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## EMERALD ASH BORER DEVELOPMENT ACROSS A LATITUDINAL GRADIENT: IMPLICATIONS FOR BIOCONTROL

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EMERALD ASH BORER DEVELOPMENT ACROSS A LATITUDINAL GRADIENT:  
IMPLICATIONS FOR BIOCONTROL

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Agriculture, Food and Environment  
at the University of Kentucky

By

Sarah Pellecchia

Lexington, Kentucky

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2020

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## ABSTRACT OF THESIS

### EMERALD ASH BORER DEVELOPMENT ACROSS A LATITUDINAL GRADIENT: IMPLICATIONS FOR BIOCONTROL

Emerald ash borer, EAB, (*Agrilus planipennis* Fairmaire) is an invasive beetle that kills ash trees. It was accidentally introduced from China, and has rapidly expanded across North America, now occupying much of the eastern US. Four classical biocontrol parasitoids have been released to help mitigate its spread and impact: *Tetrastichus planipennisi* Yang, *Spathius agrili* Yang, *S. galinae* Belokobylskij and Strazanac, and *Oobius agrili* Zhang and Huang. These parasitoids have been deployed throughout EAB's invaded range, but there has been limited recovery of the parasitoids from their release sites in southern states. I evaluated whether this lack of establishment might be linked to life cycle asynchrony between the parasitoids and EAB. EAB can have a 2- or 1-year life cycle, with the 2-year appearing more common in the north ( $\sim >40^{\circ}\text{N}$ ) and 1-year more common in the south ( $\sim <40^{\circ}\text{N}$ ). I evaluated EAB development across a latitudinal gradient from Georgia, Kentucky, and Michigan, and assessed parasitoid establishment in Kentucky.

Evidence of a 2-year life cycle was found as far south as  $34^{\circ}\text{N}$  in Georgia. There is little evidence of parasitoid establishment in Kentucky. These findings suggest that latitude may not be the sole factor influencing EAB life cycle in North America.

KEYWORDS: *A. planipennis*, *Fraxinus*, Natural Enemies, Biological Control, Establishment

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## CHAPTER 1.

### **Introduction**

Ash (*Fraxinus* spp.) is a common component of many forest ecosystems and residential settings (Cappaert et al. 2005). It was one of the most commonly planted trees in urban and suburban landscapes, frequently used along roadsides and in parks (Schlesinger 1990). Ash was historically used to make baseball bats, furniture, and other hardwood products (Gucker 2005, Schlesinger 1990). In woodland forests, more than 280 species of arthropods are associated with ash, 44 of which are highly specific to *Fraxinus* (Gandhi and Herms 2010). It is a prolific seed producer, which wood ducks, finches, squirrels, and other animals consume, and is an important source of shelter for many bird and mammal species (Gucker 2005, Schlesinger 1990). There are 22 species of ash found throughout the contiguous US, as well as into Canada and Mexico, 16 of which are native and many of which are now listed as endangered (IUCN 2020).

Ash is being threatened by an invasive wood boring beetle called emerald ash borer, or EAB (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae). EAB creates serpentine galleries under the bark of ash, cutting off the flow of water and nutrients for the tree. In its native range, EAB only attacks weakened *Fraxinus* and is not considered a pest (Orlova-Bienkowskaja and Volkovitsh 2018), however, North American ash have no host plant resistance, and EAB is able to utilize healthy trees. Orlova-Bienkowskaja and Volkovitsh (2018) reported documented outbreaks of EAB starting in the 1960s, resulting from North American ash, such as *F. americana* and *F. velutina*, being brought to China and grown in plantations. EAB can complete its development on all North American *Fraxinus* evaluated, including *F. americana*, *F. pennsylvanica*, *F. nigra*, *F. profunda*, and

*F. quadrangulata* (Haack et al. 2015), though *F. quadrangulata* has been found to have some putative resistance (Spei and Kashian 2017).

High densities of EAB can result in the rapid death of ash, able to take a tree from healthy to dead in 1-3 years (Poland and McCullough 2006). Millions of ash trees have died in North America since the introduction of EAB (Klooster et al. 2014), with areas near the epicenter experiencing >99% mortality (Herms and McCullough 2014). The external symptoms of an EAB infestation are crown die-back, epicormic shoots, bark splitting, and D-shaped exit holes, which are not discernable until the infestation is severe (Smitley et al. 2008).

To mitigate the EAB invasion, four Asian parasitoids have been screened and released in the US. *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) were released in 2007 (USDA APHIS 2007), and *S. galinae* Belokobylskij and Strazanac in 2015 (USDA APHIS 2015).

*Spathius agrili* is a gregarious idiobiont ectoparasitoid that parasitizes late instar larvae (Yang et al. 2010), overwintering in galleries of their host as pre-pupae. In China adults emerge in late-May, living 3-4 weeks after emergence (Yang et al. 2010). *Spathius agrili* have up to four generations per year (Yang et al. 2005, Yang et al. 2010, Gould et al. 2011). In Virginia, *S. agrili* adults emerge at the beginning of May through mid-June and live an average of five weeks (Ragozzino et al. 2020). The conspecific *S. galinae* is also a gregarious idiobiont larval ectoparasitoid that was discovered at ~43°N in Russia. Because the *S. galinae* collected for rearing for North American biocontrol originates from higher latitudes with colder temperatures, it is anticipated that *S. galinae* will be

more suited to establishing in the northern portions of EAB's invaded range and is being released north of 40°N (Duan et al. 2014b, USDA APHIS 2015). *Tetrastichus planipennisi* is a gregarious koinobiont larval endoparasitoid first discovered in China in 2004, where parasitism rates of EAB are 32-65% (Yang et al. 2006). Under laboratory conditions, *T. planipennisi* requires 4 weeks to develop (Duan et al. 2011), with four generations per year. Adults first emerge in spring; females live a median of 8 weeks, males live 5 weeks (Duan et al. 2011). Finally, the solitary, parthenogenetic egg parasitoid *O. agrili* was originally discovered in China in 2003 (Zhang et al. 2005). Females live 5.5-7 weeks and parasitize 50-70 EAB eggs in her lifetime (Larson and Duan 2016). Larval *O. agrili* consume the embryonic EAB, and parasitized eggs turn from a healthy amber color to black (USDA APHIS/ARS/FS 2017). Adults emerge May through July when EAB eggs are most abundant and they have one or two generations per year, with non-diapausing generations requiring ~1 month to develop (Bauer et al. 2015). At the end of summer *O. agrili* enters diapause until the following summer to align with EAB oviposition activity (Larson and Duan 2016).

In addition to the releases of Asian biocontrol parasitoids, there have been many native parasitoids utilizing EAB as a host, including Hymenoptera such as native *Spathius* spp. (Braconidae), *Atanycolus* spp. (Braconidae), *Phasgonophora sulcata* (Chalcididae), *Balcha indica* (Eupelmidae), *Leluthia astigmata* (Braconidae), and others (Bauer et al. 2015, Davidson and Rieske 2016). Woodpeckers also impose a substantial amount of predation on immature EAB (Jennings et al. 2013). These natural enemies of EAB, including the Asian parasitoids, are our brightest hope for protecting the next generation of ash within our forests.

## CHAPTER 2.

### **Assessing effects of latitude on emerald ash borer development and parasitoid synchrony**

#### **Introduction**

Emerald ash borer (EAB), *Agrilus planipennis*, is a wood boring buprestid invasive in North America. Its native range spans northeastern China, the Korean peninsula, Japan, and a small section of the Russian Far East (Orlova-Bienkowskaja and Volkovitsh 2018). It was accidentally introduced via solid wood packaging material shipped from China in the 1990s (Siegert et al. 2014), though was not discovered until 2002 in Michigan and Ontario (Haack et al. 2015). It spread rapidly over the next decades and is now found throughout much of the eastern United States and Canada (USDA APHIS).

Larval EAB feed on phloem beneath the bark, creating serpentine galleries and damaging the cambium, cutting off the flow of water and nutrients and affecting the tree's ability to heal (Herms and McCullough 2014). External symptoms of an EAB infestation are crown die-back, epicormic shoots, bark splitting, and D-shaped exit holes; these are not discernable until the infestation is severe, making conventional management techniques difficult to use as control (Smitley et al. 2008). EAB has caused the death of tens of millions of ash trees since its introduction (Klooster et al. 2014), and areas near the epicenter of the EAB introduction have experienced >99% mortality (Herms and McCullough 2014), requiring extensive, costly efforts to maintain or remove trees infested or at risk (Kovacs et al. 2010).

The backbone of EAB management in North American forests is biological control. Four Asian parasitoids have been screened and released in the US. *Spathius*

*agrili* (Hymenoptera: Braconidae), *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), and *Oobius agrili* (Hymenoptera: Encyrtidae) have been released since 2007 (USDA APHIS 2007), and *S. galinae* since 2015 (USDA APHIS 2015). *T. planipennisi*, *S. agrili*, and *S. galinae* are gregarious larval parasitoids, while *O. agrili* is a solitary egg parasitoid. *T. planipennisi* first emerges in the spring and has several generations in a year, while *S. agrili* and *O. agrili* emerge in the summer (Yang et al. 2010, Bauer et al. 2015).

*Oobius agrili* and *T. planipennisi* have been recovered from sites in north-central Kentucky at ~38°N latitude in years following releases (Davidson and Rieske 2016). Four years after initial releases in Michigan, *T. planipennisi* was recovered in 92% of sampled trees at ~42 °N latitude, with parasitization rates of 13-21% and successful dispersion with expanding EAB populations (Duan et al. 2013). In more recently invaded regions *T. planipennisi* reportedly travels >10 km in four years (Jones et al. 2019). Despite its dispersal ability, with its short ovipositor *T. planipennisi* is unable to penetrate thick bark and is therefore limited to protecting relatively small trees and branches (<12 cm diameter) (Abell et al. 2012). Nevertheless, it can reduce EAB's population growth in ash saplings by >50% (Duan et al. 2017), and so it remains an important factor in fostering the next generation of ash.

*Spathius agrili* establishment has been problematic. Adults have been recovered in low numbers, usually only in the year of release, suggesting poor establishment (Jennings et al. 2016). Releases of *S. galinae*, which were only initiated in 2015, are still too recent to determine its establishment or efficacy. However, native North American

*Spathius* species and a variety of other endemic hymenopterans have been found parasitizing EAB (Bauer et al. 2015, Davidson and Rieske 2015).

The majority of work evaluating EAB biocontrol has focused on the historic invaded range in northern latitudes, and while some of the parasitoids have been effective regulating EAB populations in the north, the parasitoids appear to be struggling to establish in the south ([www.mapbiocontrol.org](http://www.mapbiocontrol.org)). Little is known about the phenological synchrony between EAB and its classical biological control parasitoids in the south. In addition to the few recoveries of *T. planipennisi* and *O. agrili* in Kentucky (Davidson and Rieske 2016), there were few successful recoveries of *T. planipennisi* and *S. agrili* in a 2-year study in Tennessee, suggesting poor establishment at southern latitudes (Palmer 2018). A major reason for this lack of establishment could be phenological asynchrony between the parasitoids and EAB.

EAB can have either a semivoltine or univoltine life cycle (Figure 2.1), with the 2-year life cycle being more prominent in the north and the 1-year more common in the south (Duan et al. 2014a, Herms and McCullough 2014). It is unknown where the transition between the semi- and univoltine life cycles occurs, and whether the parasitoids are able to synchronize.

With the 2-year life cycle (Figure 2.1A), EAB larvae are present year-round and available to the larval parasitoids. Adult EAB emerge in May and June and feed on ash foliage (Duan et al. 2014a). Within a few weeks they mate and oviposit, laying 60-80 eggs on the bark of ash which hatch June to August (Poland et al. 2015). The larvae then bore into the tree and progress through four larval instars as they feed. They begin diapause in October to November as immature larvae, then continue feeding and

developing the following spring and summer (Wang et al. 2010). When fourth instar larvae finish feeding the second summer, they excavate an overwintering chamber in the sapwood or in the bark, depending on bark thickness, and form “J-larvae” by folding their body into a J shape. In the spring they pupate, emerging as adults in ~1 month. The 1-year life cycle (Figure 2.1B) differs in that all larvae reach their final instar before overwintering, and therefore in the spring there are only J-larvae and pupae, which are unsuitable for *T. planipennisi* parasitization (Duan et al. 2014a, Orlova-Bienkowskaja and Bieńkowski 2016, Poland et al. 2015). This asynchrony may be less of a concern for *Spathius* since it emerges in the summer when larvae are present again. If *T. planipennisi* are unable to synchronize with the 1-year life cycle, then utilizing it in releases for sustained control of EAB will be ineffective. A study conducted in Syracuse, New York evaluated the synchrony of *T. planipennisi*, *S. galinae*, and *S. agrili* with EAB life cycle and found that *T. planipennisi* and *S. galinae* were well synchronized with the prominent 2-year life cycle occurring there and were able to weather the winters, while *S. agrili* emerged when ambient temperatures were not ideal for survival, suggesting poor synchrony (Jones et al. 2020).

This research seeks to determine whether the classical biological control releases of *T. planipennisi* for EAB management are a viable option for sustainable mitigation of EAB in southern portions of the invaded range in North America. My first objective was to evaluate the EAB life cycle across a latitudinal gradient in Georgia (34°N) and Kentucky (38°N), and compare to the EAB life cycle in Michigan (42°N) (Duan et al. 2010). The second objective was to assess the presence of the Asian parasitoids and endemic hymenopteran communities in field sites in Kentucky in which the Asian

parasitoids were released from 2010 to 2017 ([www.mapbiocontrol.org](http://www.mapbiocontrol.org)). The final objective evaluates the changes in plant communities in Kentucky over the course of the EAB invasion.

## **Methods**

### *Latitudinal variation in EAB life cycle and parasitism*

Collection Sites. Sites were selected in Anderson, Fayette, Jessamine, and Spencer counties in Kentucky (one site per county) at ~38°N latitude, in a region characterized by a Western Mesophytic forest type consisting of a variety of deciduous species (Wharton and Barbour 1973) and with a significant ash component (Davidson and Rieske 2015). Emerald ash borer was initially reported at our sites in Anderson and Spencer counties in 2009–2010, and in Fayette and Jessamine counties in 2014. Anderson, Fayette, and Spencer counties were part of the parasitoid release efforts conducted initially by the Kentucky State Entomologist’s Office, followed by the UK Forest Entomology Laboratory, but releases were terminated in 2017. At the onset of our study in 2018, EAB-induced overstory ash mortality was nearly complete, but smaller diameter (<12 cm at 1.37 m above ground level, or diameter at breast height (DBH)) and regenerating ash seedlings remained plentiful. Collections at 34°N latitude occurred in Cobb county, GA, again in an area characterized by a mesophytic forest with a variety of deciduous species. EAB was initially reported at this site in 2017 but there have been no reports of parasitoid releases. During our initial collections (winter 2019) overstory ash mortality was becoming evident.

Sampling. In the winter of 2019 and 2020, small diameter (4-12 cm DBH) living ash trees showing signs of EAB infestation were selected at 38°N (n = 8 trees) and at 34°N (n = 6). Selected trees were felled March 12-13 in Kentucky and April 10 in Georgia in 2019, and February 11-13 in Kentucky and February 21 in Georgia in 2020. The trees were cut into 60 cm sections and numbered sequentially starting from the base (Davidson and Rieske 2016). Logs were stored at 4°C until processing. Felled trees were used to evaluate EAB life stages and the incidence of parasitization.

Adult sampling. Even numbered log sections were placed into cylindrical rearing bins (52 cm x 70 cm) with lids fitted with 500 ml clear plastic bottles to collect emerging EAB and associated arthropods. Collection jars were checked daily for 9 weeks, and rearing bin contents were inspected weekly to recover any insects that did not enter the collection jars.

Larval sampling. Odd numbered logs were measured (length and diameter), debarked using a draw knife, and inspected for EAB and parasitoids (Davidson and Rieske 2015). Life stage and larval instar were noted for each encountered EAB, as well as their fate: alive, parasitized, removed by woodpeckers, or dead from unknown causes. Alive EAB were classified by the presence of living immature stages or exit holes indicating a successful adult emergence. Woodpecker predation was evidenced by bark and sapwood destruction in the galleries or overwintering chambers. Parasitized EAB were classified by the presence or evidence of parasitoids within an EAB gallery. Galleries which lacked living EAB and exit holes whose fates could not be determined were classified as “dead from unknown causes.” Galleries that were severely calloused over or were interrupted by log sectioning before the gallery could terminate were not

included in counts. Larval instars were determined based on gallery width (Duan et al. 2014a). As sampling was conducted in the winter months, any larvae found were considered to require a 2-year life cycle (Figure 2.1). Those insects found alive were placed in petri dishes with moistened filter paper to monitor for development or parasitoid emergence.

Egg sampling. To assess EAB egg parasitization by *O. agrili*, the outer bark of the basal 600 cm<sup>2</sup> of the log section corresponding to a height of 1.3 m was shaved using a draw knife (Parisio et al. 2017). Bark shavings were sifted through a 1 mm sieve and sorted for EAB eggs. Unhatched eggs were assessed as healthy when golden in color and parasitized by *O. agrili* if dark in color, hatched eggs were considered parasitized if a circular hole was visible in the egg (Liu et al. 2007, Davidson and Rieske 2016).

#### *Parasitoid recovery and endemic hymenopteran community*

In June, July, and August of 2018 and 2019, the hymenopteran community at the Kentucky sites (38°N) was monitored using yellow pan traps (YPT) (USDA-APHIS/ARS/FS 2017) in Fayette (n = 16 traps), Anderson (n = 9), and Spencer (n = 15) counties. Traps consisted of nested 355 mL yellow plastic bowls (Creative Converting, Lancaster, PA) fastened onto ash trees at 1.5 m high with right-angled shelf brackets. The outer bowl was fastened to the shelf brackets using cable ties (Utilitech, N. Wilkesboro, NC) and the inner bowl was secured with binder clips. Inner bowls were filled with ~250 ml of soapy water. At 7 d intervals, trap contents were filtered through 190 micron nylon mesh paint strainers (Auto Body Now, Tea, SD) which were then placed in Ziploc bags with 70% ethanol and stored at 4°C until processing (USDA APHIS/ARS/FS 2017). Trap

contents were sorted and counted, and the presence of the classical biological control agents was noted. Hymenopterans were sorted into Apoidea, Ichneumonoidea (Ichneumonidae and Braconidae), Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea), and Other Hymenoptera (Vespoidea (including Formicidae), Evanoidea, Chrysidoidea, and Symphyta) (Johnson and Triplehorn 2005, Davidson and Rieske 2016). Endemic hymenopterans were counted to determine hymenopteran abundance, richness, and evenness (Ichneumonoidea, parasitic non-ichneumonoids, Apoidea, other hymenopterans) at each site, and diversity was derived using Shannon's and Simpson's diversity indices (Magurran 1988, Southwood and Henderson 2009). Endemic hymenopteran abundance, richness, evenness, and diversity were used to characterize hymenopteran communities across sites.

#### *Ash assessment*

To assess changes in ash composition associated with the EAB invasion, woody vegetation was censused using the Common Stand Exam (Coleman et al. 2008, USDA Forest Service 2015) at sites in Fayette, Anderson, and Spencer counties (n = 12 plots per site). Circular 400 m<sup>2</sup> plots were established to evaluate overstory and midstory vegetation (stems >12.7 cm DBH). Within each whole plot, five subplots (40 m<sup>2</sup>) and five microplots (4 m<sup>2</sup>) were positioned in the center and at each cardinal direction (7.7 m from plot center) to evaluate understory woody vegetation. In subplots, saplings and shrubs <12.7 cm DBH and >1.37 m high were recorded, while the microplots, which were centered within the subplots, were evaluated for woody vegetation <1.37 m tall (Coleman et al. 2008). Plants were identified to species and their height measured. The

DBH of trees in whole plots and subplots was also measured, and each tree was classified as living or dead.

### *Data Analysis*

Analysis was performed using SAS v. 9.3 (SAS Institute 2011). Latitudinal variation in EAB life stages (L1-L2, L3-L4, J-larvae, prepupae, and pupae) was assessed using Fisher's exact test, combining data from 2019 and 2020. Hymenoptera taxonomic group abundance, diversity, richness, and evenness were analyzed using a linear mixed model (PROC GLIMMIX); data were transformed using a  $\ln(x + 1)$  transformation when assumptions for normality were not met. These data were analyzed using two methods. First by analyzing years and sites using individual trap counts, then by analyzing means of each site-year combination. Two outliers resulting from an unusually high number of Formicidae were adjusted to the average ant abundance for those specific sites. Ash canopy decline was compared across Kentucky sites using analysis of variance (ANOVA).

## **Results**

### *Latitudinal variation in EAB life cycle and parasitism*

Adult sampling – Rearing bins. Thirty-three *T. planipennisi* were present from a single tree from Fayette Co., KY in 2019, representing at least one parasitized EAB. No classical biocontrol agents were found in Georgia in 2019, or from either latitude in 2020. Fifteen EAB were recovered from the Kentucky rearing bins within five weeks in 2019, but only one was recovered in 2020. One EAB was recovered from Georgia rearing bins

in 2019, but no EAB were recovered in 2020. One *Atanycolus* sp. emerged from Georgia rearing bins in 2020, likely parasitizing EAB. Bark beetles were abundant in both Kentucky and Georgia trees, particularly the eastern ash bark beetle *Hylesinus aculeatus* (Coleoptera: Curculionidae).

Larval sampling – Debarking. One-hundred EAB galleries were uncovered from eight trees (40 logs) in Kentucky 2019, and 35 galleries from 6 trees (24 logs) in Georgia 2019. In 2020, 99 galleries were found in eight trees (36 logs) in Kentucky, and 52 from 6 trees (33 logs) in Georgia. Three live larvae, the presence of which indicates a 2-year life cycle, were found from one tree collected from Fayette Co., KY and one from Georgia in 2019; in 2020 one live larva was recovered from Spencer Co., KY and two live larvae were found from one tree in Georgia (Table 2.1). Other live immature stages present, which could have either a 1- or 2-year life cycle (Figure 2.1), include three prepupae and four pupae from Kentucky in 2019, two J-larvae and 11 prepupae from Kentucky in 2020, and one J-larvae and six prepupae from Georgia in 2020 (Figure. 2.2). All life stages were evident during the winter from trees in Kentucky, with J-larvae appearing most frequently (41% of samples). No live J-larvae were uncovered at either latitude in 2019; individuals were preyed upon by woodpeckers or were dead from unknown causes. Woodpecker predation accounted for 37% of EAB mortality in Kentucky and 31% in Georgia in 2019, and 30% in Kentucky and 52% in Georgia in 2020. Woodpeckers consumed larvae from galleries in addition to those in overwintering chambers. In Michigan in 2009, 88% of total living EAB were larvae, while in 2019 30% in Kentucky and 100% (with only one live individual uncovered) in Georgia were larvae. In 2020, only 7% in Kentucky and 20% in Georgia of live EAB were larvae. There was

significant difference between the three latitudes in the relative abundance of each life stage ( $P = 0.0001$ , Fisher's exact test). Between Kentucky 2019 and Michigan 2009, there was a significant difference in the proportion of life stages present ( $P < 0.0001$ , Fisher's exact test). There was no significant difference between the life stages present in Kentucky and Georgia in 2020 ( $P = 0.80$ , Fisher's exact test). When data from 2019 and 2020 were combined, there was a significant difference in the proportion of life stages present between Kentucky and Michigan ( $P < 0.0001$ , Fisher's exact test) and Georgia and Michigan ( $P < 0.0001$ , Fisher's exact test). There was no significant difference between the life stages present in Kentucky and Georgia ( $P = 0.39$ , Fisher's exact test).

No classical biocontrol parasitoids were found when debarking trees either year, however in 2019 one EAB overwintering chamber from Kentucky contained an unidentified larva, which failed to be reared. This individual was classified as a parasitoid since it did not have a gallery of its own that would imply it was a predator. Additionally, in 2020 one parasitoid cocoon was found within an EAB gallery from Spencer Co., KY which failed to be reared, but the dissected cocoon revealed five parasitoid wasps in the family Pteromalidae. Two *Atanycolus* (Hymenoptera: Braconidae) cocoons were present in EAB galleries from Georgia in 2020. Of the 28 EAB eggs recovered from Kentucky, and three recovered from Georgia, none were parasitized by *O. agrili*.

#### *Parasitoid recovery and endemic hymenopteran community*

None of the four classical biocontrol parasitoid species were recovered in YPTs from any of the release sites in Kentucky in either 2018 or 2019. A total of 1,819 hymenopterans were captured in YPTs over the 2-year study, including several endemic parasitoids which have been found to parasitize EAB (Bauer et al. 2015, Davidson and

Rieske 2016), including 57 individuals of *Spathius* spp. On one occasion, a single trap recovered nine *Spathius floridanus*, indicating that these individuals may have emerged from the ash tree on which the trap was placed. Ichneumonoids comprised 30% of the trap catch, Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea) 29%, Apoidea 3%, and Other Hymenopterans (Vespoidea, Evanoidea, Chrysoidea, and Symphyta) 38%.

When assessing differences in abundance of each taxonomic group (Total Hymenoptera, Ichneumonoidea, Non-Ichneumonoid Parasitoids, Apoidea, and Other Hymenoptera) between sites in 2018, there was no statistical difference. However, in 2019 there were significantly more Non-Ichneumonoid Parasitoids in Fayette Co. compared to the other two sites ( $P = 0.03$ ) (Table 2.2). There were significantly more Other Hymenoptera in Fayette Co. from 2018 to 2019 ( $t_4 = 3.00$   $P = 0.04$ ), and significantly fewer Total Hymenoptera in Anderson Co. from 2018 to 2019 ( $t_4 = 3.00$   $P = 0.04$ ) (Table 2.3). However, when individual trap catch between years was analyzed, Ichneumonoidea abundance was weakly significantly greater in 2018 ( $P = 0.1$ ) and 2019 ( $P = 0.09$ ) in Spencer Co. relative to the other two sites (Table 2.4). Fayette had significantly greater Non-Ichneumonoid Parasitoid abundance in 2019, and there was a significant increase between 2018 and 2019 (Table 2.4). Fayette also had significantly higher numbers of Apoidea and Total Hymenoptera in 2019 (Table 2.5), in spite of lower numbers of Other Hymenoptera ( $P = 0.06$ ). From 2018 to 2019, there was a significant increase in abundance of Apoidea in Fayette ( $t_{187} = -3.87$ ,  $P = 0.0001$ ), but a significant decrease in Other Hymenoptera ( $t_{187} = 3.54$ ,  $P = 0.0005$ ) (Table 2.5).

Hymenopteran community diversity did not vary significantly among sites or between years in both sets of analysis (Tables 2.6 and 2.7), nor did richness or evenness (Tables 2.8 and 2.9). A general negative trend was found between hymenopteran abundance captured per day and years since EAB invasion when log transformed ( $F_{1,191} = 2.41$ ,  $P = 0.12$ ), while Simpson diversity remains relatively constant ( $F_{1,178} = 0.40$ ,  $P = 0.53$ ).

#### *Ash assessment*

Ash mortality was highest in Spencer Co. in 2018 with 89% of canopy trees dead, and lowest in Fayette in 2019 with 35% ash mortality (Table 2.10). Mortality has increased at all three sites over the past five years (Davidson and Rieske 2015). In both 2018 and 2019, Anderson Co. had the greatest abundance of ash seedlings and understory.

#### **Discussion**

Classical biological control has been the goal of sustainable management of EAB in forest systems since its discovery in North America in 2002 (Cappaert et al. 2005, Van Driesche and Reardon 2015). However, as the invaded range of EAB in North America has expanded southward, successful biocontrol establishment has been problematic. These difficulties may be attributable to several factors. First, latitude effects phenology; development of plants and insects are dependent upon ambient temperatures which are lower at higher latitudes. This can impact critical times in the insects' life cycles, and may affect EAB differently than the parasitoids, with consequences for parasitoid establishment. Second, latitudinal differences in ash distribution affect forest diversity,

complicating parasitoid host location. Both green and white ash are widely distributed in wildland forests of eastern North America, but the prominence of both species declines with decreasing latitude, as more southerly species take their place. The spottier distribution of ash, and consequently EAB, could have consequences for parasitoid establishment. Third, the distribution of the regionally important *F. quadrangulata*, a poor host for EAB (Spei and Kashian 2017), may also have consequences for parasitoid establishment in the southern US. Blue ash is not found in the northern portions of EAB's invaded range in the US, but becomes more common further south. Lastly, green ash was heavily used in urban landscapes prior to the introduction of EAB, and was a numerically dominant urban street tree in northern regions. Urban areas in the south are less reliant on ash, as a greater diversity of southern species are available for use. An additional facet associated with urban EAB populations is the presence of ornamental white fringetree, *Chionanthus virginicus*, in urbanized areas, which can serve as an alternative host for EAB (Cipollini 2015) yet provides it with enemy free space from *T. planipennisi* (Olson and Rieske 2019), potentially confounding parasitoid establishment. While several factors may be at play influencing the success of classical biological control against EAB in the south, this study was undertaken to determine whether phenological asynchrony between the life cycle of EAB and the classical parasitoids was the cause of this biocontrol failure.

While sampling using YPT, I found no classical biocontrols, suggesting that they are either absent or present in low abundances. This is supported by the destructive sampling, in which only a single brood of *T. planipennisi* was recovered in 2019 from Fayette Co., Kentucky; no additional classical biocontrols were found in debarked logs.

Also in 2019, live EAB larvae were present during winter sampling only at Fayette Co., indicating that any *T. planipennisi* emerging in the spring would have the opportunity to find a suitable host. These findings suggest that perhaps latitude isn't the sole factor influencing the EAB life cycle; perhaps host species, host quality, or site-specific characteristics play a significant role. The classical biocontrols are not reported to have been released in Georgia, so it is not surprising that no biocontrol parasitoids were recovered, however it's not out of the question that the parasitoids could have travelled to Georgia the same way that EAB did (e.g. in infested firewood).

Some of the trees used for destructive sampling uncovered no EAB and others showed no active galleries as the trees healed over old EAB damage. This is a hopeful sign that there are trees that managed to survive the initial wave of EAB invasion and may allow us more time to save the ash.

Emerald ash borer larvae were present at all three latitudes evaluated during winter and spring months. This provides evidence that EAB development is not dependent solely on latitudinal differences. While there is evidence of a 2-year life cycle as far south as 34°N latitude, this does not discount the presence of a 1-year life cycle at each latitude. The proportion of 2- to 1-year life cycles is still unknown, though data from Michigan would suggest a greater propensity for the 2-year life cycle (Duan et al. 2010). Given that *T. planipennisi* is reliant upon the presence of EAB larvae in the spring in order to have further generations through the summer, emphasizes the importance of the 2-year life cycle of EAB. This has potential importance for how we can manage EAB in regards to parasitoid releases. Our findings suggest that decisions regarding biological

control programs must go beyond latitude and consider site specific factors to maximize their effectiveness.

**Table 2.1** Partial life table showing stage-specific mortality of emerald ash borer in Michigan (42°N latitude) estimated based on Duan et al. 2010 from ten trees felled in April-May 2009, and from eight trees felled in March 2019 in Kentucky (38°N latitude) and six trees felled in April 2019 in Georgia (34°N latitude). Values for ‘Adults’ at 38° N and 34° N are represented by the presence of exit holes.

Latitude	Stage	Galleries (n)	Survival (%)	Parasitized (%)	Disease (%)	Woodpecker (%)	Tree resis (%)	Unknown (%)
42.7° (MI)	L1	21	48	0	0	not assessed	52	not assessed
	L2	133	85	0	4		11	
	L3	220	97	0	2		1	
	L4	185	96	0	3		1	
	J-larvae	3	100	0	0		0	
	Prepupae	20	100	0	0		0	
	Pupae	48	100	0	0		0	
	<b>Total</b>	<b>630</b>	<b>93</b>	<b>0</b>	<b>2</b>		<b>5</b>	
38° (KY)	L1-L2	6	33	0	not assessed	0	not assessed	67
	L3-L4	15	7	0		0		93
	J-larvae	41	0	2		61		37
	Prepupae	9	33	0		0		67
	Pupae	5	80	0		0		20

**Table 2.1 (continued)**

	Adults	24	96	0		0		4
	<b>Total</b>	<b>100</b>	<b>33</b>	<b>1</b>		<b>25</b>		<b>41</b>
34° (GA)	L1-L2	2	0	0	not assessed	0	not assessed	100
	L3-L4	7	14	0		43		43
	J-larvae	9	0	0		89		11
	Prepupae	0	0	0		0		0
	Pupae	0	0	0		0		0
	Adults	17	100	0		0		0
	<b>Total</b>	<b>35</b>	<b>51</b>	<b>0</b>		<b>31</b>		<b>17</b>

**Table 2.2** Abundance of (A) Ichneumonoidea and (B) Non-Ichneumonoid Parasitoids (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude). Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ); asterisks indicate between year differences, where \* indicates  $P < 0.1$ ; \*\* indicates  $P < 0.001$ . Analysis performed using means from each site-year combination.

		(A) Ichneumonoidea <sup>a</sup>			(B) Non-Ichneumonoid Parasitoids <sup>b</sup>		
Site	N	2018	2019	$t_4 / P$	2018	2019	$t_4 / P$
Fayette	17	2.2 (0.4)a	1.9 (0.3)a	0.25 / 0.81	1.9 (0.3)a	4.4 (0.7)a	-2.08 / 0.12
Spencer	15	3.0 (0.5)a	2.8 (0.4)a	0.23 / 0.83	1.7 (0.4)a	1.0 (0.2)b	0.66 / 0.54
Anderson	9	2.0 (0.3)a	1.5 (0.4)a	1.15 / 0.31	2.8 (1.3)a	1.2 (0.2)b	1.00 / 0.37
F <sub>df</sub>		F <sub>2,6</sub> = 0.71	F <sub>2,6</sub> = 1.25		F <sub>2,6</sub> = 0.29	F <sub>2,6</sub> = 6.67	
<i>P</i>		0.53	0.35		0.76	0.03	

<sup>a</sup> Ichneumonoidea includes: Braconidae and Ichneumonidae

<sup>b</sup> Non-Ichneumonoid Parasitoids includes: Proctotrupomorpha and Ceraphronoidea

**Table 2.3** Abundance of (A) Apoidea, (B) Other Hymenoptera, and (C) Total Hymenoptera (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude). Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ); asterisks indicate between year differences, where ns indicates not significant; \* indicates  $P < 0.1$ ; \*\* indicates  $P < 0.001$ . Analysis performed using means from each year-site combination.

		(A) Apoidea			(B) Other Hymenoptera <sup>a</sup>			(C) Total Hymenoptera		
Site	N	2018	2019	$t_4$	2018	2019	$t_4$	2018	2019	$t_4$
Fayette	17	0.15 (0.05)a	0.63 (0.17)a	ns	3.7 (0.6)a	1.4 (0.2)a	*	7.9 (0.7)a	8.4 (1.0)a	ns
Spencer	15	0.12 (0.05)a	0.11 (0.06)a	ns	3.3 (0.4)a	2.8 (0.5)a	ns	8.1 (0.9)a	6.6 (0.7)a	ns
Anderson	9	0.04 (0.04)a	0.07 (0.05)a	ns	3.0 (0.5)a	2.3 (0.3)a	ns	7.9 (1.3)a	5.1 (0.7)a	*
F <sub>df</sub>		F <sub>2,6</sub> = 0.92	F <sub>2,6</sub> = 1.83		F <sub>2,6</sub> = 0.16	F <sub>2,6</sub> = 2.01		F <sub>2,6</sub> = 0.02	F <sub>2,6</sub> = 2.47	
<i>P</i>		0.45	0.24		0.86	0.21		0.98	0.16	

<sup>a</sup> Other Hymenoptera include: Vespoidea, Evanoidea, Chrysidoidea, and Symphyta

**Table 2.4** Abundance of (A) Ichneumonoidea, and (B) Non-Ichneumonoid Parasitoids (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude). Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ); asterisks indicate between year differences, where \* indicates  $P < 0.1$ ; \*\* indicates  $P < 0.001$ . Analysis performed using individual traps from each year-site combination.

		(A) Ichneumonoidea <sup>a</sup>			(B) Non-Ichneumonoid Parasitoids <sup>b</sup>		
Site	N	2018	2019	$t_{189} / P$	2018	2019	$t_{189} / P$
Fayette	17	2.2 (0.4)a	1.9 (0.3)a	-0.59 / 0.56	1.9 (0.3)a	4.4 (0.7)a	-4.14/ <0.0001**
Spencer	15	3.0 (0.5)a	2.8 (0.4)a	0.23 / 0.82	1.7 (0.4)a	1.0 (0.2)b	0.88 / 0.38
Anderson	9	2.0 (0.3)a	1.5 (0.4)a	0.72 / 0.47	2.8 (1.3)a	1.2 (0.2)b	1.48 / 0.14
F <sub>df</sub>		F <sub>2,37</sub> = 2.45	F <sub>2,38</sub> = 2.59		F <sub>2,37</sub> = 0.69	F <sub>2,38</sub> = 12.37	
P		0.1	0.09		0.51	<0.0001	

<sup>a</sup> Ichneumonoidea includes: Braconidae and Ichneumonidae

<sup>b</sup> Non-Ichneumonoid Parasitoids includes: Proctotrupomorpha and Ceraphronoidea

**Table 2.5** Abundance of (A) Apoidea, (B) Other Hymenoptera, and (C) Total Hymenoptera (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude). Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ); asterisks indicate between year differences, where ns indicates not significant; \* indicates  $P < 0.1$ ; \*\* indicates  $P < 0.001$ . Analysis performed using individual traps from each year-site combination.

		(A) Apoidea			(B) Other Hymenoptera <sup>a</sup>			(C) Total Hymenoptera		
Site	N	2018	2019	$t_{189}$	2018	2019	$t_{189}$	2018	2019	$t_{189}$
Fayette	17	0.15 (0.05)a	0.63 (0.17)a	**	3.7 (0.6)a	1.4 (0.2)a	**	7.9 (0.7)a	8.4 (1.0)a	ns
Spencer	15	0.12 (0.05)a	0.11 (0.06)b	ns	3.3 (0.4)a	2.8 (0.5)a	ns	8.1 (0.9)a	6.6 (0.7)b	ns
Anderson	9	0.04 (0.04)a	0.07 (0.05)b	ns	3.0 (0.5)a	2.3 (0.3)a	ns	7.9 (1.3)a	5.1 (0.7)b	ns
$F_{df}$		$F_{2,37} = 0.81$	$F_{2,38} = 6.30$		$F_{2,37} = 0.06$	$F_{2,38} = 3.04$		$F_{2,37} = 0.49$	$F_{2,38} = 3.59$	
$P$		0.45	0.004		0.95	0.06		0.62	0.04	

<sup>a</sup> Other Hymenoptera include: Vespoidea, Evanoidea, Chrysidoidea, and Symphyta

**Table 2.6** Hymenoptera taxonomic group diversity per trap (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude) in 2018 and 2019. Diversity was calculated using Shannon’s diversity index and Simpson’s index. Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ). Taxonomic groups include Ichneumonoidea, Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea), Apoidea, and Other Hymenoptera (Vespoidea, Evanoidea, Chrysidoidea, and Symphyta). Analysis performed using means from each year-site combination.

Site	N	Shannon			Simpson		
		2018	2019	$t_4 / P$	2018	2019	$t_4 / P$
Fayette	17	0.76 (0.05)a	0.76 (0.05)a	-0.03 / 0.96	0.58 (0.03)a	0.60 (0.04)a	-0.61 / 0.57
Spencer	15	0.74 (0.06)a	0.70 (0.05)a	0.47 / 0.66	0.60 (0.03)a	0.56 (0.04)a	1.06 / 0.35
Anderson	9	0.78 (0.05)a	0.76 (0.08)a	0.21 / 0.84	0.64 (0.04)a	0.63 (0.06)a	0.14 / 0.90
F <sub>df</sub>		F <sub>2,6</sub> = 0.09	F <sub>2,6</sub> = 0.62		F <sub>2,6</sub> = 0.76	F <sub>2,6</sub> = 0.98	
<i>P</i>		0.92	0.57		0.52	0.43	

**Table 2.7** Hymenoptera taxonomic group diversity per trap (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude) in 2018 and 2019. Diversity was calculated using Shannon’s diversity index and Simpson’s index. Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ). Taxonomic groups include Ichneumonoidea, Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea), Apoidea, and Other Hymenoptera (Vespoidea, Evanoidea, Chrysidoidea, and Symphyta). Analysis performed using individual traps from each year-site combination.

Site	N	Shannon			Simpson		
		2018	2019	$t_{182} / P$	2018	2019	$t_{176} / P$
Fayette	17	0.76 (0.05)a	0.76 (0.05)a	0.05 / 0.96	0.58 (0.04)a	0.60 (0.04)a	-0.47 / 0.64
Spencer	15	0.74 (0.06)a	0.70 (0.05)a	0.59 / 0.56	0.60 (0.04)a	0.56 (0.04)a	0.66 / 0.51
Anderson	9	0.78 (0.05)a	0.76 (0.07)a	0.19 / 0.85	0.64 (0.04)a	0.63 (0.06)a	0.15 / 0.88
F <sub>df</sub>		F <sub>2,37</sub> = 0.13	F <sub>2,38</sub> = 0.42		F <sub>2,37</sub> = 0.64	F <sub>2,38</sub> = 0.56	
<i>P</i>		0.88	0.66		0.53	0.58	

**Table 2.8** Hymenoptera taxonomic group richness and evenness (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude) in 2018 and 2019. Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ). Taxonomic groups include Ichneumonoidea, Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea), Apoidea, and Other Hymenoptera (Vespoidea, Evanoidea, Chrysoidea, and Symphyta). Analysis performed using means from each year-site combination.

	N	Richness			Evenness		
		2018	2019	<i>t</i> <sub>4</sub> / <i>P</i>	2018	2019	<i>t</i> <sub>4</sub> / <i>P</i>
Fayette	17	2.57 (0.11)a	2.45 (0.14)a	0.45 / 0.68	0.86 (0.02)a	0.87 (0.02)a	-0.40 / 0.71
Spencer	15	2.46 (0.13)a	2.22 (0.14)a	0.76 / 0.49	0.89 (0.02)a	0.87 (0.02)a	1.55 / 0.20
Anderson	9	2.48 (0.12)a	2.30 (0.19)a	0.99 / 0.38	0.88 (0.03)a	0.90 (0.02)a	-0.63 / 0.56
<i>F</i> <sub>df</sub>		<i>F</i> <sub>2,6</sub> = 0.07	<i>F</i> <sub>2,6</sub> = 0.72		<i>F</i> <sub>2,6</sub> = 0.54	<i>F</i> <sub>2,38</sub> = 0.86	
<i>P</i>		0.94	0.53		0.61	0.47	

**Table 2.9** Hymenoptera taxonomic group richness and evenness (mean (SE)) captured in yellow pan traps at three sites in central Kentucky (~38°N latitude) in 2018 and 2019. Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ). Taxonomic groups include Ichneumonoidea, Non-Ichneumonoid Parasitoids (Proctotrupomorpha and Ceraphronoidea), Apoidea, and Other Hymenoptera (Vespoidea, Evanoidea, Chrysididae, and Symphyta). Analysis performed using individual traps from each year-site combination.

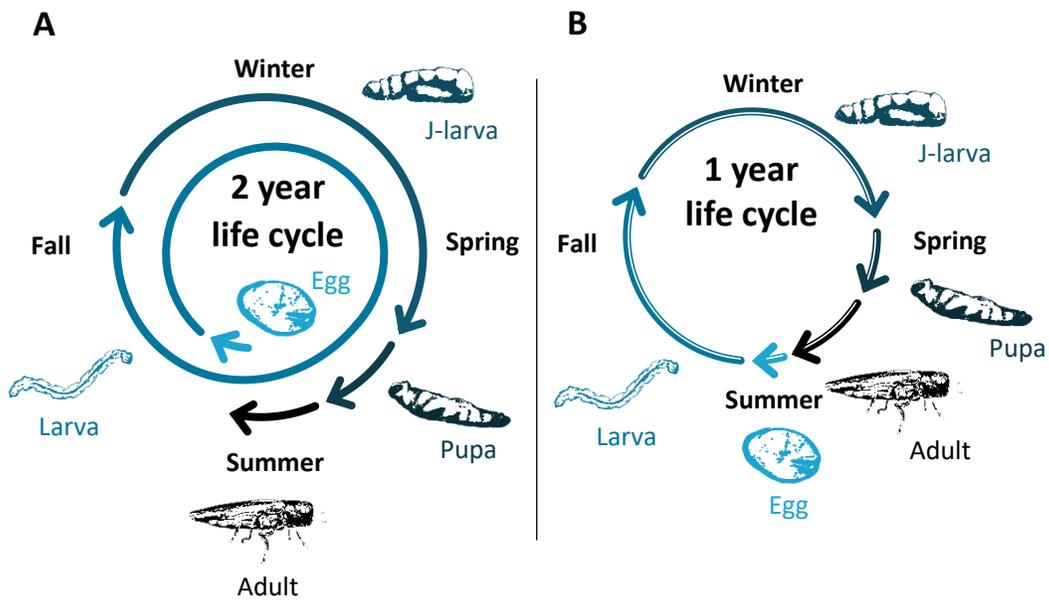
	N	Richness		$t_{189} / P$	Evenness		$t_{157} / P$
		2018	2019		2018	2019	
Fayette	17	2.57 (0.11)a	2.45 (0.14)a	0.64 / 0.52	0.86 (0.02)a	0.87 (0.02)a	-0.31 / 0.76
Spencer	15	2.46 (0.13)a	2.22 (0.14)a	1.28 / 0.20	0.89 (0.02)a	0.87 (0.02)a	0.88 / 0.38
Anderson	9	2.48 (0.12)a	2.30 (0.19)a	0.76 / 0.45	0.88 (0.03)a	0.90 (0.02)a	-0.64 / 0.52
$F_{df}$		$F_{2,37} = 0.22$	$F_{2,38} = 0.70$		$F_{2,37} = 0.53$	$F_{2,38} = 0.54$	
$P$		0.81	0.51		0.59	0.59	

**Table 2.10** Ash composition at three study sites in Kentucky.

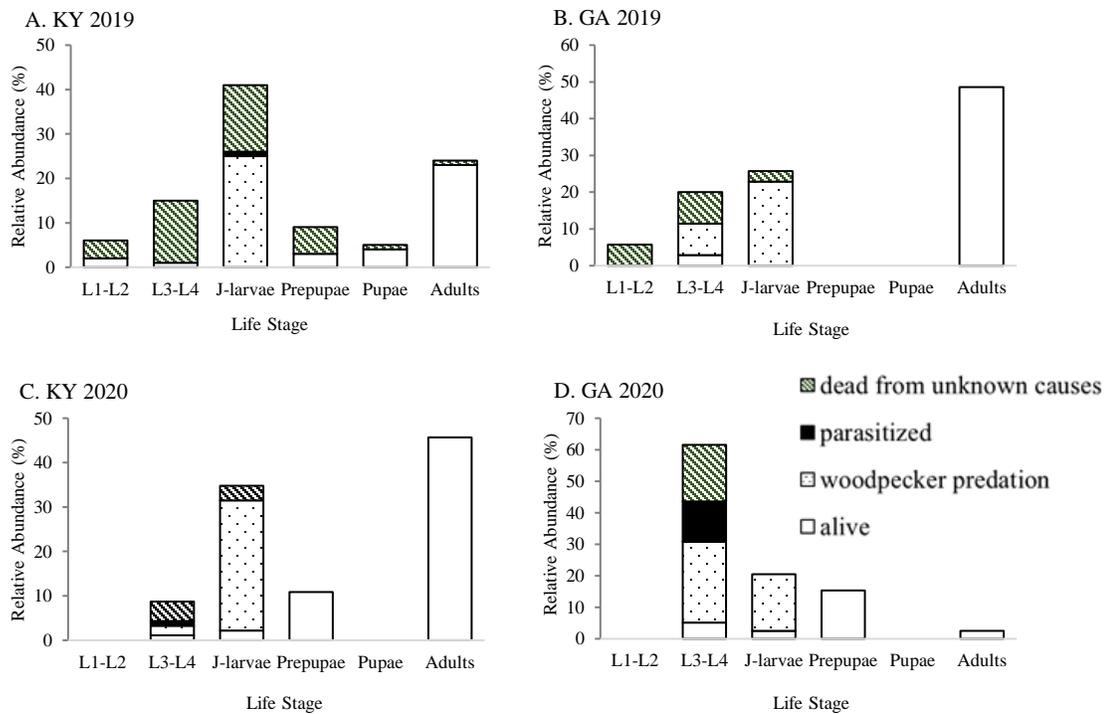
Year	Site	No. Canopy Stems	No. Understory Stems <sup>b</sup>	No. Seedlings	Ash Mortality (%)
2018	Fayette	40	2	229	65.0
	Spencer	51	3	177	89.2
	Anderson	44	280	614	37.3
2019	Fayette	37	4	512	34.7
	Spencer	49	3	125	84.1
	Anderson	39	326	779	84.6

<sup>a</sup> DBH>12.7cm

<sup>b</sup> DBH<12.7, height>1.37m



**Figure 2.1** EAB can have either a 2-year life cycle (A), where developing larvae are present throughout the year and readily available as potential hosts for emerging parasitoids, or a 1-year life cycle (B), where developing larvae are present only in the summer and fall, reducing the time period in which they could serve as potential hosts for emerging parasitoids.



**Figure 2.2** Relative abundance of EAB life stages and mortality factors as observed from trees felled during the winters of 2019 (A and B) and 2020 (C and D) in Kentucky (A and C) and Georgia (B and D). Alive EAB were living immature EAB or exit holes indicating a successful adult emergence. Woodpecker predation was evidenced by bark and sapwood destruction in the galleries or overwintering chambers. Parasitized EAB were classified by the presence or evidence of parasitoids within an EAB gallery. Galleries which lacked living EAB and exit holes whose fates could not be determined were classified as “dead from unknown causes.”

## APPENDICES

APPENDIX A

**Vegetation censusing coordinates**

Site	Stand	Plot	Latitude	Longitude
Anderson	1	1	N 38°00.474'	W 84°57.570'
	1	2	N 38°00.489'	W 84°57.594'
	1	3	N 38°00.485'	W 84°57.525'
	1	4	N 38°00.509'	W 84°57.635'
	2	1	N 38°00.565'	W 84°57.594'
	2	2	N 38°00.535'	W 84°57.588'
	2	3	N 38°00.516'	W 84°57.567'
	2	4	N 38°00.515'	W 84°57.592'
	3	1	N 38°00.568'	W 84°57.470'
	3	2	N 38°00.539'	W 84°57.437'
	3	3	N 38°00.536'	W 84°57.383'
	3	4	N 38°00.563'	W 84°57.426'
	Fayette	1	1	N 37°53.723'
1		2	N 37°53.732'	W 84°23.432'
1		3	N 37°53.779'	W 84°23.456'
1		4	N 37°53.681'	W 84°23.448'
2		1	N 37°53.799'	W 84°23.576'
2		2	N 37°53.727'	W 84°23.466'
2		3	N 37°53.700'	W 84°23.470'
2		4	N 37°53.795'	W 84°23.558'
3		1	N 37°53.672'	W 84°23.465'
3		2	N 37°53.701'	W 84°23.496'
3		3	N 37°53.709'	W 84°23.421'
3		4	N 37°53.736'	W 84°23.490'
Spencer		1	1	N 38°01.143'
	1	2	N 38°01.240'	W 85°16.811'
	1	3	N 38°01.280'	W 85°16.715'
	1	4	N 38°01.262'	W 85°16.716'
	2	1	N 38°01.284'	W 85°16.680'
	2	2	N 38°01.254'	W 85°16.658'
	2	3	N 38°01.275'	W 85°16.604'
	2	4	N 38°01.297'	W 85°16.557'
	3	1	N 38°01.141'	W 85°16.865'
	3	2	N 38°01.201'	W 85°16.783'
	3	3	N 38°01.049'	W 85°16.738'
	3	4	N 38°01.187'	W 85°16.799'

## APPENDIX B

### **Ichneumonoid morphospecies**

#### Methods

Yellow pan traps were fixed onto the bark of ash trees at three sites in central Kentucky and contents were sorted, as described in Chapter 2. Ichneumonoidea were further classified into morphospecies. Morphospecies were classified based on overall shape, color and pattern, and wing venation. Each morphospecies was photographed through the dissection microscope lens and a description of the key features and differences was noted. With each additional ichneumonoid, the individual was compared to morphospecies previously photographed and described from the trap samples. Morphologically similar individuals were considered the same morphospecies, while morphologically distinct individuals were then photographed and described as a new morphospecies.

#### Results

A total of 60 Ichneumonidae and 20 Braconidae morphospecies were identified. Three of the braconid morphospecies were *Spathius floridanus*, *S. elegans*, and *S. evansi* and another braconid was *Atanycolus* sp. The most abundant morphospecies contained 60 individuals and was identified to the ichneumonid subfamily Campopleginae. Campopleginae was found at all three field sites. There was no significant difference between abundance, evenness, richness, or either measure of diversity.

**Table B.1.** Ichneumonoid morphospecies abundance, evenness, richness, and diversity (mean (SE)) captured in yellow pan traps at three sites in central Kentucky in 2019. Diversity was calculated using Shannon’s diversity index and Simpson’s index. Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ ).

Site	Traps			Shannon	Simpson	
	( <i>n</i> )	Abundance	Evenness	Richness	Diversity	Diversity
Fayette	51	1.9 (0.3)a	1.0 (0.0)a	1.6 (0.2)a	0.6 (0.1)a	0.9 (0.1)a
Spencer	45	2.8 (0.3)a	0.9 (0.0)a	2.0 (0.2)a	0.7 (0.1)a	0.7 (0.0)a
Anderson	27	1.6 (0.4)a	0.9 (0.0)a	1.2 (0.2)a	0.4 (0.1)a	0.9 (0.1)a
		<i>F</i> <sub>2,38</sub> ; <i>P</i>	<i>F</i> <sub>2,32</sub> ; <i>P</i>	<i>F</i> <sub>2,38</sub> ; <i>P</i>	<i>F</i> <sub>2,38</sub> ; <i>P</i>	<i>F</i> <sub>2,33</sub> ; <i>P</i>
		2.76; 0.08	0.68; 0.52	2.69; 0.08	1.89; 0.17	1.87; 0.17

## Discussion

Ichneumonoid abundance and richness was greater at Spencer relative to the other sites, though this increase was only weakly significant (abundance and richness,  $P = 0.08$ ). There were no differences in the remaining ichneumonoid community parameters between sites. It is possible that some morphospecies were the same taxonomic species despite being phenotypically distinct. It is also possible that some individuals in a morphospecies were taxonomically different species despite their phenotypical similarities.

APPENDIX C

**Arthropods found in rearing bins**

Table C.1 Insects found in Kentucky and Georgia rearing bins in 2019 and 2020.

Year	Latitude	Site	Taxa	Abundance
2019	KY	Fayette Co.	<i>T. planipennis</i>	33
			EAB	2
		Jessamine Co.	EAB	5
		Spencer Co.	EAB	8
2019	GA	Cobb Co.	EAB	1
2020	KY	Jessamine Co.	EAB	1
		Fayette Co.	EAB	1
		Spencer Co.	EAB	6
2020	GA	Cobb Co.	<i>Atanycolus</i> sp.	1
			EAB	2

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**Pellecchia, S.** Latitudinal differences in emerald ash borer life cycle and parasitoid

synchrony. Oral presentation. Southern Appalachian Forest Entomology and Pathology Seminar (SAFEPS). Newland, NC. 5 March 2020.

**Pellecchia, S.** and L.K. Rieske. Effects of latitudinal variation on emerald ash borer

development and parasitoid efficacy. Oral presentation. Entomological Society of America (ESA), St. Louis, MO. 18 November 2019.

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