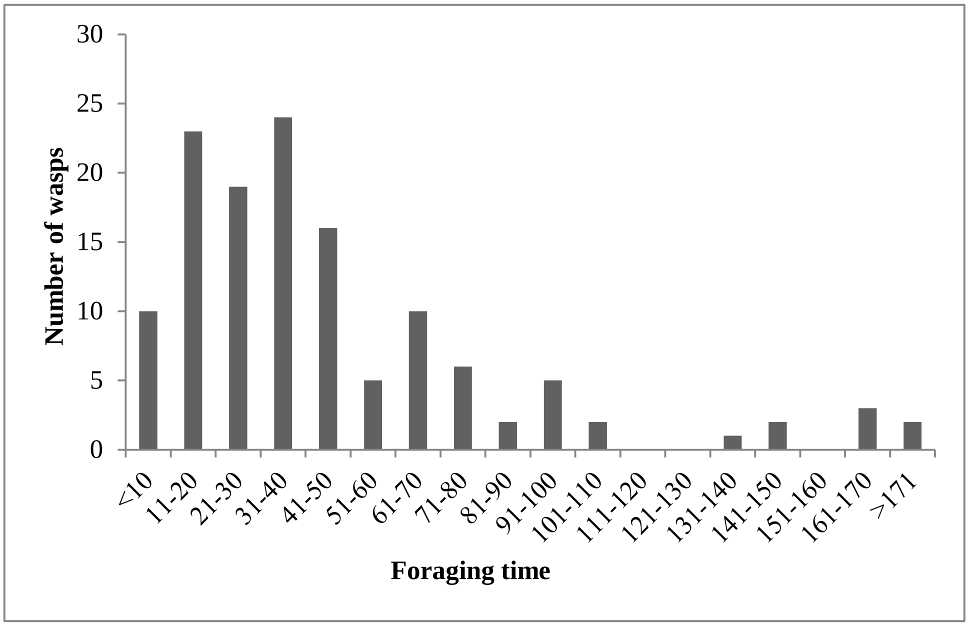


Figure 16: Individual wasp foraging times in increments of 10 minutes.

4.5 Prey Paralyzation

Specimens of 11 of the 35 prey species exhibited thanatosis, apparent death, in which individuals initially appear to be paralyzed, but after a short period they became active and it was evident they had not been paralyzed. Thanatosis is commonly displayed in certain species of leaf beetles (Coleoptera: Chrysomelidae) and other beetle groups, but here it was also observed in 10 species of buprestids. Three of these species were not used in the paralyzation analysis due to low abundance throughout the season (*Agrilus ruficollis* Fabricius, *Dicerca pugionata* Germar, and *Poecilonata cyanipes* Say $\leq$ 4 individuals). Percent paralyzation was calculated for the remaining eight species (Figure 17): *Neochlamisus bebbianae* had the lowest rate of paralyzation at 48%; *Eupristocerus cogitans* was paralyzed at 53%; *Agrilus anxius* at 74%; *A. bilineatus* at 76%; both *A. arcuatus* and *Chrysobothris femorata* at 87%; *C. sexsignata* at 96%; and *D. divaricata* at 99% (Figure 16). Average dry weight (g) of the eight most frequently unparalyzed species was graphed on the secondary y-axis to determine if dry weight and rate of paralyzation were correlated, producing an r^2 value of 0.47 (Figure 16). Individuals of all other species collected by *C. fumipennis* (25 species) were paralyzed 100% of the time.

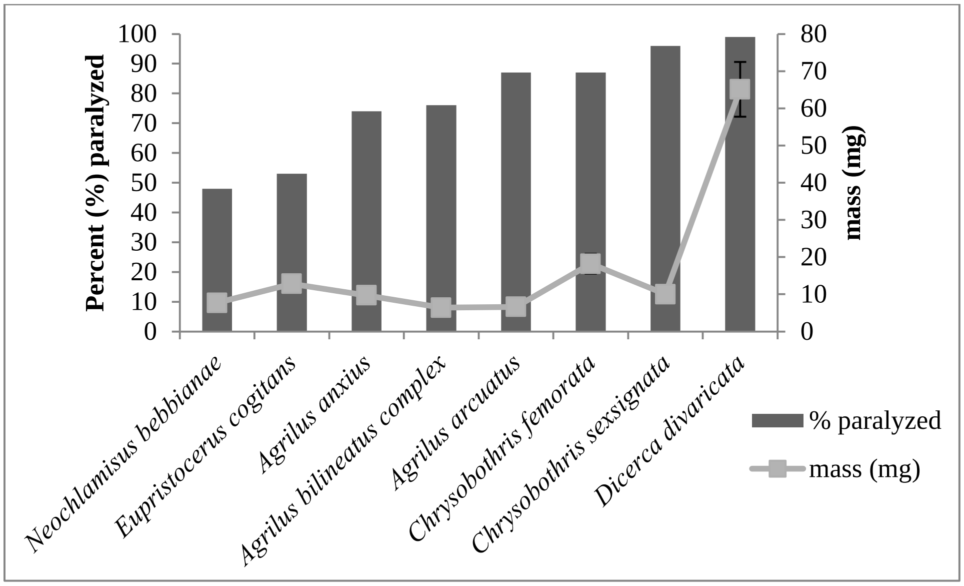


Figure 17: Percent of wasp prey that were successfully paralyzed correlated with mass of eight species of *Cerceris fumipennis* prey, r2 = 0.47. Standard error for mass (mg) is shown with error bars. (from Dube & Chandler 2017)

4.6 Prey Preference

During the 2014 monitoring season thirteen wasps returned with prey more than once throughout the season and eight of those wasps returned with the same species of prey each time, having a prey diversity index of 1 meaning they had no diversity within their collect prey. Only five wasps returned to the nest with more than one species throughout the monitoring period, and their prey diversity index ranged from 0.5 to 1 (Table 3).

Table 3: Individual wasps, their total prey collection and number of different species for one day used to calculate each wasps prey diversity index.

Wasp #	# of prey total	# of species	Prey diversity index
1	3	1	0.333333333
2	4	1	0.25
3	2	1	0.5
5	3	1	0.333333333
7	2	1	0.5
11	2	1	0.5
20	3	2	0.666666667
23	4	3	0.75
25	4	2	0.5
27	3	1	0.333333333
28	2	2	1
31	3	1	0.333333333
33	2	2	1

CHAPTER 5

Discussion

5.1 Rare, Uncommon, & New Prey Discoveries

Of the 35 beetle species collected by *C. fumipennis*, 17 are uncommon, rare, or infrequently to rarely collected as defined by Bright (1987) and/or Paiero *et al*. (2012). Three of the species were new NH state records (*Actenodes acornis* Say, *Agrilus carpini* Knull, and *Chysobothris verdigripennis* Frost). These three species had not yet been recorded from NH according to Paiero *et al*. (2012), but were expected to occur there. These beetle species are not undescribed, nor are they considered invasive. They are uncommon and difficult to survey with conventional methods because they occur in low-density populations. Trapping for species in low-density populations is normally cumbersome and time consuming using large or bulky traps and lures that are deployed over long periods of time, making much of their biology and distribution difficult to determine. Fortunately, the skill and seamless ease that *C. fumipennis* exhibits in hunting and catching these beetles can enhance understanding of the biology of species to which we normally lack ease of access.

5.2 Prey Preference in Different Forest Types

I hypothesized that by documenting forest composition for each of the sites a prediction could be made about which beetle prey would be most commonly collected by foraging *C. fumipennis* females. The *Tsuga canadensis* feeding beetle species abundances were greater at BSF compared to EAL (50:39 individuals), but *T. canadensis* feeding beetle species richness was actually lower at BSF compared to EAL (5:6 species).

There was also very little apparent correlation of abundances of host specific beetles with the other prevalent tree species. Though there was greater abundance of *Acer* spp. feeding beetles collected at EAL (275:226 individuals), there was only a slight difference in species richness between the two sites. *Pinus* feeding beetle species were only slightly more abundant at EAL than BSF (42:36 individuals) and species richness was greater by only one species at EAL than BSF (10:9 species). In fact the opposite of the prediction occurred for the *Quercus* feeding beetle species. There were 73 individuals collected at BSF and only 60 *Quercus* feeding beetles collected at EAL where there is a higher percentage of *Quercus* present (16% EAL, 9% BSF).

5.3 Seasonality of *C. fumipennis* and Buprestid Prey

Seasonality of C. fumipennis

Results from the 2013 field season at BSF and EAL suggest that *C. fumipennis* would be a useful tool for bio-surveillance of buprestids from July 11th through August 22nd. Monitoring the wasp aggregations is not productive for a short period of time (approximately 7-12 days) after initial emergence, which appears to be synchronous with 150 nests opening within 12 days. During this time *C. fumipennis* females are busy orienting themselves to the geography of the area, mating, and feeding (Hook & Evans, 1991; Careless, 2009). Time would be most productively spent collecting prey from *C. fumipennis* females once the majority of nesting females are actively foraging. This protocol should be conducted again in subsequent seasons and paired with the degree day model produced by Rutledge *et al.* (2015) so that data predicting emergence times and periods of activity can be tested. A better understanding of *C. fumipennis* seasonality can benefit government organizations and groups that rely on limited personnel to use the most effective tools and times to monitor and control the newly introduced EAB, which is predicted to severely diminish ash tree populations throughout the northeastern United States.

Seasonality of Buprestid prey

Seasonality of the species in the family Buprestidae was highly variable. Species such as *Dicerca divaricata* and *Buprestis striata* were some of the first prey items collected by *C. fumipennis*, suggesting that these prey species emerged before *C. fumipennis* became active (Figure 13). *Dicerca divaricata*, *Neochlamisus bebbianae*, *Agrilus arcuatus, and A. bilineatus* were frequent prey items for *C. fumipennis* during the final monitoring days of the season in mid- and late-August, suggesting that these beetles were probably present after *C. fumipennis* aggregations decline. For these five species, the overlap between predator and prey may not be enough to qualify as an accurate estimate of complete seasonality. We are able to estimate some peaks in activity, such as that of *D. divericata*, that had strong peaks during week two and seven (Julian days 198 and 233) and could suggest large separate peaks of emergence in NH, occurring early in the season and again later. The activity pattern shown by *B. striata* definitely indicates it is active earlier in the season, but, unfortunately, how early is yet to be determined except from label data of specimens in collections. Although some prey were abundant (especially *D. divericata* and *N. bebbianae*), we can cover only part of their total activity period as adults using *C. fumipennis* as a survey tool. Clearly, for those species that emerge earlier in the season, use of *C. fumipennis* is not a productive method for determining seasonality.

Species such as *Agrilus anxius*, *Chrysobothris femorata*, *C. rotundicollis*, *C. sexsignata*, *Eupristocerus cogitans*, *Phaenops aeneola* and *P. fulvoguttata* were not collected on the first monitoring day and abundances were in decline or absent by the last sampling day. The active foraging period of *C. fumipennis* seems to overlap almost completely with these seven prey species, giving us accurate seasonality for more than half of the common prey items collected by *C. fumipennis* wasps. The time of activity of these 7 species is boundaried by the *C. fumipennis* field season and a more accurate model of their emergence and activity could be determined using *C. fumipennis* by monitoring aggregations more frequently than once or twice a week. For species such as *E. cogitans*, which was only documented at EAL, it is likely that collection of this species by *C. fumipennis* females is due only to the population of alder, the primary host plant, that is abundant along the Suncook River just east of the research site. There was no alder documented near the research site at BSF.

While most species collected by *C. fumipennis* females are summer species, with one or two peaks, all seem to be absent or in decline by mid-August. The one prey species of Chrysomelidae, *N. bebbianae*, seems to be quite active during the end of the *C. fumipennis* hunting season and likely is present for some time after the wasps.

Dicerca divericata, while seeming to burden *C. fumipennis* females when clasped beneath, is by far the most frequently collected species, composing of approximately 50% of the total individuals caught at both sites. *Dicerca* species tend to be larger and are possibly all one larva requires for successful development. Frequent capture of *D. divericata* may also mean they were very abundant and relatively common throughout each forest.

Emerald ash borer was not collected during this study but research demonstrates that adults begin to emerge in Michigan in mid-May at around 230-260 degree days (base temperature 10°C), and peak EAB adult activity is from late June to early July (Brown-Rytlewski & Wilson, 2005). Although *C. fumipennis* females are not active as early as EAB, adults of both are active during most of the month of July suggesting that this would be the most efficient time period to monitor for EAB using *C. fumipennis*. Less is known about the other non-native *Agrilus* spp. that were mentioned in Chapter 1, but using *C. fumipennis* as a bio-surveillance tool for presence/absence data could assist in determining the seasonality of these foreign species that, when first introduced, are likely to occur in difficult to detect, low density populations.

5.4 Provisioning Rate & Foraging Times

Provisioning rate

Successful *Cerceris fumipennis* females averaged 1.9 prey items per monitoring day. Monitoring of the wasps occurred during the most productive time period of the day, not the entire day. Some female wasps prrobably start foraging prior to the start of the monitoring period, and some likely continued foraging after the monitored period ended, therefore 1.9 prey per day is probably an underestimate of *C. fumipennis* provisioning rates. A more accurate determination of provisioning rate can be developed either by extending the monitoring periods or by nest excavations.

Foraging times

The range of foraging times for the successful wasps was very large (6 to 217 minutes). The average foraging time for a female *C. fumipennis* was 47 minutes. McCabe (2017) recently conducted *C. fumipennis* releases at distances of 0.2 km, 0.4 km, and 1.0 km away from the wasp's aggregation. The average time it took these wasps to return was 2.11 hours, 2.98 hours, and 3.9 hours respectively. Comparing the average amount of time spent foraging (47 min) with McCabe's average time it takes a wasp to return to her nest from a distance of 0.2 km (2.11 hours), we can estimate that *C. fumipennis* females normally might not travel very far from their nests to forage for prey, probably less than 0.1 km. McCabe's data also documents the remarkable return rate for a few female wasps. One female returned to the nest from 1.0 km distance in 21 minutes and there were shorter flight times from releases at closer distances.

Unfortunately, these data are not complete enough to support a full understanding of the foraging behaviors of *C. fumipennis*. To better understand these behaviors a tracking or transmitting technology small enough to be mounted on a female wasp without interfering with her biology would be an excellent way to determine wasp activity more definitively.

5.5 Prey Paralyzation

Predatory Crabronidae wasps are known for their hunting, stinging, and paralyzing abilities, yet the data here reveal that 99 of the 853 prey individuals collected (11%) were not successfully paralyzed, at least at the time when they were caught and transported back to the nest. Whether continued stinging attempts occur once the prey has been brought into the nest is uncertain. Dry weight (g) was plottedagainst percent paralyzation (Figure 16), and only a moderate correlation of 0.47 between the two was observed, suggesting that individuals with a lower body mass are less likely to be successfully paralyzed. It is also evident that one species is less likely to be successfully paralyzed than others (only 51% of *Neochlamisus bebbianae* were paralyzed), but it is not known why. Suggestions by Nalepa & Swink (2015) link failed paralyzation to the prey carriage mechanism that is used to prevent the elytra from opening. A successful, balanced, uninterrupted flight back to their nest could allow further stinging attempts once back inside their burrows. Differences in paralyzation rate of the chrysolmelid, *N. bebbianae*, compared to that of the buprestid prey, suggest that there may be strong selection for the unique body form of buprestids rather than the differing body forms of other available prey. *Neochlamisus bebbianae*'s body configuration (small, shorter, and more cylindrical) is dramatically different than that of the family Buprestidae, and could lead to difficulty in locating and then penetrating the correct area for successful paralyzation with the sting (Careless, 2009) (Figure 17). These warty leaf beetles have specialized ventral grooves designed for tucking in their legs and rolling off a leaf when disturbed or threatened (Shin *et al*., 2012). Their form is similar to that of caterpillar frass, and the coxal joints, where *C. fumipennis* females are known to sting their prey, may be quite difficult to access once the beetle has retracted its legs (Careless, 2009, Shin *et al*., 2012).

Figure 17: An adult *Neochlamisus bebbianae*. Photo credit: T. Murray

5.6 Prey Preference

Eight of the monitored wasps during the 2014 field season collected only one type of prey species, which could result from hunting in the same location or by returning to the same host tree during each foraging period. The other six monitored wasps had a prey diversity index of 0.25 to 1 showing little to no particular affinity for one species of prey over another. This could be attributed to the broad host range or low host specificity of some buprestid beetles because many of the species will feed on multiple coniferous or deciduous tree species. Species such as *C. sexsignata*, for example, feeds on more than 25 deciduous and coniferous tree and shrub species.

Nest excavation is another method to determine prey preferences, but is destructive to developing larvae and multiple excavations at one aggregation could quickly diminish its size and potential for use in bio-surveillance.

CHAPTER 6

Conclusion

In this study forest composition of the two sites was generally similar, and did not allow successful prediction about which species would be the most common prey items for *C. fumipennis* based on known host tree species.

Closer monitoring of other factors such as forest age (the abundance of dead or dying trees) and disease could also help predict which prey species would be collected by foraging female wasps because most buprestid beetles target trees that are already under stress (Dunn *et al.*, 1986; Moraal & Hilszczanski, 2000; Evans et al., 2007).

Prey seasonality was determined for 10 species of prey, approximately half of the commonly collected species. For these species, more frequent monitoring would permit a more complete and accurate description of their adult activity period, emergence patterns, and time of ultimate demise. Other introduced or invasive buprestid beetles are likely to overlap at least partially with the foraging time period of *C. fumipennis,*providing a unique opportunity to apply this technique to a new and unfamiliar species.

The level of unsuccessful paralyzation raises questions regarding the competitive success of some prey species such as body shape and size (e.g. *Neochlamisus bebbianae*) and the failure to evolve the mechanisms of other species. While *N. bebbianae* is an atypical prey item, being from a different family, generally it is the smaller species of buprestids that have lower paralyzation rates (*Eupristocerus cogitans* ~ 0.0128 g and *Agrilus* sp. ~ 0.008 g) (Hellman & Fierke, 2014). Research should continue to study thanatosis in *C. fumipennis* prey so that we can better evaluate the wasp's effectiveness, and determine what, if not mass or size, is the causal agent for low paralyzation rates. EAB has a similar body shape to other members within the genus *Agrilus*, and could occur in the group that has a lower paralyzation rate based on size characteristic rather than mass (*A. planipennis* mass = 0.0428 ± SE 0.0008 g), which is much greater than that of other *Agrilus* species (Rutledge, 2012).

This project provided an opportunity to develop protocols for wasp surveillance, and establish the basis for authoritative identification of Buprestidae species in New Hampshire. Despite the numerous projects involving *C. fumipennis* in the recent past, our knowledge about the life history of this species was incomplete. This project helped increase our understanding about wasp productivity, seasonality, prey preference and paralyzation rates. Studying *C. fumipennis* has shown that it can be a useful tool in detecting rare or uncommon species of buprestids in low density populations, especially since the wasps show relatively little prey specificity. Due to this ability, *C. fumipennis* will aid in the detection of new populations of invasive species or help monitor the spread of known infestations even though provisioning rates may be low at times. Monitoring the spread of EAB, a species that is already in NH, while it is still at low densities is important for quarantine decisions, success of best management practices, and implementation of other control techniques used to manage this invasive pest.

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