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Ecology of Emerald Ash Borer (*Agrilus Planipennis*: Buprestidae) in Louisiana

Balwinder Kaur

Louisiana State University and Agricultural and Mechanical College

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ECOLOGY OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*: BUPRESTIDAE)
IN LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Balwinder Kaur
B.S., Punjab Agricultural University, 2015
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To Gurmail Singh: my idol, my inspiration.

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ABSTRACT

In 2015, emerald ash borer (EAB) was detected in North Louisiana (LA). There is no information on wood inhabiting insects of green ash (*Fraxinus pennsylvanica* Marshall) trees, phenology and parasitoid establishment in LA. The first objective of the study was to quantify the insect assemblages and to compare their diversity, abundance and evenness within the epicenter of the EAB infestations in North LA including regions in Central and South LA where EAB has not been detected yet. We established four treatments, based on log diameter: small and large; and log condition: dead and live for green ash. All the insects collected bimonthly from emergence traps were identified to family level. A total of 25, 420 insects belonging to 104 families and 11 orders were collected. Assemblages differed among live large trees (Wald test = 8.016, $P < 0.01$), which composed of greater percentages of Mycetophilidae (Diptera), Megaspilidae (Hymenoptera), Ceratopogonidae (Diptera), and Liposcelididae (Psocodea). Total abundance for live small logs was 11.5-times higher than live large, and 7.7-times higher than dead small logs at non- EAB 1 site due to niche differences. Shannon's diversity and evenness were found to be higher in dead small, live large, and live small logs at non-EAB 2 site. The potential changes to local ash bottomlands after the EAB invasion in LA are also discussed.

The second objective was to determine the phenology of EAB and establishment of non-native parasitoids. Results focused on life history of EAB suggested the fast development of EAB in LA along with its one generation life cycle in LA. Adults emerged from April to June. EAB larvae appeared earlier in

season including June for L1, L2, L3 and August for L4, Then, J larvae were collected in July until March. These J larvae later pupate (January- March) while some overwinter. Additionally, parasitoid recovery studies did not yield any released parasitoid except one specimen of *Oobius agrili* (an egg parasitoid) in northern LA. EAB's fast development, one generation cycle along with regional differences such as subtropical climate in LA compared to the native range of parasitoids would affect EAB biocontrol in LA.

CHAPTER 1. BACKGROUND AND LITERATURE REVIEW

1.1. Importance of ash trees & emerald ash borer (EAB) distribution, biology, and ecology

In the United States, *Fraxinus* accounts for 8.7 billion trees and saplings (Flower et al. 2013) and is a dominant and co-dominant trees species among 150 forest communities (Cippolini 2015). *Fraxinus* species, such as *F. americana* L. (white ash) and *F. pennsylvanica* Marshall (green ash), are typical of hardwood forest, famous for their strength, high disease resistance, hardiness to insect pests and stressful environmental conditions-and are widely used as beautification trees in parks, yards and along streets (Burns and Honkala 1990). Ash is highly flammable and is essential as fuelwood (Elias 1987). In addition, Robust and flexible ash wood is used for flooring, crate packaging, tool handles, paneling, furniture and baseball bats (Elias 1987). Importantly, white ash is preferred wood for Louisville Slugger – a popular baseball bat with professional players (Burns and Honkala 1990).

Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera, Buprestidae), hereafter referred as EAB, is a wood boring green beetle which has emerged as a significant pest of ash trees (*Fraxinus* spp.) in North America and Canada. *Agrilus planipennis* Fairmaire (1888) is also named in the literature as *A. marcopoli* Obenberger (1930) in China, *A. marcopoli ulmi* Kurosawa (1956) in Japan, and *A. feretrius* Obenberger (1936) in Taiwan (Jendek 1994). Based on the morphology of the scutellum, pronotal medial sulcus, and robust body, *A. planipennis* resembles more closely the Asian species *A. auristernum* Obenberger, *A. cyaneoniger* Saunders, and *A. lubopetri* Jendek (Haack et al. 2002).

EAB adults are metallic, coppery-green, elongate beetles with size 0.95cm long and 0.42cm wide (Yu 1992). As typical of *Agrilus*, EAB larvae are white, flat, slender, and have two sclerotized, pincer-like appendages (urogomphi) on the terminal abdominal segment (Peterson 1960). Like other insects, EAB is poikilothermic; their development events such as oviposition, pupal development, and emergence are temperature dependent (Yurk and Powell 2010). Thus, the date of events in the life cycle of EAB is dependent on the accumulation of specific heat units to complete each stage of development (Growing Degree Days), which depends on the local weather. In southwestern Ontario, the first adult's emergence begins in the last days of May, and peak emergence in mid-June (Lyons and Jones 2005). Peak flight activity in southwestern Ontario was in mid-July (Lyons and Jones 2005).

In the Great Lake region of North America, emerald ash borer adults begin their flight in May or June with the peak in July- August and end up by September (Cappaert et al. 2005). In Canada and North America, adults start mating and ovipositing two weeks after emergence (Cappaert et al. 2005). Various volatiles and visual cues help in the orientation of adults towards trees for mating and laying eggs. Scientists predicted that EAB primarily use visual cues in mating (Domingue and Baker 2012), and adults are highly attracted to purple and green color (Crook et al. 2009). On the contrary, another study showed that males are attracted to mate by volatile chemical cues (Silk et al. 2009).

Following successful single or multiple mating, females oviposit in cracks or crevices under the bark of a tree, on a tree trunk, and main branches of trees

(Jennings et al. 2014). Females lay 70-90 eggs in a year with maximum up to 307 eggs (Jennings et al. 2014). Eggs are initially light yellow then turn brownish yellow and hatch after 2-3 weeks depending on temperature and local environment conditions (Cappaert et al. 2005). After eclosion, neonate larvae chew the cambial region and start making serpentine galleries filled with frass in the phloem region of a tree (Wang et al. 2010). EAB larvae are white, bell-shaped and develop through four instars before they turn into pre-pupa or pupa (Cappaert et al. 2005). Additionally, mature fourth instars chew pupation chambers in the outer sapwood or bark and develop a folded “J” appearance and are termed as “J-larva” (Wang et al. 2010). Pupation occurs in early spring with adult emergence from late spring through early summer (Wei et al. 2007).

EAB completes development either in one year by overwintering as mature fourth instar which becomes adult the following spring (Wang et al. 2010) or in two growing seasons by overwintering as an immature J-larva which further undergoes development (Cappaert et al. 2005). Newly formed adults chew their way out building a D-shaped exit hole (Wang et al. 2010). Adults live from three to six weeks feeding on mature ash leaves and do not cause any significant damage to the host tree (Bauer et al. 2004). However, larvae feed for many months on the phloem and outer sapwood, creating extensive serpentine galleries that eventually girdle, disrupting the nutrient and water flow, and hence killing the tree.

Adult males and females live for 43 days and 7-9 weeks respectively. Depending on climate conditions and tree health, EAB has 1-2 generations per year

(Wei et al. 2007). However, in southern regions of USA as in Louisiana with long growing days and high humidity, phenology of emerald ash borer is poorly known.

Emerald ash borer has been found to complete larval development on 16 *Fraxinus* spp. of North America (Anulewicz et al. 2008). EAB can infest readily on all sizes of ash tree from sapling 2-5cm dbh to mature tree (Haack et al. 2002) and kills healthy trees within 2-6 years once infected (Knight et al. 2013). On the contrary, most native wood-boring buprestids are limited to stressed trees (Cappaert et al. 2005). The natural spread of EAB occurs through an adult flight, which usually expand several kilometers each year (Taylor et al. 2008). However, the dispersal of EAB over large distances of tens or hundreds of kilometers mostly occurs through anthropogenic means by movement of infested host material such as ash nursery stock, wooden logs, and firewood (Cappaert et al. 2005; Poland and McCullough 2006). Once a tree gets infested, it shows symptoms of canopy decline, canopy dieback, defoliation, low crown ratio, and low crown density (Cappaert et al. 2005).

EAB is native to East Asian countries including China, Japan, Korea, Mongolia and Taiwan (Chammarro et al. 2015), and Russian Far East (Alekseyev 1979). EAB was introduced into North America in 1990's and was first detected in Southeast Michigan, the United States and Ontario, Canada in 2002 (Haack et al. 2002). However, the movement of EAB infested firewood and timber, coupled with lack of natural parasitoids in its invaded regions, allowed the beetle to be able to invade ash growing areas quickly in North America (Cappaert et al. 2005). By June 2018, EAB has been found in thirty-three states of US; Arkansas, Colorado, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland,

Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin and three Canadian provinces; Manitoba, Ontario and Quebec (EAB Info 2018). In February 2015, Louisiana was the 25th state to confirm the presence of EAB. EAB is currently found in Bossier, Claiborne, Lincoln, Webster, Union, Bienville, Jackson, and Morehouse Parishes (EAB Info 2018). Most recently in 2016, EAB was confirmed in Texas (EAB Info 2018). There are a federal quarantine and protocols to prevent its further spread (Cappaert et al. 2005).

In its native range, the ash species reported as host of emerald ash borer includes Chinese ash (*F. chinensis* Roxb.) and *F. rhynchophylla* [Hance] A. E. Murray), and Manchurian ash (*F. mandshurica* Rubr.) (Wei et al. 2004; 2007). Out of sixteen *Fraxinus* spp. (Knight et al. 2013), four *Fraxinus* spp. including green ash (*F. pennsylvanica* Marsh.), white ash (*F. americana* L.) ash, black ash (*F. nigra* Marsh.) and Oregon ash (*F. latifolia* Benth) constitute 82% of these groups in the United States (NatureServe Explorer 2014). In Southeast Michigan, EAB has killed 99% of green ash (*F. pennsylvanica* Marsh.), white ash (*F. americana* L.), black ash (*F. nigra* Marsh.). Additionally, EAB attacks other *Fraxinus* spp., including pumpkin ash (*F. profunda* Bush) (Knight et al. 2013), blue ash (*F. quadrangulata* Michx.) (Anulewicz et al. 2008). In Russia, EAB has emerged as a major mortality factor to European ash (*F. excelsior* L.) (Orlova- Beinkowskaja 2013). In Japan, *Juglans* spp. (butternuts and walnuts), *Ulmus* spp. (elms), *Pterocarya* spp. (wingnuts), as well as, *Fraxinus* spp. have been reported as a host of emerald ash borer (Akiyama and Ohmomo 1997). In

2015, white fringe tree (*Chionanthus virginicus* L.) was confirmed as a susceptible larval host for emerald ash borer besides ash in the United States (Cipollini 2015).

1.2. Management of EAB

Despite eradication efforts, EAB has continued to spread throughout North America. Several management tools have been deployed to conserve ash trees that include the use of classical biological control, chemical insecticides, and the development of resistant ash cultivars (Herms and McCullough 2014). An estimate found that the cost of treating, removing, and replacing EAB-infested ash trees in urban areas for 2010 to 2020 sums to \$12.5 billion (Kovacs et al. 2011). Emerald ash borer is controlled by several management tactics including monitoring, eradication of infested trees, biological and chemical control. Due to cost limitations, biological control is the most common approach in North American forests.

1.2.1. Monitoring of EAB

Green and purple traps baited with leaf volatiles are being used by United States Department of Agriculture- Animal Plant Health Inspection Service (USDA-APHIS) for area-wide EAB detection surveys (Crook and Mastro 2010). These canopy traps are three sided prisms made up of dark purple corrugated plastic (Coroplast Inc., Dallas, Texas) containing an insect trapping glue and Manuka oil lures released at 50mg/ day. Other canopy traps are Lindgren multiple funnel traps (Chemtica Internacional, San Jose, Costa Rica) coated with Fluon and baited with cis- 3- hexanol with release rate of 50mg/ day (Francese et al. 2011). These are widely used to monitor emerald ash borer adults in an area (Crook and Mastro 2010).

Other management efforts involve removing the dead and dying ash trees in core infested areas (Herms and McCullough, 2014). The eradication projects cover a regulatory area bounded by a perimeter 800 m beyond the furthest infested ash tree (Herms and McCullough, 2014). Additionally, it involves removing all the trees greater than 2.5 cm in diameter whether infested or non-symptomatic in that regulatory area. This also include restricting the movement of firewood and other ash material out from quarantined area (Herms and McCullough 2014). Eradication for EAB was done in six counties of southeast Michigan but it does not provide any practical solution to slow/ stop the EAB infestations (Herms and McCullough 2014). Bark removal, crown loss assessment, branch sampling, deploying artificial pheromone lure traps, bio surveillance using predatory wasp and remote sensing are some of the detection tools for EAB.

1.2.2 Biological control of EAB

Biological control of EAB using exotic parasitoids is widely employed in North America. USDA-APHIS-PPQ (United States Department of Agriculture- Animal and Plant Health Inspection Service- Plant Protection and Quarantine) Biological Control Production Facility in Brighton, Michigan, produces EAB parasitoids for field release of these parasitoids in the USA (Duan et. al 2012). Releases of *Tetrastichus planipennisi* Yang, *Spathius agrili* Yang, and *Oobius agrili* Zhang and Huang are initiated in infested areas when growing degree-days are approached (Gould et al. 2016). *Oobius agrili* is an egg parasitoid that can parasitize about 61.5% and 28% of EAB eggs in China and USA respectively (Liu and Bauer 2007; Duan et. al 2012). *Oobius* spp. lays a single egg inside the EAB egg and develop parthenogenetically

(Liu and Bauer 2007). These are shipped in oobinators, which are pill vials with screening containing mature pupae of *Oobius* inside EAB eggs. (Gould et al. 2016). *Spathius agrili* is a larval ectoparasitoid that has been found to parasitize 90% of EAB pupae in Tianjin, China and is adapted to cold regions (Yang et. al 2010). *S. galinae* is a larval ectoparasitoid that has been found to parasitize around 71% of EAB larvae in China and USA and is released in warmer regions of USA (Duan et. al 2012). Both *Spathius* spp. drill hole into the bark, inject venom in the larvae and lay approx. 5 eggs/ larvae (Gould et al. 2016). These *Spathius* spp. are shipped as adults in plastic cups (Gould et al. 2016). *Tetrastichus planipennisi* is a gregarious endoparasitoid that can parasitize 50% and 24% of the EAB larval population in China and USA, respectively (Duan et al. 2012). These are shipped as mature pupae in small ash bolts, and upon emergence, these parasitoids drill into the bark and lay 4-172 eggs/ EAB larvae, hence feeding internally (Yang et al. 2010).

1.2.3. Chemical control

Several systemic insecticides are used to treat the ash trees infested with EAB in urban settings. Products such as emamectin benzoate, azadiractin, imidacloprid are applied to outer sapwood by injecting these chemicals around the base of tree trunk or to the soil around the base of tree (Herms and McCullough 2014). Economic analysis of treating ash trees with chemicals showed that protecting ash trees with products containing active gradient emamectin benzoate and sold as TREE-äge™ could provide 100% control up to 2-3 years (McCullough et al. 2009). This insecticide affects EAB adults which would feed on ash leaves and neonate/ early instar larvae (Mercader et al. 2011). Chemical control of EAB using emamectin benzoate is

highly effective in the form of tree injections and sprays in urban landscapes but would not be economically or environmentally sustainable in forests (Herms and McCullough 2014).

1.3. Ecological effects of EAB invasion

In addition, large-scale mortality of ash has negative ecological impacts on plant communities and insect biodiversity in ash communities (Gandhi and Herms 2010, Koenig et al. 2013). Previous studies on ecology showed that EAB infestations have caused a change in forest ecosystem structure, habitat fragmentation, local climatic changes, species distribution and the establishment of alien plant species (Gandhi and Herms 2010). Vacant areas earlier occupied by ash trees were occupied by new plants like glassy buck thorn (*Frangula alnus* Mill) and exotic honeysuckles (*Lonicera tatarica* Mill, *L. morrowii* Mill) as occurred in Indiana and Ohio (Tallamy and Shropshire 2009). Similarly, gaps created by the loss of ash in Michigan have been filled by spicebush (*Lindera benzoin* L.), pawpaw (*Asimina triloba* Dunal), and prickly ash (*Zanthoxylum americanum* Mill.) (*Carex* L.) (Knight et al. 2013).

EAB disturbance impacted the abundance of vertebrate' predators, specifically four insectivorous species of birds including downy woodpecker (*Picoides pubescens* Linnaeus), hairy woodpecker (*Picoides villosus* Linnaeus), red-bellied woodpecker (*Melanerpes carolinus* Linnaeus) (Koenig et al. 2013), and the white-breasted nuthatch (*Sitta canadensis* Latham). Following the ash tree mortality, relative abundance of red-bellied woodpeckers and white-breasted nuthatches increased whereas populations of downy and hairy woodpeckers declined significantly initially and then increased in Michigan (Koenig et al. 2013). Additionally,

ash tree loss also impacted other vertebrates such as white-tailed deer and moose due to habitat loss; and beavers, rabbits, and porcupines due to the scarcity of food (Hirschfeld and Heyd 2005).

EAB infestation also negatively affected the insect fauna of ash ecosystems. For example, canopy loss reduced leaf litter moisture, which impacts the litter-dwelling arthropod communities (Yi and Moldenke 2008). Gaps created by the loss of ash trees can also reduce ground beetle populations (Gandhi et al. 2014). A recent study by Wagner and Todd (2016) listed specialist herbivores that are threatened by loss of ash in the northern USA and Canada. This list includes members of five insect orders Lepidoptera (32), Coleoptera (24), Diptera (9), Hemipteran (25), Hymenoptera (3), and Acari (5). Species at high risk of endangerment includes two Hercules beetles (*Dynastes*), two rhinoceros beetles (*Xyloryctes*), and nine species of hawk moths (Sphingidae) (Wagner and Todd 2016). The impact of EAB invasion on ash-associated arthropods in Louisiana is unknown.

1.4. Importance of wood type, wood size and location to predict insect assemblages, and effects of an invasive EAB in a community

Study of saproxylic insects including bark feeders, fungi feeders, parasitoids, predators, and detritivores is important to understand the forest trophic structure, and hence to predict effects of an invasive insect (Grove 2001). Wood type including live wood, dead fallen tree or dead standing tree is a key to studying saproxylic insect diversity because different wood types or levels of wood decay support different insect assemblages (Hammond et al. 2001). Insects colonizing freshly dead/ live wood have narrow host specificity (Hamilton 1978) and wood size, i.e. log diameter

also affects the insect composition (Palm 1959). Many studies suggested a positive relationship between tree or dead-wood diameter and species richness, or abundance (Warren & Key 1991). This might be because larger-diameter trees and dead wood are highly heterogeneous habitats, undergoing slower decomposition, and hence allowing many specialist species and different kinds of species to colonize at the same time (Kolstrom & Lumatjarvi 2000). Saproxylic insect fauna may also vary depending on the disturbance event including deforestation, fires, and invasive species (Spies et al. 1988). Invasive species can endanger local insect species by changing local environment and ecosystem. For example, the invasion of Asian long horn beetle (*Anoplophora glabripennis* Motschulsky) in the United States shows how an invasive species can alter an ecosystem function (Meng et al. 2015).

Hence, the first objective of the study was to compare the diversity, and abundance of wood- inhabiting insects in EAB invaded and non-invaded regions in northern, central and southern regions of Louisiana. Since EAB has been reported in only eight parishes including Webster, Bossier, Union, Claiborne, Lincoln, Bienville, Jackson, and Morehouse, this project helped to know how EAB invasion might change the wood- inhabiting insect abundance, richness and diversity after its attack and which species are threatened and may be displaced by loss of ash stands. Insect fauna from small diameter, large diameter, live, and dead standing trees were compared to understand the community composition of wood-inhabiting insect herbivores in ash bottomlands of Louisiana.

1.5. Monitoring the establishment of released parasitoids of EAB in Louisiana

Natural enemies including hymenopteran parasitoids: *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), an egg parasitoid (Zhang et al. 2005); *Tetrastichus planipennis* Yang (Hymenoptera: Eulophidae), a larval endoparasitoid (Yang et al. 2006); and the larval ectoparasitoids *Spathius agrili* Yang (Yang et al. 2005), and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) (Belokobylskij et al. 2012) have been released by United States Department of Agriculture- Animal and Plant Health Inspection service (Bauer et al. 2015) in North America. This release program was initiated in 2007 for *O. agrili*, *T. planipennis*, and *S. agrili* followed by *S. galinae* in 2015 (Bauer et al. 2015).

In Michigan, following parasitoid releases, a study on parasitism rates showed that for *O. agrili* (egg parasitoid) parasitism increased from 0.7% to 22 % and *T. planipennis* (larval parasitoid) parasitism increased from 1.2 to 21% from 2008 to 2012 (Abell et al. 2014). *S. agrili* (pupal parasitoid) is found to parasitize 45% of EAB larvae in Michigan after one year of release. *S. galinae* is a larval ectoparasitoid that has been found to parasitize around 71% of EAB larvae in Michigan (Duan et al. 2012). *Spathius agrili* are not adapted for release in the northern portions of the United States and is only restricted for release to areas below the 40th parallel (Bauer et al. 2014) but *S. galinae* can be released in the northern portions of the United States.

Several studies have monitored the establishment of introduced parasitoids of EAB by different sampling methods such as (1) the use of sentinel eggs under flaps of bark or sentinel egg logs (small ash logs bearing EAB eggs) and visual surveys for

EAB eggs on ash trees to estimate rates of egg parasitism, (2) deploying emergence traps using ash logs or bark samples for parasitoid emergence (Bauer et al. 2011) and (3) using yellow pan traps for monitoring adult EAB parasitoids populations (*T. plannipenisi* and *S. agrili*) (Duan et al. 2014).

Given the current scale of infestation in Louisiana, a biological control program was initiated in 2015 to reduce the population of EAB. A total of 16,300 of *O. agrili*, 21,135 of *T. planipennisi*, 2,167 of *S. agrili* were released at two field sites in Webster Parish in Louisiana. Since then, parasitoid recovery programs involving felling EAB-infested ash trees for larval parasitoids, deploying YPTs (yellow pan traps) to collect emerging adult parasitoids and collecting emerald ash borer eggs for egg parasitoids began in the United States (Gould et al. 2016) to determine the establishment as well as successful reproductive ability of introduced parasitoids in the United States. A study was conducted from 2016- 2017 to test the establishment of released exotic parasitoids in the southern USA (Louisiana) using similar parasitoid recovery methods.

1.6. Phenology of emerald ash borer in southern USA (Louisiana)

Understanding the basic population dynamics of emerald ash borer is important to effectively target its different life stages using parasitoids in USA (Jennings et al. 2013). Since the EAB's first invasion in 2002 in Michigan, studies on life table analysis have been initiated. In Michigan, a detailed study on EAB life cycle found that EAB adult flight begins in May or June and ends towards September. Additionally, (L1, L2, L3, and L4) were found from July till October (Cappaert et al. 2005). Several methods such as establishing experimental cohorts of EAB or

accessing wild populations of EAB all around the year have been utilized to study the population dynamics of EAB in the northern USA (Duan et al. 2010).

Louisiana has a subtropical climate that is vastly different from the temperate regions of the native range of EAB. Hence, I predicted this temperature variation from temperate areas of EAB origin, will affect the lifecycle of EAB and its released parasitoids. Therefore, studies were conducted to study phenology of EAB in North Louisiana. Observations were made by sampling ash trees for different life stages every two months in infested areas. Adult EAB observations for the first emergence, peak emergence was done deploying purple prism traps and lindgren funnel traps.

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CHAPTER 2. WOOD-INHABITING INSECT COMMUNITIES ASSOCIATED WITH GREEN ASH (*FRAXINUS PENNSYLVANICA* MARSHALL) TREES IN LOUISIANA

2.1. Introduction

Biological invasions and other anthropogenic changes are the main reasons for loss of biodiversity (Lovett et al. 2006; Pimental 2005). One such invasion involves emerald ash borer, which has brought significant alterations in natural ecosystems causing ecological and economic effects (Cappaert et al. 2005). Several species groups including insects, birds, litter dwelling arthropods, etc. have been studied in EAB invaded areas in the northern parts of the United States. However, there is poor knowledge on the ecological changes, and the potential negative consequences of emerald ash borer on insects and other invertebrate groups in southern regions of the US.

Emerald ash borer, *Agrilus planipennis* Fairmaire (Buprestidae) (hereafter referred to as EAB), is a significant pest of ash (*Fraxinus* spp.) trees in North America (Knight et al. 2013). Since its initial discovery in Detroit, Michigan in 2002, EAB has killed millions of ash trees in both landscape plantings and natural ash communities (Haack et al. 2002) causing an economic loss of > \$10 billion. It can attack all the 16 of dominant and co-dominant ash species across the US (Cappaert et al. 2005), along with olive and white fringe tree (*Chionanthus virginicus* L.), recently confirmed as a larval host for EAB (Cipollini 2015). EAB larvae feeding causes stress symptoms on trees, such as epicormic shoots, crown loss, canopy dieback, defoliation, low crown ratio, low crown density, and eventually kills the tree in two to six years (McCullough and Katovich 2004; Knight et al. 2013) due to infestations of 100-1000

of larval mines inside the tree. Stressed ash trees can be identified based on signs such as D- shaped exit holes, S-shaped galleries and woodpecker flecking (Knight et al. 2013). Anthropogenic movement of infested plant material including firewood and nursery stock has accelerated its long-distance spread (McCullough et al. 2009) confirming its presence in thirty-three states of US and three Canadian provinces in North America (EAB Info 2018).

EAB infestations can cause changes in forest ecosystem structure, such as habitat fragmentation, canopy formation, increase in coarse woody debris, species distribution and establishment of alien plant species (Gandhi and Herms 2010). In northern Ohio, the Net Primary Production of forest was reduced by 31 % due to loss of ash trees along with compensatory growth of non-ash trees in those open spaces (Flower et al. 2013). Following ash mortality in Indiana and Ohio, vacant areas were occupied by new plants such as glassy buck thorn (*Frangula alnus* Mill) and exotic honeysuckles (*Lonicera tatarica* Mill, *L. morrowii* Mill) (Tallamy and Shropshire, 2009). Similarly, gaps created due to ash loss were filled by other native plants including spicebush (*Lindera benzoin* L.), pawpaw (*Asimina triloba* Dunal), and prickly ash (*Zanthoxylum americanum* Mill.) (*Carex* L.) in Michigan (Knight et al. 2013). EAB infestations negatively affect litter-dwelling arthropod communities due to canopy loss and reduced leaf litter moisture (Yi and Moldenke 2008). Gaps created by the loss of ash trees also reduce the ground beetle populations (Gandhi et al. 2014). Another study in Michigan, Ohio, Indiana, and Ontario estimated that 98 ash specialist invertebrates are at risk of extinction due to EAB invasion (Wagner and Todd 2015). Species at high risk of becoming endangered includes two Hercules

beetles (*Dynastes granti* Horn and *Dynastes tityus* L.), two rhinoceros beetles (*Xyloryctes jamaicensis* Drury and *Xyloryctes thestalus* Bates), and nine species of hawk moth (Sphingidae spp.) (Wagner and Todd 2015).

Ash dependent insect communities, especially saproxylic and wood inhabiting insects including wood decomposing fungus feeders, parasitoids, associated predators, detritivores feeding on their waste, and other associated insects (Speight 1989) are functionally important component of ash ecosystems. Estimating the loss of species diversity and abundance in managed invaded bottomlands can predict the effects of an invasive insect (Grove 2001). Insect faunae vary among different host types such as hardwood and softwood (As 1993). Insect community composition is affected by type of wood substrate such as live wooden logs, dead logs or leaf litter debris. Within dead wood itself, different wood types or levels of wood decay support different insect assemblages (Hammond et al. 2001). Additionally, wood diameter also affects the insect composition (Palm 1959). Previous studies showed small and medium diameter decaying wood (11 ± 4 cm: Brin et al. 2011) contained higher saproxylic species richness (Kappes & Topp, 2004). Another study also showed a positive relationship between dead-wood diameter and species richness, or abundance (Warren & Key 1991). This might be because larger diameter trees and dead wood are highly heterogeneous habitats, undergoing slower decomposition, hence supporting the colonization by different kinds of species (Kolstrom & Lumatjarvi 2000).

Forest site types also influence the susceptibility of species to a natural or anthropogenic disturbance (Kolstrom & Lumatjarvi 2000). Invasive species can

endanger local insect species by changing local environment and ecosystem.

Moreover, based on the competitive theory which states that invasive insects usually compete with native insect communities and thereby reducing their diversity (Begon et al. 2005), I proposed that invasive EAB will compete with other wood inhabiting insects of ash trees and hence its abundance will be lower in invaded areas than non-invaded. However, the insect species groups and their habitat requirements in bottomland ash trees in southern areas of USA are poorly known. Therefore, this study has following objectives. 1) To describe the insect abundance, diversity, and evenness and species assemblage patterns of wood- habiting insects among different forest sites (EAB/ non-EAB) in north, central and southern regions of Louisiana. 2) It described insect assemblages in different wood types including small diameter, large diameter, live, and dead logs. 3) It also explored the wood- inhabiting insect patterns highly associated with green ash trees in southern US. Wooden logs from live and dead standing green ash trees from EAB and non-EAB locations in different regions of Louisiana were harvested. Furthermore, wooden logs were classified into treatments of small and large diameter to discern the insect compositions in different wood size. Medium and small diameter wood (11 ± 4 cm) has maximum species richness and easily transportable for experimental study (Brin et al. 2011). Hence, we selected wood diameter in this size range. From June 2016- October 2017, the wood-inhabiting insect communities of these logs were collected using emergence traps (Ferro and Carlton 2011). Insects were identified to taxonomic family level and analyzed for total abundance, Shannon diversity, and

evenness among different sites and wood treatment types using several ecological models at experimental sites from 2016 to 2017.

2.2. Materials and methods

2.2.1. Study sites in Louisiana

Four green ash forests were selected in northern, central, and southern Louisiana. Two EAB-infested sites were located near the cities of Shongaloo, (N 33.0167°, W -93.3152°), and Andreas, (N 32.6625°, W -93.3693°) in Webster Parish in North Louisiana. Two sites without EAB presence were in Baton Rouge, (N 30.2644°, W 91.0404°); and Jean Laffite National Park, Barataria Preserve, (N 29.7839°, W-90.1176°) in Central and South Louisiana respectively. Study sites in Shongaloo, Andreas, Baton Rouge, Jean Laffite National Park are denoted by EAB 1, EAB 2, non-EAB 1, and non-EAB 2 respectively. The climate data from 2015-17 at EAB study sites showed maximum, minimum precipitation as 12.15 mm and 0.01 mm in northern Louisiana. The maximum, minimum temperature at both EAB sites was 38.8° C and 3.8° C (climdata.srcc.lsu.edu). Similarly, maximum and minimum precipitation rates for non-EAB 1 were (7.08 mm, 0.01 mm) and non-EAB 2 were (5.06 mm, 0.01 mm). The maximum and minimum temperature at these sites were 37.7° C, 2.7° C for non-EAB1 and 38.8° C, 3.8° C for non-EAB 2 (climdata.srcc.lsu.edu).

EAB 1 and EAB 2 sites were about 1.01 ha and 5.6 ha in area respectively, both showing external signs and symptoms of EAB attack such as dieback, woodpecker flecking, and D-exit holes (Herms and McCullough 2014). The forest species present at these northern sites include red maple (*Acer rubrum* L.), sweet

gum (*Liquidambar styraciflua* L., and hickory (*Carya sect. Carya* Nutt.) along with green ash (*Fraxinus pennsylvanica* Marshall) (W. Johnson 2016, personal communication). The Baton Rouge site called as Bercham was 74 ha in area owned by Vil Bents Company. This site contains 2.5 ha area covered by monoculture plantation of green ash (*Fraxinus pennsylvanica*). Jean Lafitte National Park (JELA) is a 7446 hectare preserve approximately 15 km south of New Orleans in Jefferson Parish, LA. The dominant tree species at JELA include bald cypress (*Taxodium distichum* Rich.), water tupelo *Nyssa aquatica* L., drummond red maple *Acer rubrum* var. *drummondii* (Hook. & Arn. ex Nutt.), pumpkin ash *Fraxinus profunda* (Bush), dwarf palmetto (*Sabal minor* Pers.) and green ash (*Fraxinus pennsylvanica* Marshall) (Denslow and Battaglia 2012).

2.2.2. Tree selection, log harvesting and setting up treatments (2016)

Standing green ash trees with > 5 cm diameter dbh were randomly selected and harvested using a chainsaw in June 2016. Logs were cut down based on log diameter a) large diameter: 14 to 24 cm, and b) small diameter: 2 to 12 cm logs and log condition a) dead logs in decay classes (class 2: >10% decay; class 3: 10 to 25% decay) (Hunter 1990), and (b) live. Also, decay class 2: has loose bark, and low sapwood degradation. Decay class 3: has little or no bark, and initiation of sapwood decay. Logs were placed in traps until reaching a weight of approximately 20 Kg. A total of eighty traps with five traps per treatment per study site were deployed.

2.2.3. Construction and deployment of emergence traps (2016)

Emergence traps were 16 l tote box (61 × 47 × 40 cm). Several modifications were done on each tote box to build emergence traps (Ferro and Carlton 2011). One

ventilation hole (~3 cm diameter) drilled at the front wall of tote box about ~10 cm above the bottom of the tote. Then, two ventilation holes ~3-cm diameter each drilled on the top lid of tote box. Ventilation holes were covered with double layer of Weedblock® landscape black fabric and secured with Liquid Nails® brand (LN-901) Heavy Duty Adhesive to prevent any insect escape. Another ~6 cm diameter hole drilled at the bottom on tote box and a Kerr® wide-mouth ~240 ml mason jar was secured around this opening with three screws (truss washer lath). A liquid nail adhesive was applied to tightly fix the mason jar and fill any gaps between jar and tote. Mason jars filled with propylene glycol were attached at the base of trap and traps were then filled with different log treatments.

Emergence traps from quarantined EAB locations were placed in the Kisatchie National Forest in Pineville (N 31.2497°, W -92.8174°). However, traps with the wooden substrate from non-EAB locations were placed at the LSU Agcenter Reproductive Biology Center, in St. Gabriel (N 30.2699°, W -91.1057°). The working principle of emergence chamber is that arthropods emerging from the substrate such as wooden logs, are attracted to the light coming through clear mason jar, hence fall in the mason jar and are collected and identified to the lower family levels at Entomology Department at LSU.

2.2.4. Insect collection and taxonomic identification (2016, 2017)

Sampling for the study was initiated in June 2016 and continued for 16 months. Insect samples were collected from emergence traps every two months until October 2017, when logs were discarded. For sample collection, contents of each glass jar were strained through a nylon mesh filter grit 300 Easter®. Samples

collected using mesh filter were then transferred to 20 ml vials containing 70% ethyl alcohol. Each scintillation vial was labelled in the field with date, location, collector name and respective trap number from which the sample is taken. Glass jars were filled with residual and new Propylene glycol antifreeze as needed. At the end, each mason jar was secured tightly to respective emergence trap for further collection. All insects were identified to family level using the appropriate taxonomic literature (Triplehorn et al. 2005). Voucher specimens were preserved according to guidelines and, deposited in the Louisiana arthropod museum, LSAM.

2.2.5. Statistical analysis

Analyses of insect distribution among large diameter, small diameter, live and dead logs were analyzed using multivariate analyses for assemblage composition, and into several generalized linear model, and linear mixed model analyses of total abundance (the number of individuals per family per trap), Shannon's diversity index (H') (the total number of different families in a community), and evenness (measure of the distribution of individuals among families). Specific hypotheses proposed that assemblages and ecological measures would respond to whether a log was alive or dead and small or large in diameter. For all analyses, the data were examined to determine whether analytical assumptions could be met and when multiple analyses were possible, each analysis was performed and assessed against diagnostic measures of model fit or appropriateness.

Total abundance, Shannon diversity, and evenness were analyzed as log-transformed response variable in a linear model that included treatment and presence of EAB (Program R, vers. 3.3.4, R Core Team 2017). All assumptions

assessed for the linear model, and the log-transformation were applied to address non-linearity. A total general linear (regression) model was used to find the effects of the presence of emerald ash borer on other insect communities of green ash trees. Shannon's diversity index and evenness were analyzed as a response variable in generalized linear models with treatment and presence of borer as fixed effects and then with log link functions using either Poisson, Poisson with a biomass offset, or a quasi-Poisson probability distribution (Program R, vers. 3.3.4, R Core Team 2017). Abundance of Curculionidae and Cerambycidae were analyzed per unit experimental site and among treatments for the study period (2016, 2017) using GLM. The model with the lowest deviance/degree of freedom selected for interpretation.

Hypothesis-based multivariate techniques PERMANOVA (Anderson 2001; vegan package, Program R, vers. 3.3.4, Oksanen et al. 2018; R Core Team 2017) and multivariate generalized linear models (MGLM; Wharton et al. 2012; mvabund package; Program R, vers. 3.3.4, Wang et al. 2018) with study site and treatment (large dead, large live, small dead, and small live) as fixed effects were evaluated. After exploratory analyses, multivariate generalized linear models were selected because PERMANOVA failed the test for homogeneity of dispersion (Anderson 2006; Wharton et al. 2012; Wang et al. 2012). MGLMs performed with log link functions, as well as, Poisson and negative binomial probability distributions. The MGLM with the lower residual likelihood values for response variables selected for interpretation. Contributions of insect families to total insect assemblage (all the different insects pooled together) were assessed by the analysis of similarity percentages (vegan packages; Program R, vers. 3.3.4, Oksanen et al. 2018; R Core Team 2017).

2.3. Results

2.3.1. Insect composition

65,614 arthropods were collected from emergence traps from July 2016 to October 2017. Of these, 12,022 arthropods were collected in June- December 2016, followed by 53,592 in January- October 2017. From these arthropods, 25,420 insects were collected in 2016 and 2017 comprising 11 orders and 106 families. Additionally, sixteen emerald ash borer adults were collected in the emergence traps. Staphylinidae, and Elateridae were the primary predaceous Coleoptera families and Formicidae the primary Hymenoptera family collected. Cecidomyiidae, Silvanidae, Sciaridae were the fungivorous families.

Twenty-seven hymenopteran families were collected. The highest proportion was Formicidae (54%) and composed mainly of arboreal ant species, followed by Braconidae (12%), and other Hymenoptera (34%). Among Coleoptera, about thirty-nine families were collected, with the most abundant family being Curculionidae (41%), which mostly consists of Scolytine subfamily including bark beetles and eastern ash bark beetles, followed by Cerambycidae (15%) and other beetle families. Fifteen families were collected from the Diptera, with Cecidiomyiidae (43%) collected at the highest proportion, followed by Sciaridae (21%), and other dipteran families (Appendix, A.1).

2.3.2. Effect of site on insect abundance

Insect abundance varied among study sites. In 2016, abundance did not differ significantly among study sites (Figure 2.1). However, in 2017, mean insect

abundance was significant at EAB 1 (1.452 ± 0.349 SE, $t = 4.157$, $P < 0.001$) and non-EAB 1 (1.366 ± 0.353 SE, $t = 3.864$, $P < 0.001$) sites than EAB 2 site (2.951 ± 0.250 SE, $t = 11.801$, $P < 0.001$). Insect abundance was 1.1-folds higher at EAB 1 than non-EAB 1 site. These are the log transformed values.

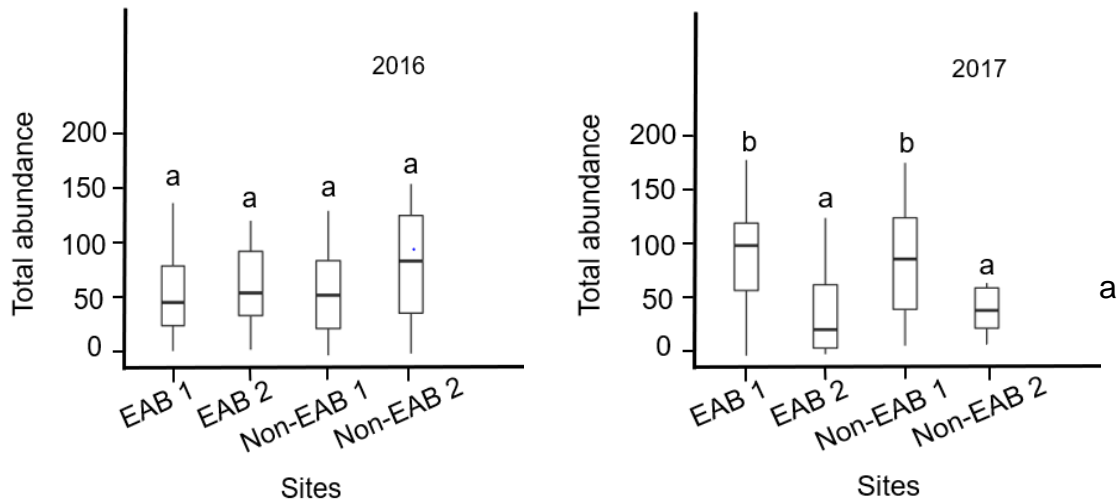


Figure 2.1. Total abundance of insects collected per site (EAB and Non-EAB sites) in Louisiana during 2016 and 2017. Bars with different letters were statistically significant ($P < 0.001$).

2.3.3. Effects of log diameter and condition on insect abundance

Insect abundance was compared among different years, sites, and treatments (Figure 2.2). All the values obtained were log transformed values. The mean insect abundance was significantly higher at EAB 1 site (-1.462 ± 0.694 SE, $t = -2.108$, $P < 0.05$) than non- EAB 1 site (0.194 ± 0.694 SE, $t = 0.280$, $P < 1$) and non- EAB 2 site (-0.305 ± 0.694 SE, $t = -0.441$, $P < 1$). Among EAB 1 site itself, abundance varied among treatments and was significantly different in small live logs (2.03 ± 0.98 SE, $t = 2.075$, $P < 0.05$) than other treatments in 2016. The insect abundance also varied

among sites and treatments in 2017. The mean insect abundance was significantly higher at non- EAB 1 site (2.765 ± 0.667 SE, $t = 4.144$, $P < 0.0001$) than EAB 1 site (2.114 ± 0.667 SE, $t = 3.169$, $P < 0.001$) and non- EAB 2 site (1.748 ± 0.667 SE, $t = 2.620$, $P < 0.01$). For non-EAB 1 site, insect abundance was found higher in live small logs (-3.362 ± 0.943 SE, $t = -3.563$, $P < 0.001$) than other treatments. Live small logs have 11.5-times higher abundance than live large, and 7.7-times higher than dead small logs at non- EAB 1 site.

2.3.4. Effects of log diameter and condition on diversity and evenness

Analysis for Shannon diversity indicated that diversity varied significantly among different treatments, but not sites (Figure 2.3a). All the values obtained were log transformed. In 2016, the treatments including dead small logs (2.384 ± 0.488 SE, $z = 4.879$, $P < 0.0001$) and live small logs (3.113 ± 0.461 SE, $z = 6.743$, $P < 0.0001$) have significantly higher diversity than live large logs (-0.0897 , ± 0.477 SE, $z = -0.188$, $P < 1$). However, among treatments and sites interaction, insect diversity was significantly higher in live small (-3.482 ± 0.701 SE, $z = -4.964$, $P < 0.0001$), dead small (-2.725 ± 0.729 SE, $z = -3.735$, $P < 0.0001$) and live large logs (-1.743 ± 0.705 SE, $z = -2.472$, $P < 0.1$) at non- EAB 2 site.

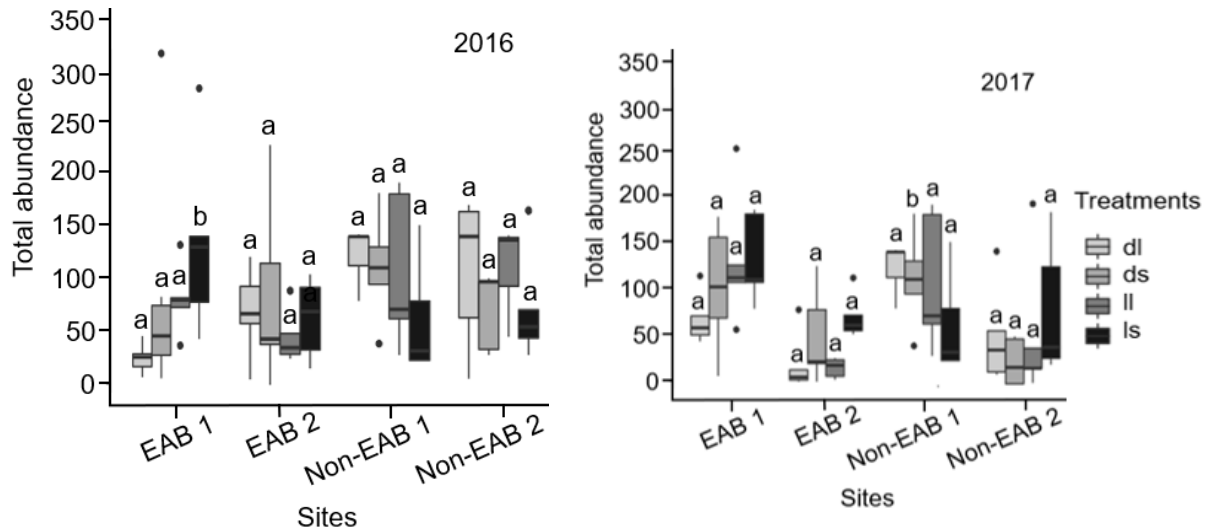


Figure 2.2. Total abundance of insects collected from EAB and non-EAB sites among different treatments in Louisiana during the study period. Treatments (dead, live, small diameter and large diameter) are represented by bars of different colors. Bars with different letters are significantly different.

In 2017, diversity was significant among dead small logs (2.613 ± 0.603 SE, $z = 4.332$, $P < 0.0001$) and live small logs (3.565 ± 0.548 SE, $z = 6.502$, $P < 0.0001$). However, among treatments and sites interaction, insect diversity was significantly higher in dead small (-3.611 ± 0.906 SE, $z = -3.985$, $P < 0.0001$), live large (-2.274 ± 0.829 SE, $z = -2.742$, $P < 0.01$) and live small logs (-4.100 ± 0.794 SE, $z = -5.163$, $P < 0.0001$) at non-EAB 2 site.

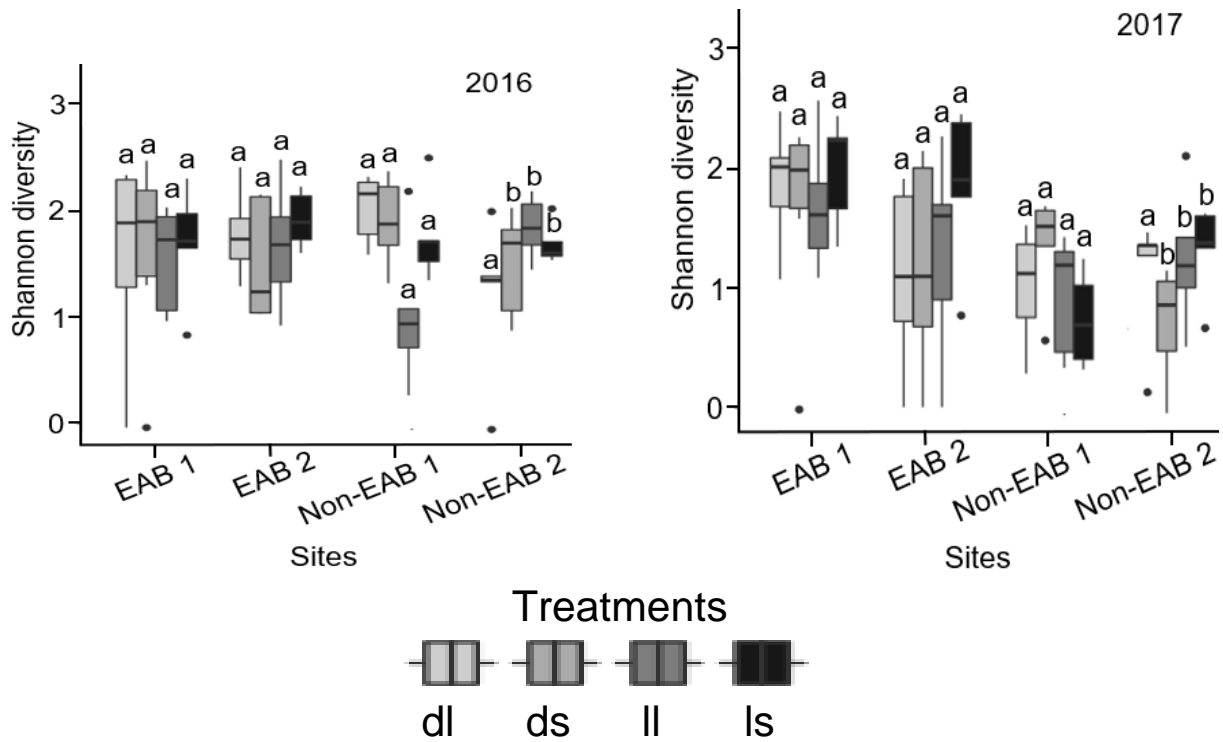
Insect evenness followed the similar pattern as Shannon diversity (Figure 2.3b). In 2016, evenness was significantly different in live small logs (3.113 ± 0.729 SE, $z = 4.269$, $P < 0.0001$) and dead small logs (2.384 ± 0.772 SE, $z = 3.088$, $P < 0.01$). Then, on comparing treatments at each of the site, evenness was significantly higher in live small (-3.48 ± 1.108 SE, $z = -3.142$, $P < 0.01$), live large (-1.74 ± 1.11 SE, $z = -1.565$, $P < 0.05$) and dead small logs (-2.72 ± 1.15 SE, $z = -2.364$, $P < 0.1$).

Then, in 2017, evenness was higher in treatments including dead small (2.613 ± 0.953 SE, $z = 2.740$, $P < 0.01$) and live small logs (3.565 ± 0.866 SE, $z = 4.113$, $P < 0.001$). Insect evenness among the treatments at each site were found higher at dead small (-3.61 ± 1.432 SE, $z = -2.521$, $P < 0.05$), live small (-4.10 ± 1.255 SE, $z = -3.266$, $P < 0.01$) and live large (-2.274 ± 1.311 SE, $z = -1.735$, $P < 0.1$).

2.3.5. Effects of treatments and sites on Cerambycidae and Curculionidae abundance

Curculionidae and Cerambycidae were among the most abundant beetle families collected from the emergence traps. Site-specific linear model analysis produced log transformed values. In 2016, there were no differences in Cerambycidae population among different treatments (Figure 2.4). In 2017, Cerambycidae abundance were significantly higher in live large trees (1.8 ± 0.79 SE, $t = 2.268$, $P < 0.05$) than live small logs (0.8 ± 0.793 SE, $t = 1.008$, $P < 1$) at non-EAB 2 site. Hence, in 2017 at non-EAB 2 site, Cerambycidae abundance were 2.7-folds greater at live large logs than live small logs.

(a)



(b)

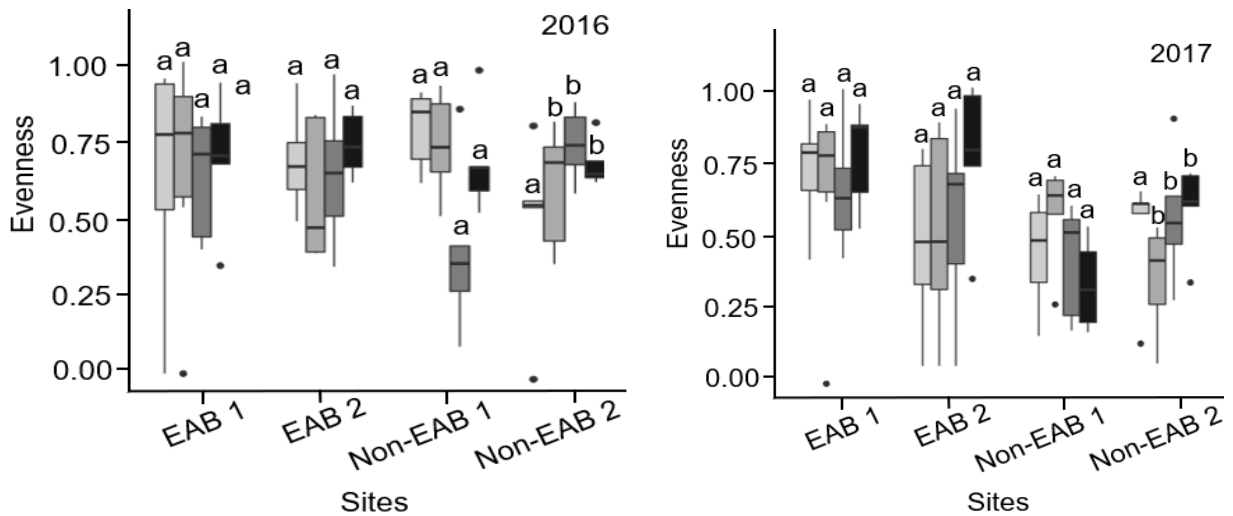


Figure 2.3. (a) Shannon diversity (H') and (b) evenness of insects collected from EAB and non-EAB sites among different treatments in Louisiana during the study period (2016, 2017). Treatments (dead, live, small diameter and large diameter) are represented by bars of different colors. dl = dead large, ds = dead small, ll = live large and ls = live small logs. Bars with different letters are statistically significant.

Abundance of Curculionidae did not vary among sites and treatments in 2016 (Figure 2.4). However, Curculionidae abundance was higher in only live small trees at non- EAB 1 (-4.200 ± 1.136 SE, $t = -3.698$, $P < 0.001$), non-EAB 2 (-4.200 ± 1.136 SE, $t = -3.698$, $P < 0.001$), and EAB 1 (-4.600 ± 1.136 SE, $t = -4.050$, $P < 0.001$) for 2017. Abundance of Curculionidae was higher at small live logs; approx. 1.5-fold greater at both non-EAB sites than EAB 1 site. Additionally, the abundance of both cerambycids and curculionids declined in 2017 compared to 2016.

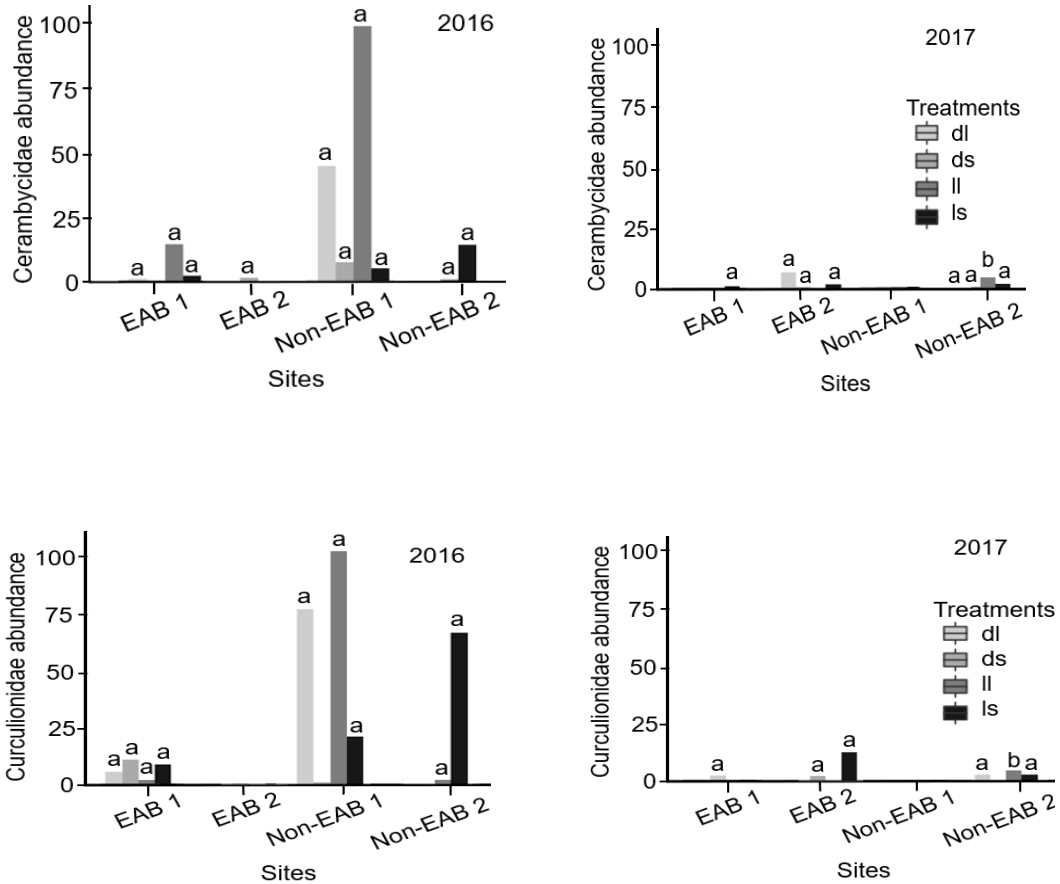


Figure 2.4. Total number of (a) Cerambycidae and (b) Curculionidae specimens per study site in each treatment and year of sampling. Treatments (dl= dead large, ds= dead small, ll= live large, and ls= live small) are represented by bars of different colors. Bars with different letter are significant.

2.3.6. Effect of EAB on insect assemblages among different treatments

Assemblages differed between live and dead, small and large trees (permutation test statistic = 23, $P < 0.01$). Specifically, among treatments large, live trees supported significantly different assemblage than all other treatments (Wald test statistic, $\chi^2 = 8.016$, $P < 0.01$). When compared by analysis of similarity percentages, large, live trees assemblage composition was composed of a higher proportions of Mycetophilidae, Megaspilidae, Ceratopogonidae, and Liposcelididae than the other treatments. Cumulative contribution of insect families suggested higher proportions of Ceratopogonidae (0.6517), Mycetophilidae (0.6779), Megaspilidae (0.7036) and Liposcelididae (0.6197) in live large trees (Table 2.1).

2.4. Discussion

2.4.1. Insect composition

The abundance and community composition were significantly different among treatments (dead, live, small diameter, and large diameter) of wooden logs. We collected a total of 25,420 insects belonging to 11 orders and 104 families from green ash logs through emergence traps in Louisiana. The insects were captured from narrow selection of wood, including only few microhabitats of saproxylic beetles. Moreover, collections were done from emergence traps charged with wooden logs in June 2016 with no additional wood added later. The emergence traps have been used in previous studies as an effective method to collect or rear the saproxylic or wood inhabiting insects associated with wooden logs, thereby reducing the chances of bycatch insects in the emergence traps (Ferro and Carlton 2011).

The most abundant orders collected were Diptera, Hymenoptera, and Coleoptera between pterygotes and Collembola among apterygotes. Among Coleoptera, the abundant beetle species collected were red-headed ash borer (*Neoclytus acuminatus* Fabricius, Cerambycidae) and eastern ash bark beetle (*Hylesinus aculeatus* Say, Curculionidae). Similarly, in another study in Maryland, insect captured from ash tree boles included red-headed ash borer (*Neoclytus acuminatus* Fabricius, Cerambycidae) (Jennings et al. 2017).

Besides Coleoptera, the most abundant hymenopteran families collected from the traps were Formicidae and Braconidae. Previous research also suggested Braconidae and Ichneumonidae as the abundant hymenopteran families of ash tree boles, with braconids containing a majority of released parasitoid that includes *S. agrili* (Jennings et al. 2017). However, we did not recover any released parasitoids in North Louisiana.

2.4.2. Forest site type and insect abundance

The insect abundance and community composition were significantly different among sites and was higher in 2016 compared to 2017. Among our EAB and non-EAB sites, it was hypothesized that non-EAB areas will have higher insect abundance, due to absence of any competition from invasive EAB, based on competitive theory which states that diversity of the native insect communities usually declines in the presence of invasive insects due to competition (Begon et al. 2005). However, total insect abundance was higher in small diameter live logs at EAB 1 site in 2016 and again was higher at EAB 1 and non-EAB 1 sites for small diameter live logs in 2017.

Table 2.1. Cumulative contribution of pterygotes (winged) insect families on assemblage among different treatments such as dead large, dead small, live large, and live small. Collembola is an apterygote (wingless) and was not included in the table. Insect families among different treatments were analyzed using Wald test statistic, $\chi^2 = 8.016$, $P < 0.01$.

Treatments					
Dead large vs dead small	Dead large vs live large	Dead large vs live small	Dead small vs live large	Dead small vs live small	Live large vs live small
Formicidae 0.4080	Curculionidae 0.2896	Formicidae 0.1716	Formicidae 0.4435	Formicidae 0.4655	Formicidae 0.1693
Curculionidae 0.5138	Formicidae 0.3968	Curculionidae 0.4191	Sciaridae 0.5198	Pompilidae 0.5510	Pompilidae 0.4371
Sciaridae 0.5805	Sciaridae 0.4796	Pompilidae 0.4938	Cecidomyiidae 0.5783	Cecidomyiidae 0.6129	Sciaridae 0.5094
Cecidomyiidae 0.6357	Mycetophilidae 0.5441	Sciaridae 0.5584	Pompilidae 0.6276	Sciaridae 0.6717	Cecidomyiidae 0.5758
Mycetophilidae 0.6902	Cecidomyiidae 0.6055	Cecidomyiidae 0.6194	Liposcelididae 0.6656	Mycetophilidae 0.7035	Liposcelididae 0.6197
Phoridae 0.7297	Liposcelididae 0.6611	Mycetophilidae 0.6784	Curculionidae 0.6941		Ceratopogonidae 0.6517
	Phoridae 0.7111	Phoridae 0.7118	Mycetophilidae 0.7208		Mycetophilidae 0.6779
					Megaspilidae 0.7036

These differences in insect abundance can be explained through two possible reasons. Firstly, different structural and geographical features of the site (i.e. EAB 1

and EAB 2 in northern, non-EAB 1 in central, and non-EAB 2 in southern regions of Louisiana) affect the insect composition and hence abundance (Minelli 2016).

Secondly, EAB was confirmed in Louisiana in 2015, and this study to quantify the insect abundance, diversity, and evenness associated with wood-inhabiting ash logs was started in 2016. Therefore, it might be too early to visualize the effects of EAB invasion on wood-inhabiting insects of ash trees in Louisiana. Additionally, selection of sampling site for non-EAB locations should be done near of EAB sites in northern regions of Louisiana to effectively compare species abundance and diversity patterns in that area.

2.4.3. Wood diameter and insect abundance

The overall abundance of insects differed among wood types (dead/ live), and wood size (large/ small diameter). From dead, live, small and large diameter wood samples, about 39 beetle families were collected. Small diameter logs (2-12 cm) have higher abundance than large diameter (14-24 cm) among live trees at EAB site in 2016 and non-EAB 1 site in 2017 respectively. A survey of beechwood forests in Europe predicted that medium diameter size (11 ± 4 cm) has more abundance than large diameter size (40-60cm) (Kappes et. al 2004), which falls in the close range of our small diameter logs (2- 12 cm). However, our study did not include environmental variables and habitat characteristics, hence it would be difficult to predict the mechanism behind the higher insect abundance in live small diameter logs at these sites. These small diameter logs inhabit specific insect fauna and hence, removing these small-diameter wood for firewood or other purposes can affect the overall species abundance in bottomland forest as found in studies (Brin et al. 2011).

2.4.4. Substrate volume, study site, wood type, and wood diameter influence insect diversity

Overall, in our study, treatments- dead small, live large and live small logs had high insect diversity compared to large dead logs during the study period at non-EAB 2 site. Diversity decreases with increases in the altitude or latitude (Lawton et al. 1987), and our results also showed high diversity at lower altitude, non-EAB 1 site than other sites. However, this finding can underestimate actual number of local species present and this is usually true for stream and terrestrial insects (Mark and Hawkins 2003). Further studies need to be done exploring the mechanisms controlling insect diversity.

Based on wood type, it was hypothesized that dead wood being a heterogenous habitat (Grove 2001) supports more species and hence has higher insect diversity than live logs. Another potential factor for insect diversity is the stage of the dead wood collected (Grove 2001). Saproxylic insect diversity is usually higher in early and medium stage dead wood along with live wood than late decay wood (Alexander 2002). However, in our study, insect diversity was higher in both live logs (small, large diameter) and dead (small diameter) logs. This might be due to different method of collection and volume of substrate for saproxylic insects, i.e., collecting insects from a bunch of logs in emergence trap compared to individual logs in bole trap besides wood types.

Wood debris size and saproxylic insect diversity are positively correlated (Ranius and Jansson 2000). However, in our study, diversity was significantly higher among small diameter (2- 12 cm) and sometimes in large diameter logs (14 to 24 cm)

at non-EAB sites. Any emergence chamber despite varied size and dimensions alter the microclimate of substrate hence would influence the emergence of species. This difference in insect diversity among wood diameter can be due to micro climate factors (temperature, humidity) of the collection trap (Minelli et al. 2002, Ferro and Carlton 2011). Additionally, insect evenness followed the similar trend as insect diversity among different treatments and sites in 2016 and 2017.

2.4.5. Curculionidae and Cerambycidae abundance

Curculionidae and Cerambycidae were trapped in higher numbers in non-EAB sites, i.e. undisturbed sites. Scolytinae population were higher in undisturbed sites after one year compared to disturbed sites in White Spruce trees (Werner 2002). The vegetation types, habitat and environmental factors also influence the abundance of species in an area (Minelli 2016). Cerambycidae were trapped in higher number from live large trees and Curculionidae from live small trees. This might be since Cerambycidae are long horned beetles and usually bigger in size while Curculionidae are small sized beetles (Triplehorn et al. 2005). Most abundant species among these were eastern ash bark beetle (*Hylesinus aculeatus* Say, Curculionidae), and red-headed ash borer (*Neoclytus acuminatus* Fabricius, Cerambycidae). These are native wood borers and highly specific to ash trees (Gandhi and Herms 2010).

2.4.6. Insect assemblage differences

Insect assemblage in large live ash trees was significantly different than other wood types and, was composed mainly of Mycetophilidae (Diptera), Megaspilidae (Hymenoptera), Ceratopogonidae (Diptera) and Liposcelididae (Psocodea) among study sites. We proposed that these four insect families might decline in number with

other wood borers as emerald ash borer moves further south in coming years. The list of threatened invertebrate herbivores in North USA and Canada includes 98 species of ash specialists arthropods (Wagner and Todd 2016).

This study provided the baseline data on wood-inhabiting insect taxa associated with green ash trees in Louisiana. Hence, can be used to predict in coming years that how the arthropod community might change as the EAB infestation proceed further down into southern Louisiana. Moreover, the collected insects and arthropods if classified to lower taxonomic level (species, subspecies), can be used to construct feeding guilds and community structures of green ash inhabiting arthropods.

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CHAPTER 3. PHENOLOGY OF EMERALD ASH BORER AND ESTABLISHMENT OF NON-NATIVE PARASITIDS IN LOUISIANA

3.1. Introduction

Emerald ash borer, *Agilus planipennis* Fairmaire (Buprestidae) (EAB), is one of the most destructive invasive forest pests in North America attacking sixteen species of ash (*Fraxinus* spp.) trees (Cappaert et al. 2005). This beetle originating from regions of China and South Asia (Yu 1992) has killed millions of ash trees in both landscape plantings and natural ash communities. Experts believe that this green beetle entered the US through wooden packaging material and was first detected in Detroit, Michigan (Haack et al. 2002). By June 2018, EAB has been confirmed in 33 states of USA and several Canadian provinces (EAB Info 2018). Anthropogenic movement of infested wood facilitate the long dispersal of EAB (Buck and Marshall 2008). Several quarantines have been implemented in infested areas to restrict the further spread of EAB.

In its native range, the life cycle of EAB, including development events such as oviposition, pupal development and adult emergence requires 1 to 2 years and is temperature dependent (Cappaert et al. 2005; Siegert et al 2009). The adult's first emergence begins in late spring, peaks in early to mid-summer, and ends in late summer when adult starts feeding on ash leaves in temperate regions of the USA (Cappaert et al. 2005). EAB adults begin their flight in May or June, peak in July-August, and ends by September in the Great Lake regions of North America (Wang et al. 2010; Cappaert et al. 2005). In spring 2015, EAB was detected in Webster Parish, Louisiana (N 33.0167°, W -93.3152°), which is currently the southern limit of

emerald ash borer distribution (Carlton et al. 2018). Climate suitability can impact the establishment of invasive species as well as released biological control agents. Hence, we predict that the difference in temperature in Louisiana from native temperate areas of emerald ash borer, will affect its lifecycle as well as its released parasitoids.

Several management methods to control EAB including sanitation of areas by removing the infested dead or live trees, monitoring of EAB adults using purple prism traps containing z-3-hexanol as a lure and chemical control using imidacloprid, and fipronil, have been initiated in urban and landscape settings (Crook and Mastro 2010).

Biological control involving the release of EAB co-evolved parasitoids is a potential tool for managing EAB in North America (Bauer et al. 2008). Four species of parasitoids from China were introduced into Michigan and are being distributed to other EAB-infested states in the USA for biocontrol of this invasive pest (Bauer et al. 2008). Research has shown that parasitoids deployed for emerald ash borer biological control program in the United States including *Oobius agrili*, *Spathius agrili*, and *Tetrastichus planipennis* can cause up to 70% parasitization in Asia (Duan et al. 2012). According to Wang et al. (2010), *Oobius agrili* (egg parasitoid) can parasitize about 60% and 28% of EAB eggs, *Spathius agrili* (pupal parasitoid) parasitize 90% and 45 % of EAB larvae, and *Tetrastichus planipennis* (larval parasitoid) can parasitize 50% and 24% of the EAB population in China and USA respectively. Efforts are concentrated in the release of exotic parasitoids as well as a post-release assessment of exotic parasitoids to manage the emerald ash borer populations

across North America (Bauer et al. 2016). Numerous studies have monitored the establishment of introduced parasitoids of EAB (Gould et al. 2013) by different sampling methods such as (1) sentinel eggs under flaps of bark or sentinel egg logs (small ash logs bearing EAB eggs) followed by visual surveys estimate egg parasitism rates, (2) emergence traps using ash logs or bark samples for parasitoid emergence (Bauer et al. 2016) and (3) yellow pan traps for monitoring adult emerald ash borer parasitoids.

In addition, Larval morphology has been studied by several researchers to understand the basic biology and ecology of emerald ash borer (Chamorro et al. 2012). The length and width of larval epicranium along with urogomphi measurements have been used to distinguish between different instars of emerald ash borer (Chamorro et al. 2012). Based on these morphological features of different life stages of EAB, life tables have been constructed in Michigan by collecting wild populations and experimental cohort populations of EAB (Duan et al. 2014). However, no information is available on population dynamics of EAB in the southern USA. Therefore, the first objective of the study was to construct the phenology of emerald ash borer in Louisiana. Observations were made by sampling ash trees for different life stages of EAB every two months in the infested areas. Mature and immature stages of EAB were collected from infested sites in Webster Parish, Louisiana. Head capsule measurements of extracted EAB larvae from trees along with adult EAB observations for the first emergence, peak emergence by deploying purple prism traps, lindgren funnel traps and green prism traps. The second objective of the study was to monitor the establishment of released exotic parasitoids in

Louisiana using yellow pan traps (YPTs), visual surveys, collecting immatures of emerald ash borer (eggs, larvae, and pupae) for parasitoid recovery.

3.2. Materials and methods

3.2.1. Study sites

Field surveys were conducted from June 2016 to November 2017 in two EAB infested sites, (Shongaloo N 33.0167°, W -93.3152° and Andreas N 32.6625°, W -93.3693°) in Webster Parish, Louisiana. Shongaloo and Andreas have been referred as EAB 1 and EAB 2 site respectively during the study. The vegetation type at both sites was mostly dominant ash trees with 12-25% mean ash cover. EAB 1 and EAB 2 sites were approx. 1.0 ha and 5.6 ha in area respectively, both showed EAB signs and symptoms on trees such as woodpecker flecking, dieback, canopy loss, low crown cover, epicormic shoots, and D- shaped exit holes (Herms and McCullough 2014). The dominant species at these sites include green ash (*Fraxinus pennsylvanica*) besides other vegetation including red maple (*Acer rubrum* L.), sweet gum (*Liquidambar styraciflua* L., and hickory (*Carya sect. Carya* Nutt.) (W. Johnson 2016, personal communication).

Additionally, parasitoids have been introduced at these sites in 2016. A total of 16,300 *O. agrili*, 21,135 *T. planipennisi*, and 2,167 *S. agrili* were released at EAB 1 site whereas 5,700 *O. agrili*, 8,500 *T. planipennisi*, and 1,067 *S. agrili* were released at non-EAB site to manage EAB populations (MapBiocontrol 2018) in Louisiana.

3.2.2. Emerald ash borer egg collection from ash trees (2016, 2017)

Early in July 2016, a total of twenty EAB infested green ash trees with damage signs such as woodpecker flecking, vertical splitting in barks, D-shaped holes, dieback, crown thinning, and epicormic shoots were selected, and ribbon tagged at both study sites. These trees have parasitoid releases of three exotic parasitoids namely *Oobius agrili*, *Spathius agrili* and *Tetrastichus planipennis* in 2015 and 2016. These tagged trees were then marked with a vertical bark area 10 x 50 cm on the south, southwest, or west side on the lower trunk (about 1-m above ground) followed by wrapping a massive plastic sheet around an individual tree about 2 m from its base like an inverted cone with duct tape. Then, by debarking this marked bark area, EAB eggs and wooden debris were collected using a drawknife and stored in a paper bag labeled with tree number, dbh, site number and date. These collected bark samples were then dried for a month at room temperature and followed by sieving through number 14 soil sieve to separate EAB eggs and small adult parasitoids from wood debris (Bauer et al. 2011). Collected eggs were examined under a microscope for parasitoid holes to detect parasitization.

3.2.3. Larval epicranium measurements and immatures collection in 2016 and 2017)

Beginning in March 2016, a total of 8-10 EAB attacked ash trees were randomly selected, debarked using a drawknife in the field to expose S-shaped galleries and EAB life stages were collected using a pair of forceps. During each sampling occasion at a two-month interval, a total of 50-70 larvae were collected per site, and notes were taken regarding the condition of immature stages, i.e., larval

instar, J-shaped larvae and pre-pupae. Collected samples were labeled with site location, tree number, and date of collection and preserved in 70% ethyl alcohol for further processing. Collected larvae were identified to different instars based on larval keys (Chamorro et al. 2012). Moreover, the width and height of the sclerotized epicranium of collected individuals were measured under dissecting microscope and image J software (Schneider et. al 2012). In October 2017, four trees were cut at each of the experimental sites and their bark was peeled off using drawknife to collect the immature larval and pupal stages to detect parasitization.

3.2.4. EAB adult surveys using pheromone traps (2015, 2016, 2017)

At each site, the United States Forest Service (USFS) personnel deployed fifteen purple prism trap, and twelve lindgren funnel traps baited with manuka, and z-3-hexanol attractant at 0.4- 5m above the ground on the south or southwest side with the help of ropes on a tree branch (Crook and Mastro 2010). Traps were placed in the lower- mid tree crown from early March to late July 2015. The lure was replaced every 60 days and adults trapped were collected every 14 days. Moreover, estimates of first adult emergence, peak emergence, and last adult emergence made from the trap counts from June 2016 to August 2017. Specimens were preserved in a Ziploc bag filled with ethanol, identified and sexed (male/female).

3.2.5. Visual surveys and yellow pan trap collections (2016, 2017)

EAB immatures collected in the previous study to estimate epicranium width of different stages were then strictly observed for attached and internal parasitoids (Appendix, A.3). Potential parasitoid specimens were collected, labeled with ID (tree

number, site name, and GPS coordinates), and reared in quarantine laboratory at Department of Entomology, LSU to recover the parasitoids.

At each site, we selected green ash trees with signs of EAB presence. YPTs consisting of two yellow bowl were constructed according to guidelines of USDA-APHIS (Bauer et al. 2012). A total of fifteen YPTs filled with a non-scented detergent solution was tied to a tree per site. YPTs were deployed at two release sites in Webster Parish from July 2017 to September 2017. After setting up, traps were labeled with site number, GPS coordinate, and date. Traps were checked every two weeks, and collected specimens were identified to family level using taxonomic literature (Triplehorn et al. 2005).

3.3. Results

Results indicate that EAB adults started to emerge in April and were collected until late June (Figure 3.1). First larval instars were collected in serpentine galleries in early June-July followed by second larval instars during early June- early August. The third instar was observed during mid-June- late October. The fourth instar was found during early August to early November. J-shaped larvae or pre-pupae were observed during July to March. It is important to note that J-larvae were present throughout the year except during the adult activity period (April- June).

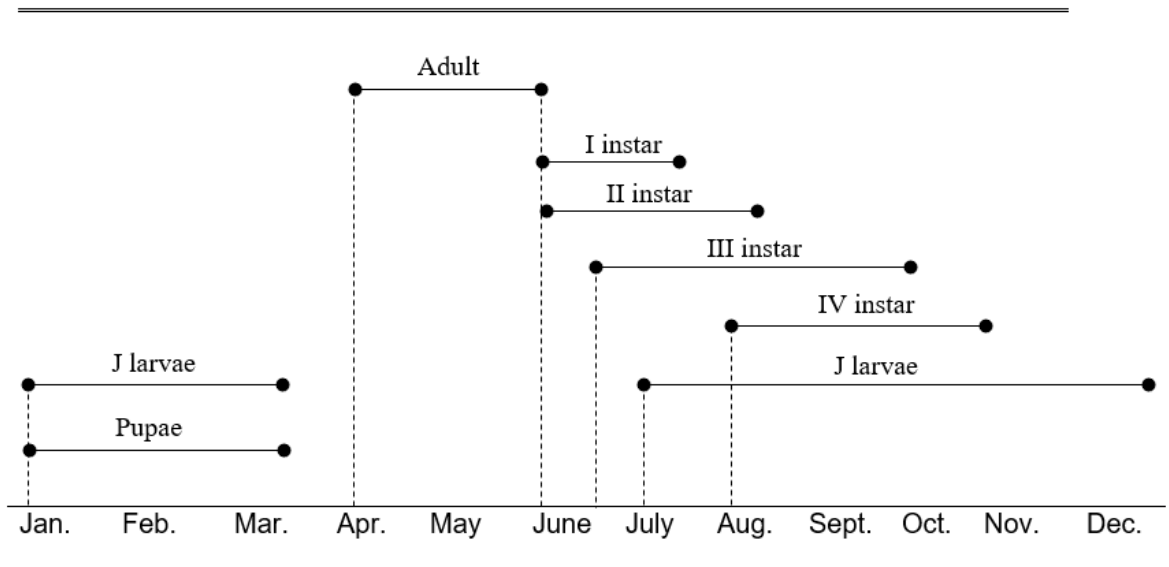


Figure 3.1. Distribution of different life stages of emerald ash borer life cycle observed in Webster Parish, Louisiana in 2017.

Width and height of head capsules have been used in several studies to distinguish among larval instars of insects (Martin et al. 2018). Larval head measurements for EAB includes length(mm) ranging from 0.2- 1.6 and width (mm) from 0.2- 1.8 mm. Head capsule size of EAB increases and became more variable as larvae matured. The relative abundance of different larval instars of EAB showed L3 and L4 have high populations throughout the study period (Figure 3.3).

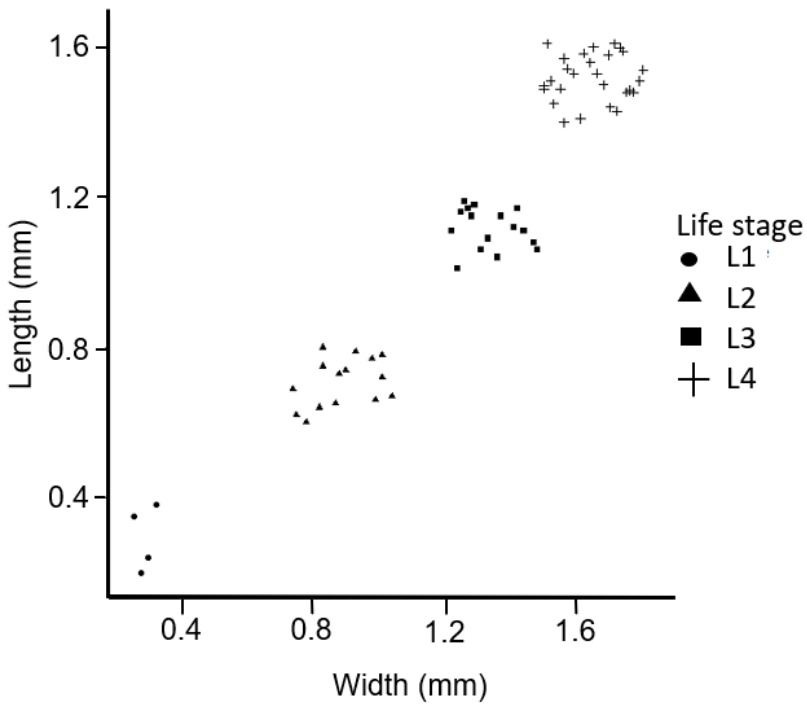


Figure 3.2. Length and width (mm) dimensions of head capsule of different larval stages (L1, L2, L3, and L4) of emerald ash borer.

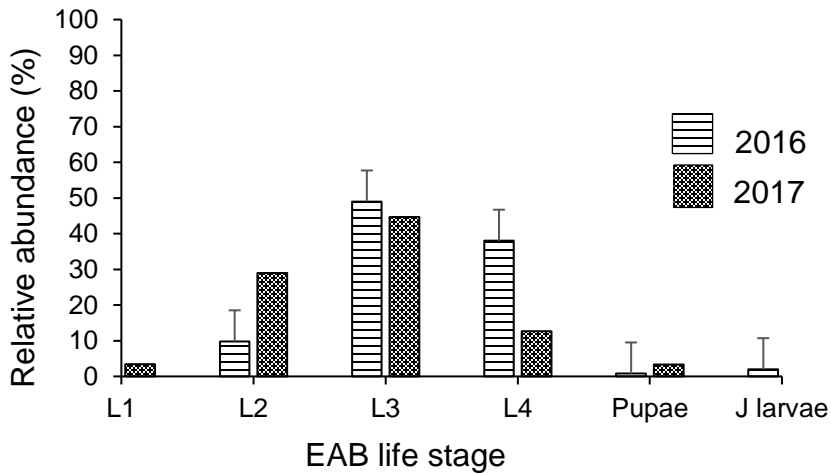


Figure 3.3. Relative abundance of different immature life stages of emerald ash borer collected during the study period (2016-2017) in Louisiana.

Adult peak emergence was found in late April at Webster Parish, Louisiana (Figure 3.4). Lindgren traps captured more males than females and vice versa (Figure 3.5). Among both traps, the total adult catch was higher in lindgren traps than purple traps for the year 2016 (Figure 3.5).

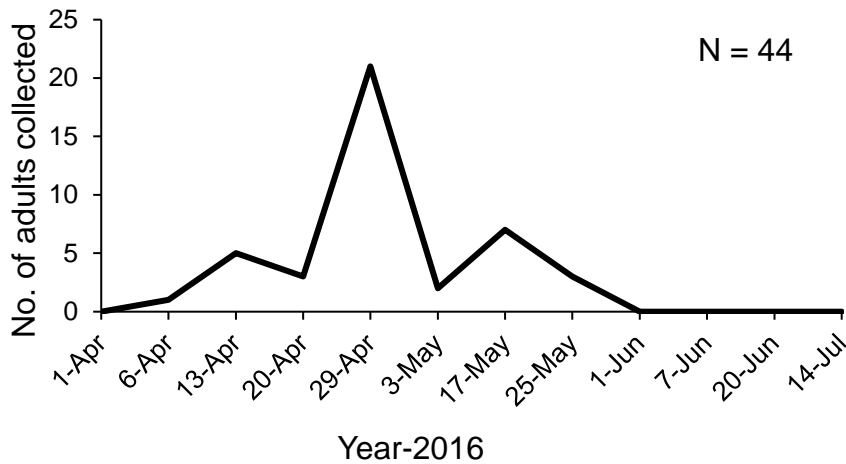


Figure 3.4. Total number of EAB adults (N) collected in lindgren funnel traps at EAB 1 site in Louisiana. Solid line indicates the peak adult emergence in late April of the year.

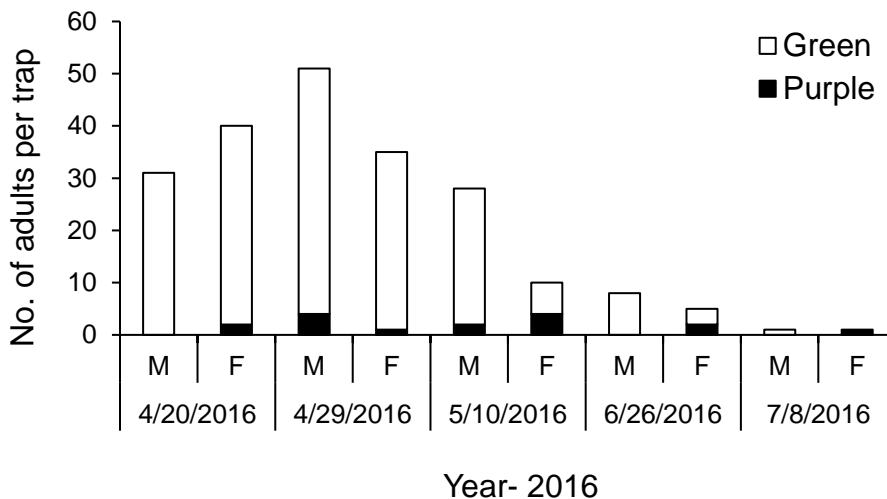


Figure 3.5. Total number of male and female of emerald ash borer trapped per lindgren and purple prism traps.

A total of 16,300 *O. agrili*; 21,135 *T. planipennis*; 2,167 *S. agrili* were released at EAB 1 site in Webster Parish June to October 2016 (Appendix, A.2). Additionally, a total of 5,700 *O. agrili*, 8,500 *T. planipennis*, 1,067 *S. agrili* with total 15,267 were released from 21 July to 20 October 2016 at EAB 2 site in Webster Parish (Appendix, A.2). Results from yellow pan traps (YPTs) did not recover any released parasitoids except – one *O. agrili* specimen at Shongaloo. No other released parasitoids *S. agrili* (Braconidae) and *T. planipennis* (Eulophidae), were recovered at any of the released site using YPTs. Ichneumonidae was amongst the abundant parasitic hymenopteran family collected in YPTs (Figure 3.6) along with non-parasitic Diapriidae and Pergidae. These ichneumonids including *Cubocephalus sp.*, *Dolichomitus sp.*, and *Orthizema sp.*, and have been found attacking emerald ash borer in northeastern North America (Duan et al. 2014). However, in our YPTs, we did not collect any of the native ichneumonids.

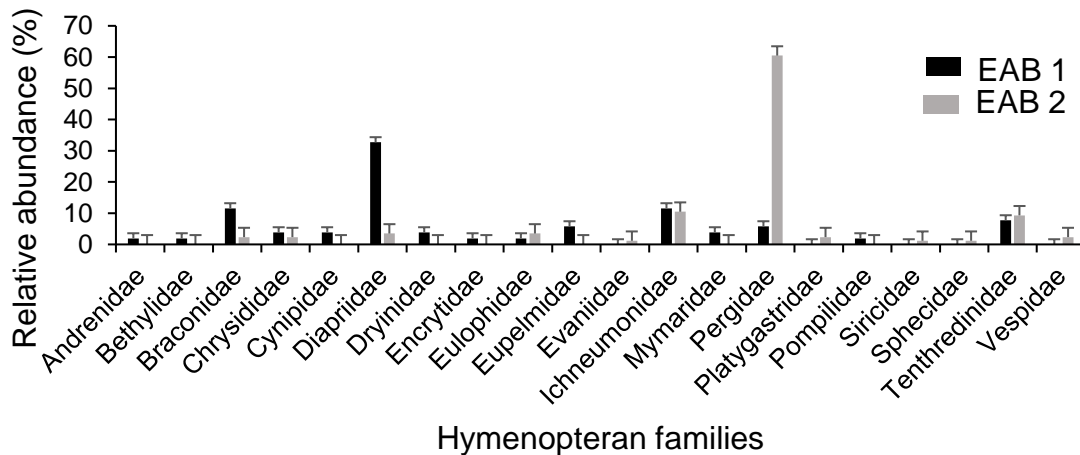


Figure 3.6. Relative abundance of different hymenopteran families collected per experimental site from yellow pan traps (YPTs) in 2017.

3.4. Discussion

3.4.1. Fast development in LA

Emerald ash borer emergence and adult flight began early in North Louisiana. The first adult emergence occurred in early April, peaked in late April- early May and the last catch was in late June in EAB 1 site. In native range of Asia and the Great Lakes region of North America, EAB flight season depending on 400–500 growing degree days (GDD) based on 10°C, begins in May or June with adult peak activity in June-July followed by ending in September (Wei et al. 2007).

EAB larvae appeared earlier in season including, June for L1, L2, L3 and August for L4, Then, pre- pupae/ or J larvae were collected in July until March. These J larvae later transform to pupae (January- March) while some survive winter as J larvae and were present mostly all through the year except the adult activity period (April- June). In Michigan, most EAB instars finished feeding in October or November to form prepupae (Cappaert et al. 2005) followed by pupation in mid-April to May. Additionally, in Michigan, some EAB overwinter as young larvae and go through the second year of development before emerging as adults (Jennings et al. 2013). Also, knowledge of the lifecycle of EAB in Louisiana is crucial to conduct its successful biological control in order to effectively attack the specific life stage of EAB according to parasitoid released. *O. agrili* (egg parasitoid) are present from June and till September synchronizing with EAB adult oviposition period in northeast China and Michigan (Liu et al. 2007). *S. agrili* are present from July through August attacking late instar EAB in native China and Michigan (Gould et al. 2011). *T. planipennisi* are

present from April or May until October parasitizing EAB larvae ranging from second through fourth instar (Liu et al. 2007).

Throughout our study, EAB developed faster in Louisiana. Most of the life stages early in Louisiana compared to Michigan based on heat accumulation units (Liu 2017). Wild cohorts and experimental cohorts study in Michigan indicated that EAB completes its life cycle in more than a year (Duan et al. 2014). In the present study, EAB larvae and pupae were collected during specific time of the year and no life stage was found twice during the life cycle except J larvae. EAB overwinters as J larvae in outer wood of ash trees in Louisiana compared to young larvae in Michigan and native regions of Asia (Yu 1992) suggesting its one generation per year in Louisiana. Additionally, adult traps deployed by USDA-APHIS also yielded EAB adults until July and no second-generation adults were captured after late summer (W. Johnson 2018, personal communication) in Louisiana, which suggest a one-year life cycle of EAB in Louisiana.

Our sampling (June 2016- December 2017) was based on field populations of EAB and more research is needed to confirm voltinism of EAB in Louisiana. Hot and humid climate might be responsible for shortened development time for EAB in Louisiana.

3.4.2. Head capsule measurements to identify larval instars

In this study, larval measurements were done to identify different instars based on head epicranium length and width. The head capsule sizes range become broader for fourth instars, which might be due to sexual dimorphism in adult body size between the males and females in L4, as found in EAB morphology study in

Michigan (Cappaert et al. 2005). The head capsule size varies among different instars and can be used along with urogomphi, other larval features (Chamorro et al. 2012) to distinguish different instars.

3.4.3. Lack of parasitoid establishment

No exotic parasitoids were captured in yellow pan traps, except for – one *O. agrili* at the EAB 1 site. Surveys of emerald ash borer attacked trees along with larvae collection from galleries did not yield any larval parasitoids and did not provided any signs of parasitism. Additionally, emerald ash borer larvae collected by girdling trees in October 2017, also failed to capture any of the released parasitoids.

No egg parasitoids were recovered which might be due to insufficient EAB densities in the forest to attract egg parasitoids (*O. agrili*). In a study in Michigan, EAB eggs also failed to capture its egg parasitoids in initial years (Duan et al. 2012) Also, *O. agrili* has a short life span of less than 24 hours (L. Bauer, unpublished data) and hence its searching capacity for host might be less making it difficult to establish populations in forests in Louisiana.

No larval parasitoids including *T. planipennisi* and *S. agrili* were recovered. A previous study using late larval instar demonstrated parasitism by *T. planipennisi* and *S. agrili*. two years after last of parasitoid release in Michigan (Bauer et al. 2011). However, in the present study, no larval parasitism was detected by debarking tree, collecting the larval instars and manually inspecting the larvae for signs of parasitization. Lack of establishment of parasitoids at introduced sites might be due to the absence of specific EAB stage corresponding to parasitoid stage, hence affecting the reproduction and survivorship of parasitoids.

Other possible reason for lack of establishment of EAB might be timing of recovery methods. In Michigan, egg and larval parasitoids were recovered after two to three years since the last parasitoids release, then detection of parasitoids increased with subsequent years (Duan et al. 2018). For example, egg parasitism by *O. agrili* increased from 1 to 4% from 2008–2011 to 28% by 2014. However, in Louisiana, EAB was detected in Spring 2015 (Carlton et al. 2018) and releases of parasitoids to manage EAB were conducted in Fall (2015 to 2016) (EAB Info 2018, MapBiocontrol 2018) followed by monitoring program in 2017 (MapBiocontrol 2018). This study was initiated a year after the last parasitoid release in Louisiana, which was probably too early to detect parasitoid establishment using intensive sampling methods such as YPTs, girdling and debarking trees. The recovery of parasitoids should be done two or three years post the last release of parasitoids (Duan et al. 2018).

Regional differences such as high temperature and high humidity in southern United States compared to the temperate native range of parasitoids have effects on EAB bio control (Bauer et al. 2015). Different life stages of emerald ash borer develop faster in southern United States compared to North USA, because of variation in heat accumulation (Growing Degree Days) in these regions (Duan et al. 2018). Moreover, one-year life cycle of EAB in southern USA compared to more than a year life cycle in native Asia regions of EAB would have reduced the impact of parasitoids on EAB population. Exploration of parasitoids in South China is needed to discover parasitoids suitable for establishment in southern USA (Duan et al. 2018).

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CHAPTER 4. CONCLUSIONS

During the study, a total of 25,420 insects belonging to 11 orders and 104 families from green ash (*Fraxinus pennsylvanica* Marshall) were collected. The most abundant orders collected were Diptera, Hymenoptera, and Coleoptera. Among Coleoptera, the abundant colonizing beetles were red-headed ash borer (*Neoclytus acuminatus* Fabricius, Cerambycidae) and eastern ash bark beetle (*Hylesinus aculeatus* Say, Curculionidae). Formicidae and Braconidae were amongst the abundant Hymenopteran families but did not yield any released Hymenopteran parasitoids. In addition, the abundance of phloem and xylem feeders including Cerambycidae, Curculionidae declined in 2017 compared with 2016. This study further expands our knowledge about insect colonization after a disturbance or an invasion.

Another findings suggested that insect abundance varies in EAB and non-EAB sites which might be due to different geographical features of the sites instead of solely due to presence of emerald ash borer (Minelli 2016). We speculated that it might be too early to predict the effects of EAB invasion on wood inhabiting insects of ash trees in southern USA (Louisiana). This study showed the insect abundance levels in initial stages of EAB invasion and it might change in coming years as EAB moves further down South Louisiana.

Among different treatments such as dead, live, small diameter and large trees, our results suggested that small diameter logs (2- 12 cm) have higher insect abundance than large diameter (14-24 cm) woods at our study sites. This might be

since wood-inhabiting insect's abundance are usually higher in medium diameter (11 ± 4 cm) which closely matches our small diameter logs (2- 12 cm) than very large (40- 60cm) diameter wooden logs (Kappes et al. 2004). It can be concluded that abundance of small diameter wood which colonize specialists/ specific insect fauna plays a role in ash ecosystem and can affect the overall biodiversity in bottomlands.

A previous study suggested that species diversity is usually proportional to wood size (Ranius & Jansson 2000). Our results indicated that Shannon diversity (H') and evenness were higher in small diameter (2- 12 cm) dead, live logs and sometimes in live large diameter (14- 24 cm) logs in non-EAB 2 sites. These patterns in diversity might be due to temporal and spatial factors of trees besides habitat features of site (Minelli et al. 2002). This difference in insect diversity among wood diameter might be due to micro climate factors (temperature, humidity) of the emergence trap (Ferro and Carlton 2011).

Assemblage analysis suggested that community composition of large live ash trees composed mainly of Mycetophilidae (Diptera), Megaspilidae (Hymenoptera), Ceratopogonidae (Diptera) and Liposcelididae (Psocodea). We did not predict the list of threatened species in Louisiana from our two-year study, but we proposed that these four insect families might decline in number beside other wood borers and Hymenopteran parasitoids in coming years. For future studies, the collected insects/ arthropods if classified to lower taxonomic level (species, subspecies), can be used to construct feeding guilds and community structures of green ash inhabiting arthropods in a forest –a study under trophic ecology- to discern risks of EAB to insect species in Louisiana.

The phenology of EAB started with the first adult emergence in early April and the last catch in late May in Shongaloo, Louisiana. On contrary, EAB adult emergence in northeastern USA begins in May and continues till September (Wei et. al 2007). Moreover, J-instar were collected all through the year except the adult activity period (early April- early June). The phenology study suspected that EAB has faster development cycle in Louisiana with one generation per year compared to native regions of China and northeastern USA where it takes more than a year to complete the development (Wang et al. 2005). However, additional future studies comprising of wild cohorts and experimental cohorts of EAB, need to be done to validate the univoltine life cycle of EAB in Louisiana.

A better understanding of EAB phenology is required to bring the EAB population under check by releasing the stage-specific parasitoids during the specific time of the year for the better establishment of exotic parasitoids. Timing of emergence of specific parasitoids such as larval parasitoid should match with presence of emerald ash borer larval instars.

Sampling the naturally occurring larvae by bark peeling and visual observation did not confirm any instance of parasitization at any of the release sites. EAB eggs collected from ash barks lack any parasitoids (*O. agrili*, *T. planipennisi*, and *S. agrili*) (Bauer et. al 2011) and predators. Yellow pan traps (YPTs) which were highly deployed to trap the adult parasitoids, contained one egg parasitoid only. A few other parasitic species other than released species were captured. Additionally, detection traps such as Lind green funnel traps and purple

traps deployed showed the similar results as found in other EAB trapping studies. Lindgren traps captured more males than females and purple traps captured a higher proportion of females than males (Crook and Mastro 2010).

Surveys of EAB also did not yield any parasitoids and did not have any signs of parasitism. Additionally, EAB larvae collected by debarking trees also failed to capture any of the released parasitoids. Lack of establishment of parasitoids at these release sites might be due to the absence of specific emerald ash borer stage concerning parasitoid stage present. Another reason can be climate unsuitability due to temperature variation (high temperature, high humidity) in Louisiana compared to the temperate native range of parasitoids (Duan et al. 2018).

Future studies should examine other methods to trap as well as preserve parasitoids in southern USA. Peeling bark for collecting EAB larvae to detect parasitism is laborious and, damages the insect life stage. Additionally, larval parasitization is often difficult to detect in field, hence, integrating novel trapping techniques and tools to diagnose parasitization will help to make decisions in emerald ash borer biological control program in southern USA (Louisiana).

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APPENDIX: SUPPLEMENTAL FIGURES

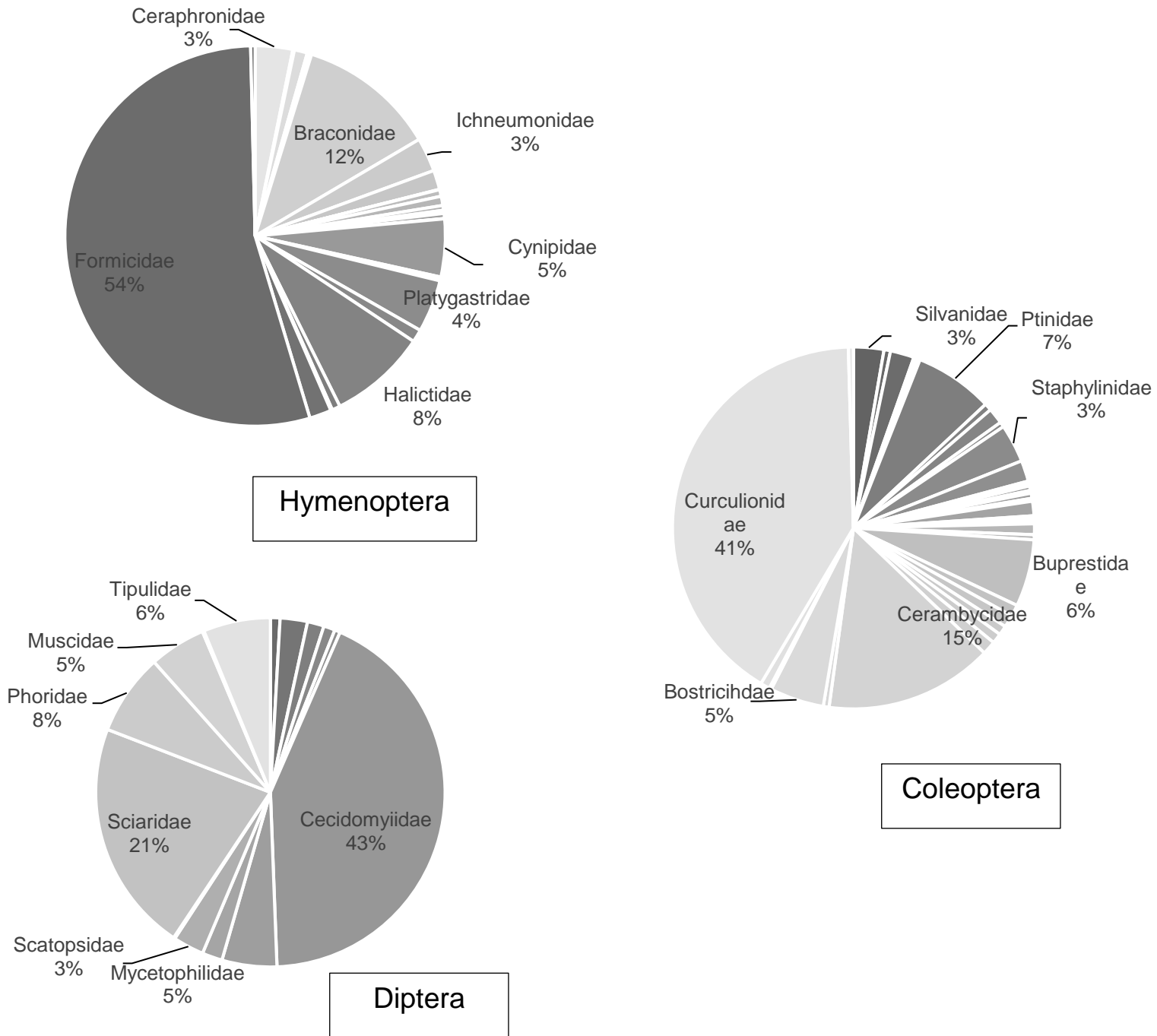


Figure. A.1. Proportion of major families collected per each insect orders; Hymenoptera, Diptera and Coleoptera from emergence traps at all study sites.

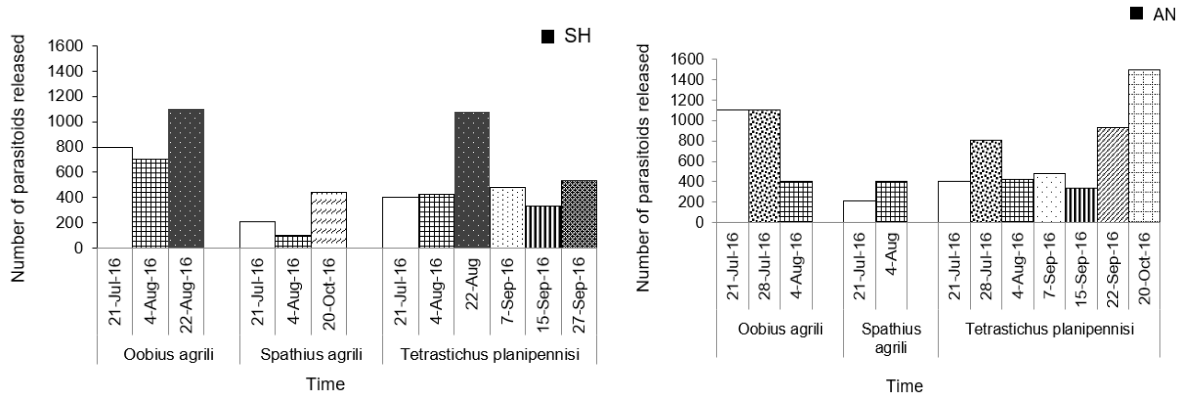


Figure. A. 2. Release of three parasitoids at (a) Shongaloo, and (b) Andreas sites in Webster Parish from July to October 2016.

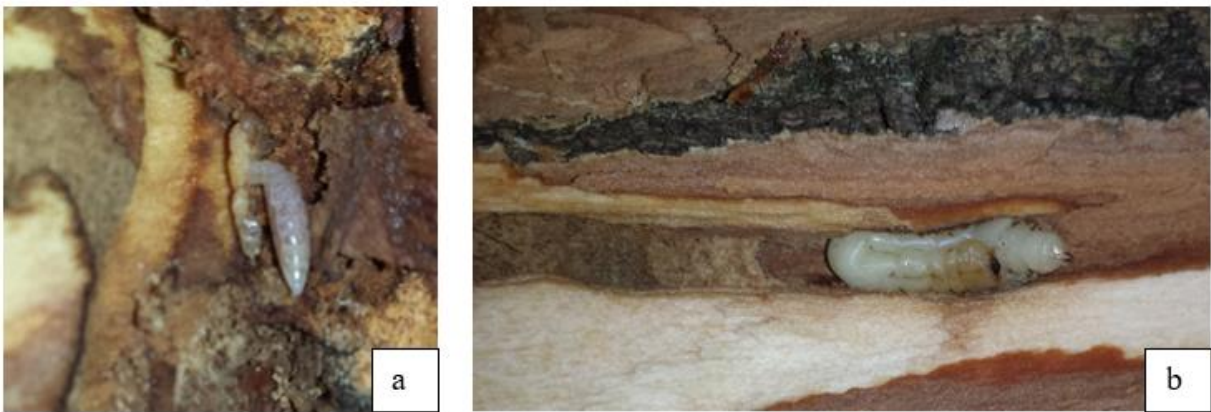


Figure A. 3. (a) Parasitoid attached to body of EAB larvae and (b) parasitized EAB larvae with black eggs of parasitoid over larval body at Shongaloo, Louisiana.

VITA

Balwinder Kaur, came from an agricultural family background in Punjab, India with interest in biological entities and agriculture. She did B.Sc. Agriculture (Honors) four-year degree with major in crop protection at Punjab Agricultural University (PAU), Ludhiana in 2015. To explore wider avenues in Entomology and continue her pursuit of research, she travelled to U.S. and started her MS in entomology at LSU in 2016. She anticipates graduating in August 2018 while working on a USDA-FS project on invasive emerald ash borer. Balwinder aspires to continue her research in insect science particularly in plant- insect interactions and biological control, along with actively involved in extension and social service activities, to live her dream of successful entomologist as well as a conscious citizen.