Characterizing Prepupal Diapause and Adult Emergence Phenology of Emerald Ash Borer

Thesis

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Abstract

Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire), is an exotic beetle native to Asia that threatens to exterminate North America's ash genus. The loss of ash resource represents a huge economic and ecological disaster, in many regions across the United States ash trees are commonly found in forests and urban landscapes. Little is known about the adult emergence phenology, the effects of temperature on development and the diapause of EAB. Understanding the seasonal biology of EAB is required to timely forecast the emergence of the adults and inform time management practices.

The main objectives of this study were to document the effects of temperature on diapause termination of prepupal EAB larvae and to characterize the adult emergence phenology of EAB across a latitudinal gradient. The first experiment documented the effects of temperature on the prepupal diapause termination of EAB. Diapausing larvae where collected from late October through late April from outdoor conditions and transferred to 18°C and 25°C. Results showed that by the second week of December, more than half of the overwintering larvae collected were able to continue development when held at 18 and 25°C. The number of insects that terminated diapause increased as the winter months advanced, suggesting the importance of chilling in diapause termination. These findings also provide evidence of the effect of diapause on synchronizing the adult emergence phenology of EAB.

The second experiment characterized the adult emergence patterns of EAB across a latitudinal north-to-south gradient compromising 500 km or 4.5 degrees in latitude. EAB adult emergence was monitored from infested ash logs from start to end in 2011 and 2012 at six locations along the latitudinal gradient. In both years, emergence started fist in the southernmost site, Cincinnati, OH and latest in the northernmost site, Midland, MI, but no clear pattern was observed for the rest of the sites (Columbus, Delaware, Wooster, and Toledo, OH).

Emergence started earlier in 2012 compared to 2011 in all sites, corresponding to the warmer temperatures in 2012, the length of emergence periods were also longer in 2012. Using the emergence data collected from all sites and years a phenological model to predict future emergence was developed. The model accurately predicted 10, 25, 50, 75 and 95% of the total emergence of EAB. The average deviation from observed and predicted emergence for first and end of emergence was ± 7 days. Thus the model provided in this study can be a useful tool for making regulatory decisions.

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Field of Study

Major Field: Entomology

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CHAPTER 1

LITERATURE REVIEW

1.1. Biological invasions: hidden cost of international trade

Biological invasions have recently become an important problem of global consequences, even though this is not a new phenomenon; the number of invasive species has increased with the advent of new ways of transport and commerce (Mack *et al.* 2000). Invasive species represent a risk to North America ecosystems; exotic species can affect ecosystem biodiversity and productivity (Mack *et al.* 2000). Biological invasions have increased in recent years, mostly because of increased international trade; many exotic species are transported with their hosts in the commodities shipped to the US (Aukema *et al.* 2011). Damage and control costs of invasive species in the United States are estimated to be more than 138 billion USD annually (Pimentel *et al.* 2005).

1.2. Emerald ash borer: threat to North America's ash resources

Insect invasive species are among the most damaging and long lasting biological invasions. There has been a historical accumulation of exotic insect species in North America since the European settlement; in the period from 1860 to 2006 approximately 2.5 new invasive forest insect species have been detected in United States per year (Aukema *et al.* 2010). Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire), is an exotic beetle in the family Bupestridae native to Asia, that was introduced to North

America on the 1980´s. First detected in Michigan in 2002 (Haack *et al.* 2002), EAB threatens to functionally extirpate the entire genus of ash in North America (*Fraxinus* spp.).

1.3. Ecological and economic impact of emerald ash borer

The loss of the ash resource in North America will have widespread ecological and economic impacts. Forest community structure and composition will be affected by ash mortality associated with EAB (Smith 2006, Gandhi and Herms 2010). There are an estimated 8 billion ash trees in North America valued at 300 billion USD (Poland and McCullough 2006, Sydnor *et al.* 2007). In Ohio forests alone, there are estimated to be 3.8 billion white ash trees (Griffith *et al.* 1991). The estimated cost of EAB to US communities over a 10 year period from 2009-2019 is about $ 10.7 billion (Kovacs *et al.* 2010). Ash trees are commonly planted in urban ecosystems and are also an important source of saw timber, firewood and furniture. As of April 2013, EAB is known to exist in nineteen US states and two Canadian provinces (EAB Info 2013), the widespread distribution of EAB provides evidence that it can adapt to a wide variety of climate regions. EAB has rapidly become in one of the most devastating invasive species in NA with ecological impacts comparable to the Chestnut blight and Dutch elm disease.

1.4. Biology of emerald ash borer

EAB can have univoltine or a semivoltine life cycle, depending on the climate, level of infestation and host vigor (Cappaert *et al.* 2005a, Tluczek *et al.* 2011). The observed life cycle of EAB in North America is similar to that described in China

(Cappaert *et al.* 2005b), as well to other buprestids native to North America such as the bronze birch borer (*Agrilus anxius* Gory) (Muilenburg and Herms 2012). In the Southeast Michigan and Northern Ohio region of the United States, adults start to emerge from mid-May to early June, which occurs at an accumulation of 230-260 degree days base 10°C as black locust (*Robinia pseudoacacia*) reaches full bloom (Brown-Rytlewski and Wilson 2005).

Adults live three to six weeks and mate within the first ten days of emergence. Beetles feed on ash leaves causing minimal damage (Bauer *et al.* 2004). Peak adult activity occurs in late June to mid-July, at 800-1,200 degree days base 10°C (DD_{10}), and 95% of emergence on trap capture period is complete by the end of July at about 1,400 degree days base 10°C (DD_{10}) (Poland *et al.* 2011). Females lay eggs on bark surface or in crevices. A single female can lay 60-90 eggs during their life span, and peak oviposition occurs in late June to early July. Eggs are beige in color and 1 mm in diameter, and hatch within 7-10 days (Bauer *et al.* 2004, Cappaert *et al.* 2005b, Lyons and Jones 2005).

Larvae feed on phloem and form s-shaped galleries as they feed. Larvae cause the most damage to trees by disrupting phloem transport; trees are killed within 1 to 3 years showing symptoms of infestation (Poland 2007). The larval stage has four instars and can last about 305-315 days (Bauer *et al.* 2004, Cappaert *et al.* 2005b, Lyons and Jones 2005). The majority of larvae overwinter as fourth instar prepupal larvae in pupal chambers, however, small numbers of larvae have been observed to overwinter as earlier instars (Bauer *et al.* 2004, Lyons and Jones 2005). Larvae develop into pupal stages as weather warms in April and pupation lasts three to four weeks.

1.5. Overwintering physiology and diapause of emerald ash borer

Glycerol is the most abundant cryoprotectant utilized by prepupal larvae of EAB (Crosthwaite *et al.* 2011). Its concentration in the hemolymph increases during the coldest months, allowing EAB to reduce their supercooling point during to less than -30°C (Crosthwaite *et al.* 2011). Cold tolerance of EAB prepupal larvae is reduced when larvae are exposed to warm periods of 10°C and 15°C for time periods of 0-14 days (Sobek-Swant *et al.* 2012). Supercooling point (SPC) of larvae are increased with higher temperatures, and this increase in SPC is not reversible; however, this increase in the SPC in larvae may be not enough to cause mortality of overwintering larvae (Sobek-Swant *et al.* 2012).

Diapause is a state of arrested insect development that allows insects to survive harsh environmental conditions such as cold, drought, rainy seasons, and or lack of resources (Denlinger 2008). The occurrence of cold hardiness and diapause can be merely coincidental or a causal linkage (Denlinger 1991, Pullin 1996), and there are instances where diapause occurs without cold hardiness, such is the case of tropical diapause (Denlinger 1986). There are also or cases where cold hardiness occurs without diapause (Young and Block 1980).

Studying the diapause of EAB can help to understand its seasonal biology (Denlinger 2008), which could improve accuracy of population dynamics models and physiological time models. For example, developmental rate models have been developed for diapause of gypsy moth (*Lymantria dispar*) eggs (Gray *et al.* 1995, Schaub *et al.* 1995) and spruce bark beetle (*Dendroctonus rufipennis*) larvae (Hansen *et al.* 2011).

1.6. Phenology models and IPM

Jarosik *et al.* (2011) determined that closely related species and populations share similar thermal requirements using a database of independent studies of thermal requirements of development times different at constant temperatures. The known values of lower developmental threshold, LDT (the temperature below which development stops), and the sum of effective temperatures (the number of degree days above the LDT necessary to complete developmental stage) of a related species can be used for a species with unknown thermal requirements (Jarosik *et al.* 2011).

Degreeday models are useful tools for predicting pest activity in IPM. Timely forecasting of EAB adult emergence has been suggested as a potential way of controlling EAB by targeting adults before they start mating (Wang *et al.* 2010). Accurate prediction of EAB adult emergence is required to time regulatory and management decisions, such as safe windows for transport of ash products and pesticide applications.

Accuracy of prediction in phenological models can vary across sites, thus different models may need to be fitted at different sites (e.g. Akers and Nielsen 1984). Akers and Nielsen (1984) developed forecasting models for the native buprestid bronze birch borer (*Agrilus anxius* Gory) in white barked birches (*Betula* spp.) across different locations in Ohio. Akers and Nielsen (1984) statistical models predicted 10% of bronze birch borer with 3.8 days mean deviation from the actual emergence dates, therefore, these models can be used to time insecticide applications to control bronze birch borer.

1.7. Research Objectives:

The main objectives of this project were to study the effects of temperature on diapause termination of prepupal EAB larvae and to characterize the adult emergence phenology of EAB. These objectives were divided into three specific objectives:

Objective 1 (Chapter 2): To determine the effects of temperature on the termination of diapause. EAB prepupal larvae were collected from infested ash trees during October 2011 through April 2012 on a monthly basis. Groups of 30-35 larvae were placed inside growth chambers at constant temperatures of 18°C and 25°C and photoperiod of 16L: 8D. Larvae were monitored weekly to observe signs of development.

Objective 2 (Chapter 3): To characterize EAB emergence patterns across a latitudinal gradient. In 2011 and 2012, EAB infested ash trees across six locations in a latitudinal gradient were felled and cut into bolts of 1.5-1.8 m length and 10-20 cm diameter. Emergence was monitored from start to end during the entire season.

Objective 3 (Chapter 3): To develop a phenological model to predict adult EAB emergence. Using adult emergence data collected and average daily temperature data events, a phenological model was fitted using a logistic regression function. Parameters for the model, the slope and the intercept were estimated using maximum likelihood and parameters estimated for each site and year were compared using a parallel line analysis.

1.8. References

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CHAPTER 2

EFFECTS OF TEMPERATURE ON EMERALD ASH BORER PREPUPAL DIAPAUSE TERMINATION

2.1. Abstract

Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire), is an invasive beetle that threatens to eliminate native ash trees (*Fraxinus* spp.) from North America. Little is known about the overwintering physiology and diapause of EAB. Investigating EAB diapause can help improve the accuracy of population dynamics and physiological time unit models to predict future insect activity. The present study was developed to study the effects of temperature on EAB prepupal larvae diapause termination. EAB prepupal larvae were collected from infested ash trees during October 2011 through April 2012 on a monthly basis. Groups of 30-35 larvae were transferred to growth chambers at constant temperatures of 18°C and 25°C and 16L: 8D photoperiod. Larvae were monitored weekly to observe signs of development. Results show that temperature is an important stimulus for diapause development; the amount of time larvae were exposed to outdoor temperatures increased the number of larvae that terminated diapause. 60% of the larvae collected in December where able to continue development when transferred to 18°C and 25°C. Diapause synchronizes EAB adult emergence. This study provides additional information to the understanding of the seasonal phenology EAB. Additional studies are proposed to address further questions that aroused from the findings of this study.

Keywords: *invasive species; seasonal phenology; overwintering biology; chilling requirements, ash trees.*

2.2. Introduction

Emerald ash borer, EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive wood boring beetle that has killed millions of ash trees since its accidental introduction to North America on the 1980's (Haack *et al.* 2002). EAB threatens to functionally extirpate the entire *Fraxinus* genus from North America, with enormous ecological and economic impacts. There are an estimated eight billion ash trees in North America valued at 300 billion USD (Poland and McCullough 2006, Sydnor *et al.* 2007). EAB has rapidly become one of the most devastating invasive species in North America with potential impacts comparable to those of the Chestnut blight and Dutch elm disease invasions.

EAB has a univoltine or a semivoltine life cycle, depending on the climate, level of infestation, and host vigor (Cappaert *et al.* 2005a, Tluczek *et al.* 2011). Life cycle of EAB described in North America is similar to that observed in China (Cappaert *et al.* 2005b). In the Southeast Michigan and Northern Ohio region of the US adults start to emerge from mid-May to early June, which occurs at an accumulation of 230-260 degree days base 10°C as black locust (*Robinia pseudoacacia*) reaches full bloom (Brown-Rytlewski and Wilson 2005). Adults feed on ash leaves during their entire life, which lasts 3 to 6 weeks, and mating occurs within 10 days. Females lay eggs on bark crevices, a single female can lay 60-90 eggs during their life span, peak and oviposition occurs in late June to early July. Eggs are beige in color and 1 mm in diameter, and hatch within 7-10 days.

Larvae feed on phloem and leave s-shaped galleries as they feed. Larvae cause the most damage to trees by disrupting phloem transport; trees are killed within one to three years after expressing symptoms of infestation (Poland 2007). The larval stage has four instars and can last 305-315 days (Bauer *et al.* 2004, Lyons and Jones 2005, Cappaert *et al.* 2005b). The majority of larvae overwinter as fourth instar prepupal larvae in pupal chambers, however, small numbers of larvae have been observed to overwinter as earlier instars (Bauer *et al.* 2004, Lyons and Jones 2005). Larvae develop into pupal stages with warmer weather, around April pupation lasts three weeks.

In 2011, Crosthwaite *et al.* documented the overwintering physiology of EAB; they found that glycerol is the most abundant cryoprotectant utilized by EAB, and its concentration in the hemolymph increases during the coldest months, allowing EAB to reduce their supercooling point below -30°C. Sobek-Swant *et al.* (2011) found that cold tolerance is reduced by warm periods of 10-15°C and is not reversible; however, this increase in the supercooling point in larvae may still be not enough to cause mortality of overwintering larvae.

Diapause is a state of arrested insect development that allows insects to survive harsh environmental conditions, such as cold, drought or rainy seasons and lack of resources. The occurrence of cold hardiness and diapause can be merely coincidental or a causal linkage (Denlinger 1972, Pullin 1996) there are instances where diapause occurs without cold hardiness; such is the case of tropical diapause (Denlinger 1986), or cases where cold hardiness occurs without diapause (Young and Block 1980).

Studying diapause can help us better understand the seasonal biology of EAB (Denlinger 2008). This information could improve accuracy of population dynamics models and physiological time models. For example, developmental rate models have been developed for diapause of gypsy moth (*Lymantria dispar*) eggs (Schaub *et al.* 1995, Gray *et al.* 1995) and spruce bark beetle (*Dendroctonus rufipennis*) larvae (Hansen *et al.* 2011).

Numerous studies have addressed diapause termination requirements in insects (Williams 1946, Denlinger 1972, Tauber and Tauber 1976). The main factors affecting the maintenance and termination of diapause are temperature and photoperiod (Tauber and Tauber 1976). Denlinger (1972) investigated effects of different environmental factors on the termination of pupal diapause in the flesh fly (*Sarcophaga bullata*). Temperature is the most important stimuli in diapause termination, while photoperiod is ineffective. The ecological reason for this is that *S. bullata* overwinters buried in the soil, which limits the amount of light received by prepupal larvae. Hence, photoperiod will not be reliable cue of favorable conditions as temperature (Denlinger 1972).

Hormones can serve as regulators of diapause; hormones involved in diapause vary among species and the developmental stage in which this process occurs (Denlinger, 1985). Larval and pupal diapauses are characterized by a failure of ecdysteroids synthesis to continue development (Denlinger, 2002). Pupal diapause in flesh flies (*Sarcophaga* sp.) can be terminated or shortened with different rates of injections of ecdysterone or 5,β-hydroxyecdysterone (Zdarek and Denlinger, 1975). The role of genes associated with the action of ecdysteroids is critical for regulating larval diapause (Denlinger, 2002). The Ecdysone receptor (EcR) and its dimerization partner ultraspiracle (USP) are well studied receptors of ecdysteroids (Henrich and Brown, 1995). In the flesh fly *Sarcophaga crassipalpis* expression of EcR and USP varies during diapause stages; receptors are highly expressed during the preparation diapause, less expressed during diapause, and highly expressed during the termination of diapause (Rinehart *et al.*, 2001). Studies in *Chironomus tentans* and *Manduca sexta* indicate that there is species variation in expression patterns of EcR and USP (Denlinger, 2002).

Based on Denlinger (1972) and Hansen *et al.* (2011) findings on the role of photoperiod in diapause termination, it was hypothesized that photoperiod would not play a major role in EAB diapause maintenance, since the amount of light received by larvae overwintering under the bark would not be a reliable indicator of favorable conditions. Instead, the present study focuses on the effects of temperature on EAB diapause termination. To determine the effects of temperature on termination of diapause, EAB prepupal larvae were collected from infested ash trees during October 2011 through April 2012 on a monthly basis. Groups of 30-35 larvae were placed inside growth chambers at constant temperatures of 18°C and 25°C and 16L: 8D photoperiod. Larvae were monitored weekly to observe signs of development. The main objective of the study was to determine the effects of temperature on termination of diapause in EAB prepupal larvae.

2.3. Materials and Methods

2.3.1. Plant material and study animals:

Twenty heavily infested with EAB ash bolts ranging from 20-40 cm in diameter and 50-70 cm in length were cut in late October and mid December 2011 at a residential area in Dublin, Ohio (40° 7'2"N, 83° 3'46"W). Bolts were held in an outdoor location away from buildings and parking lots in an upward position against other trees. Temperature of the location where the bolts were deployed was obtained from the Ohio Agricultural Research and Development Center (OARDC) weather station in Columbus (OARDC Weather System 2013). The bark of the bolts was removed using chisels, drawknives and a mallet, and in some instances the bark was loose enough to remove by hand. EAB prepupae overwintering in pupal chambers in the inner bark or outer sapwood of the bolts were carefully removed using forceps.

2.3.2. Determination of chilling requirements from field collected larvae:

Groups of 50-70 larvae were sampled from the logs monthly from late October 2011 through late April 2012. After collection prepupae were stored in closed petri dishes lined with moist paper towel with five larvae per plate. Petri dishes were brought into growth chambers and kept at 18°C and 25°C constant temperature and a photoperiod of 16L: 8D. Larvae were monitored on a weekly basis for the duration of the experiment. Larvae, pupae and adult development were monitored and recorded weekly until all animals died (Table 2.1). 150 larvae were collected from the same bolts as the first experiment on 19 December 2011. Larvae were stored on a sealed transparent plastic container lined with moist paper towels at a constant temperature of 4°C and 16L:8D photoperiod. Larvae were stored at 4°C for 6, 13, and 18 weeks. After the cold storage treatment, larvae were placed in petri dishes lined with moist paper towels and transferred to growth chambers at 18°C and 25°C constant temperature, and 16L:8D photoperiod. Larvae were monitored weekly until the end of the experiment and variables were measured the same as the previous experiment (Table 2.2).

2.3.3. Preliminary study on the hormonal regulation of diapause:

In a preliminary study, the effects of 20 hydoxyecdysone (20 HE) injections on EAB prepupal larvae diapause duration and termination were evaluated. 60 animals collected from the same logs as the previous experiments were injected with doses of 5, 1, 0.5, 0.25, 0.1 and 0.05 μL of 20 HE diluted in insect saline solution (ISS) for a total injection volume of 5 μL per larvae. Animals injected with 20 HE were compared to larvae injected with ISS and uninjected ones, and held at 25°C. The experiment was conducted in late October and repeated two weeks after.

2.4. Results

A total of 503 larvae were collected from the logs during the entire experiment; where, 64 (12.72 %) larvae developed into adults, 138 (27.44 %) died as pupae and 301 (59.84 %) remained as larvae. At the last collection in late April, only pupae were found. Development for larvae held at 18°C and 25°C was similar, thus the results are presented combining data from larvae held at both temperatures (Table 2.1). The number of larvae that pupated and developed into adults steadily increased as the months advanced. In October, the proportion of larvae that terminated diapause was only 7%, whereas in March 59% of the larvae terminated diapause (Table 2.1). 60% of the larvae collected on December 2011 broke diapause, representing a 40% increase from November (Table 2.1).

Larvae collected in February and held at 25°C, had a faster developmental rate, it took an average of 21 days to pupate and develop into adults, and on the other hand larvae held at 18°C took an average of 52 days. Developmental rates of larvae collected in March were similar for larvae held at 25°C and 18°C; it took an average of 37 days and 39 days respectively for larvae to pupate. For pupae collected in April it took two and four weeks to develop into adults when held at 25°C and 18°C respectively.

A total of 130 larvae were transferred to 18°C and 25°C growth chambers after 4°C storage, and of those only 8 (6.15%) larvae developed into adults, 81 (62.31%) died as pupae, and 41 (31.54%) remained as larvae. It took an average of four and six weeks for larvae held at 4°C to pupate after being transferred to 18°C and 25°C respectively.
All 60 larvae injected with 20 hydoxyecdysone (20 HE) with 20 HE died within two weeks regardless of the concentration administered. In contrast, all 20 larvae injected with insect saline solution and 20 uninjected larvae survived. From the 40 surviving larvae, 20% of them broke diapause, similar to the larvae collected in November and held at 25°C.

2.5. Discussion

Low temperatures are an important diapause termination agent for EAB. Exposure to low temperatures increased diapause development and the number of adults observed, especially for larvae exposed to outdoor temperatures. The number of larvae that terminated diapause increased over time. By the second week of December, 60% of the prepupae larvae collected terminated diapause, suggesting that the majority of larvae had completed their chilling requirements by mid-December. Storage at 4°C for 13 and 18 weeks increased diapause development. However, this increase in diapause development observed after 13 and 18 weeks of 4°C storage could be confounded with the time of collection. These larvae were collected in December and a high percentage of them had already completed their chilling requirements. Interestingly, EAB started pupation by the end of March, and by the end of April all EAB had pupated.

Some explanations as to why all larvae injected with 20 hydroxyecdysone (20 HE) died could be that EAB larvae may be susceptible to the stock solution of 20 HE used, larvae where not able to tolerate the contents of ethanol of the stock solution. It is possible that the ecdysone receptor EcR and its ultra-spiracle USP dimer were not being expressed at the time the 20 HE was administered (Rinehart *et al.* 2001). Therefore, it is suggested to use other forms of ecdysteroids such as 5, β hydroxyecdysterone that could be more effective, or grading the doses of 20 HE through multiple doses (Zdarek and Denlinger 1975).

A large number of larvae and pupae desiccated during the experiment, especially for larvae stored at 4°C. One explanation could be the low relative humidity in the growth chamber. Although mortality can also be attributed to the stress due to handling, desiccation of pupae and larvae was probably the main cause of mortality and the reason why very few insects were able to develop into adults. Relative humidity can play an important role in diapause regulation, and can be just as important as temperature (Tauber *et al.* 1998). Another explanation to the mortality could be that larvae developing under constant temperatures may have depleted all their energy reserves to the point of not having enough to pupate and become adults; this hypothesis was also suggested by

Sobek-Swant *et al.* (2011). They observed the effects of warm spells of 10 °C and 15 °C during winter on larval deacclimation and cold hardening of EAB larvae; and found that the deacclimation period lowered concentrations of cryoprotectants in the hemolymph of the larvae.

To reduce the amount of desiccation and reduce the amount of handling when removing larvae and subsequent mortality, the use of pieces of bark or logs could be a better approach. The use of phloem sandwiches (Kim and Miller 1981) such as the ones used for observing bark beetles would provide a better approach for handling EAB larvae and would allow observing its feeding behavior and development.

To increase the percentage of diapause development as well as reducing the length of diapause, termophases (alternating temperatures from 18°C and 25°C), acclimation periods or temperature gradients could be used (Williams 1942). Diapause is a dynamic state (Tauber and Tauber 1976) and larvae are exposed to a wide range of fluctuating temperatures during winter, exposing larvae to a constant temperature is not ecologically realistic. For instance, Denlinger (1972) using flesh flies (*Sarcophaga Sp.*) found that a combination of temperatures from 17°C and 25°C results in a shorter diapause than constant exposure to either temperature. This also raises questions about what other factors might be regulating diapause termination in EAB. Although, in our study the percentage of diapause termination was around 60-70% (from December to March), there was still 30-40% of other possible factors (e.g.photoperiod) that could be playing a role in EAB diapause termination. Previous studies with codling moths (*Carpocapsa pomonella*) and flesh flies (*Sarcophaga crassipalpis*) have demonstrated that they are sensitive to light even at the low levels perceived from their environments, inside an apple and an insect respectively (Peterson and Hamner 1968, Denlinger 1971). On the other hand, there are other studies that demonstrated that photoperiod is ineffective in diapause termination, such in the case of the case of the spruce beetle (*Dendroctonus rufip1ennis*) (Hansen *et al.* 2011) and flesh flies (*Sarcophaga bullata*.) that overwinters buried in the soil (Denlinger 1972).

One limitation of this study is the fact that we don't know exactly when the larvae entered diapause; this is particularly a problem because it is not possible to tell the total duration of diapause. This problem could be addressed by rearing EAB under laboratory conditions; however, rearing EAB under laboratory conditions would be experimentally difficult and would require developing an artificial diet.

Unfortunately in this study, very low numbers (<1%) of early instar larvae were found during sampling. Thus, the diapause termination requirements of earlier instars larvae of EAB could not be determined. This is a particularly important question to understand the voltinism of EAB. Cappaert *et al.* 2005 observed that under low densities, only 18% of 282 larvae collected were fourth instar. An explanation for the low percentage of early instars larvae found, could be that the trees used for collecting the larvae where heavily infested and with high densities of EAB. Under these conditions, larvae develop more rapidly and most of them are able to complete development the first year. The question about how EAB life cycle is affected by larval density and host resistance was addressed by Tluczek *et al.* (2011), in this study, larval development and adult emergence in ash trees with low levels of infestation was observed to take two years, and the percentage of insects that overwintered as a fourth instar larvae increased, as larval density increased and plants became more stressed.

In summary, this study provides valuable insights on EAB diapause and its termination requirements. It was found that exposure to cold temperatures increased the number of larvae that terminated diapause as the months advanced. Most EAB larvae completed their chilling requirements by mid-December. Therefore it is possible that diapause plays a role in synchronizing the emergence phenology of EAB.

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ILLUSTRATIONS

Table 2.1. Collection dates, initial number of emerald ash borer larvae collected and number of larvae that terminated diapause at the end of the experiment (4 June 2012).

Date Collected	Initial # of larvae	Diapause development
28-Oct-11	200	13 (7%)
17-Nov-11	30	6 (20%)
12-13 Dec-11	90	54 (60%)
9-Jan-12	25	16 (64%)
23-Jan-12	29	23 (79%)
29-Feb-12	59	40 (68%)
23-Mar-12	49	29 (59%)

Table 2. 2. Weeks stored at 4°C constant temperature, initial number of emerald ash borer larvae collected and number of larvae that terminated diapause at the end of the experiment (4 June 2012).

Weeks Stored at 4 °C	Initial # of larvae	Diapause development
0 weeks	40	25 (63%)
6 weeks	30	15 (50%)
13 weeks	30	25 (83%)
18 weeks	30	24 (80%)

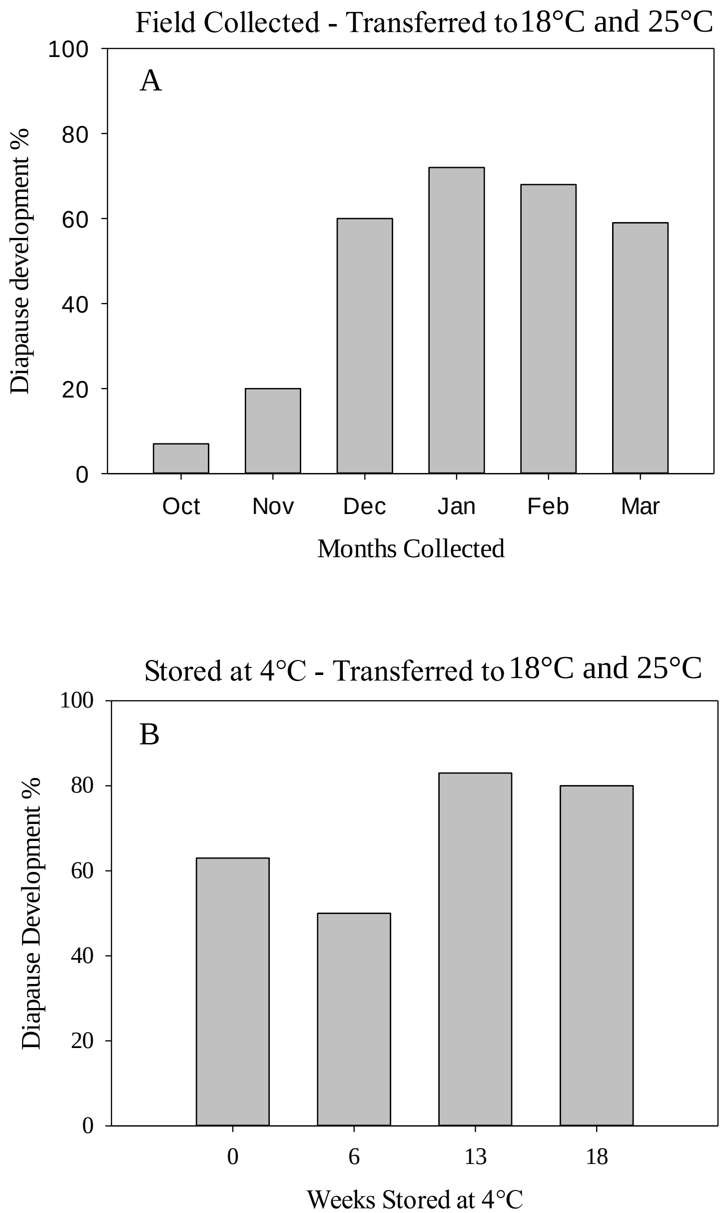

Figure 2.1. A) Percentage of diapause termination of emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) diapausing larvae collected from infested ash logs at ambient temperature during late fall and winter and then held at 18°C and 25 °C constant temperature. B) Percentage of diapause termination of EAB larvae collected from infested ash logs in December, stored for 6, 13, and 18 weeks at 4°C and then held at 18°C and 25 °C constant temperature.

CHAPTER 3

CHARACTERIZATION OF EMERALD ASH BORER ADULT EMERGENCE PHENOLOGY ACROSS A LATITUDINAL GRADIENT

3.1. Abstract

Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) is an invasive species that threatens to functionally extirpate ash (*Fraxinus* spp.) from North America with devastating economic and ecological impacts. Accurate prediction of adult emergence is required to inform regulatory and management practices. In 2011 and 2012, EAB adult emergence phenology was characterized at six sites on a north- south gradient comprising 4.5° of latitude (500 km). Adult emergence was quantified weekly throughout from early May through late August. Emergence data was combined with temperature data to generate phenological models to predict adult emergence. In 2011 and 2012 EAB emergence started first in southernmost site Cincinnati, OH and last in Midland, MI, the northernmost. Adult emergence period was longer in Cincinnati, OH than in Midland, MI. No Clear pattern was observed among the rest of the sites. Different models were fitted for each location. Results show that the models can accurately predict the start of adult emergence 275-325 degree days base 10°C (DD_{10}), 95% of adult emergence 750-1000 (DD_{10}) and the end of emergence 1200-1500 (DD_{10}). These results document that effect of latitude and weather on EAB adult phenology and the model presented in this study can be useful to accurately predict EAB emergence and time management practices.

Keywords: *invasive species; phenological models; control practices; ash trees, time management; temperature.*

3.2. Introduction

Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire), is an invasive buprestid native to Asia, first detected in southeastern Michigan in 2002 (Haack *et al.* 2002). EAB threatens to functionally extirpate the entire ash genus in North America (*Fraxinus* spp.); with severe ecological and economic impacts (Smith 2006, Kovacs *et al.* 2010). Forest community structure and composition will be affected by ash mortality associated with EAB (Smith 2006).

In Ohio forests alone, there are estimated to be 3.8 billion white ash trees (Griffith *et al.* 1991). Kovacs *et al.* (2010) estimated that the discounted cost to treat, remove and replace 17 million ash trees in urban landscapes for 25 US states over a 10 year period to be $ 10.7 billion. Ash trees are commonly planted in urban ecosystems; and are also an important source of saw timber, firewood and furniture. As of April 2013, the known distribution of EAB was known to compromise 19 US states and 2 Canadian provinces (EAB Info 2013). This widespread distribution suggests that the EAB can survive in a wide variety of climates.

EAB has a similar biology to other buprestids native North America such as the bronze birch borer (*Agrilus anxius* Gory) (Muilenburg and Herms 2012). EAB can have a univoltine or a semivoltine life cycle, depending on the climate region and host vigor (Tluczek *et al.* 2011). In Michigan and Ohio adult EAB emergence has been observed to begin in late spring at 230-260 degree days base 10°C (DD_{10}) (Brown-Rytlewski and Wilson 2005, Cappaert *et al.* 2005b), and continue into midsummer. In trap catches in

Michigan, peak of emergence has been observed in late June to mid-July, at 800-1,200 degree days base 10°C (DD_{10}), and 95% of emergence by the end of July 1,400 degree days base 10°C (DD_{10}) (Poland *et al.* 2011).

Adults feed on ash leaves causing little damage to trees and mate within 10 days, females oviposit on bark. Eggs hatch within 7-10 days; larvae feed on phloem, and leave s-shaped galleries as they feed, disrupting water, nutrient and carbohydrate transport causing tree mortality. Trees die within 1-3 years of showing symptoms. Larval stage has four instars and can last 305-315 days (Lyons and Jones 2005). By late October, EAB overwinters as fourth instar larvae and larvae enter pupal stages with warmer weather, around April (Wang *et al.* 2010).

Accurate prediction of EAB adult emergence is required to time regulatory and management decisions, such as safe windows for transport of ash products and pesticide applications, and degreeday models are useful tools for predicting pest activity (Akers and Nielsen 1984). Previous research has shown that accuracy of prediction models can vary across sites, and that different models may be required at different sites (Akers and Nielsen 1984). Akers and Nielsen (1984) developed forecasting models for the bronze birch borer in white barked birches (*Betula* spp.) across different locations in Ohio, and their models predicted 10% of bronze birch borer with a 3.8 days mean deviation from the actual emergence dates, thus these models can be used to time insecticide applications to control bronze birch borer.

Jarosik *et al.* (2011) determined that closely related species and populations share similar thermal requirements using a database of independent studies of thermal requirements of development times different at constant temperatures. The known values of lower developmental threshold, LDT, (the temperature below which development stops) and the sum of effective temperatures (the number of degree days above the LDT necessary to complete developmental stage) of a related species can be used for a species with unknown thermal requirements (Jarosik *et al.* 2011). The objectives of this study were: to characterize EAB emergence patterns across a latitudinal gradient from north to south, and to develop a degree day model to predict EAB emergence using emergence and temperature data collected from each location.

3.3. Materials and Methods

3.3.1. Study sites

EAB-infested ash trees were located at six locations on a north-south gradient representing 4.5° of latitude (500 km) from 2011 through 2012. The north-to-south latitudinal gradient included from Midland, MI, the northernmost site, and Toledo, Wooster, Delaware, Columbus and Cincinnati, OH, the southernmost site. The location and environment in which the ash trees were growing included urban street trees in Toledo, Wooster and Columbus, OH; urban landscapes, Cincinnati, OH, and forest trees, Midland, MI and Delaware, OH. The location in Columbus, OH, was added in 2012, and no data is available for this site in 2011.

3.3.2. Insect Sampling and Plant Material

At each location, three to six EAB-infested ash trees with showing severe canopy decline were harvested during April and early May before the start of adult emergence. Felled trees were cut into 12-24 bolts of 1.5- to 1.8-m in length and 10- to 20-cm in diameter. Existing exit holes were marked and counted before adult emergence began. Previous studies with bronze birch borer have shown that felling in spring has no effect on the timing of emergence (Akers 1984). Felled bolts were deployed in an upright position in full sun to partial shade, but away from heat sinks such as buildings or parking lots. EAB adult emergence was monitored by counting the number of new exit holes weekly from first emergence until three consecutive weeks of no new emergence. Phenological indicators that correspond with first emergence, peak of emergence and of emergence were also recorded.

3.3.3. Climatological Data

Quality controlled, maximum, minimum and average daily temperature records were obtained from weather stations operated by the National Oceanic and Atmospheric Administration (NOAA, data retrieved from the National Climatic Data Center, NCDC), located at Midland, MI; Toledo, Delaware and Cincinnati, OH. Climatological data for Wooster and Columbus, OH was obtained from weather stations operated by the Ohio Agricultural Research and Development Center (OARDC) weather system. Weather stations used were located within 15 km of the study sites.

3.3.4. Degree Day Computation Method

Growing degree days (GDD) were calculated using the modified sine wave method approach (Allen 1976). Development was assumed to occur only at times where daily temperature was above the minimum developmental threshold, an upper developmental threshold was not accounted. The base temperature used was 10°C and 1 January as the starting date similar to what has been used with bronze birch borer (Herms 2004).

3.3.5. Model development

3.3.5.1. Model fitting

Logistic regression analysis was used to model the proportion of beetles emerged as a function of degree days (Equation 1) (PROC LOGISTIC, SAS v.9.3; SAS Institute 1999). Probit, Logit and Cumulative Log Log link functions were used to determine the best model for each site. To improve accuracy of prediction and model fitting accumulative GDD data was log transformed ($\log_{10}(n+1)$) (Niemczyk *et al.* 1992). Best model selection was based on models with the lowest relative goodness of fit test Akaike information criterion (AIC) criterion, the highest adjusted R-square, results of the Hosmer-Lemeshow Goodness of Fit Test, deviation from the predicted emergence and observed emergence and visual fit of the data. The event /trial syntax was used to specify the binary response data. Sites and years were included in the CLASS statement.

$$F^{-1}(p) = \boldsymbol{x}'\boldsymbol{\beta} \qquad (1)$$

Where F is the cumulative distribution function used to model the probability of emergence (normal for probit models or logistic for logistic models), $\boldsymbol{x}$ is the vector of predictor values cumulative degree days, and $\boldsymbol{\beta}$ is the corresponding vector of parameters, slopes and intercepts.

3.3.5.2. Parallel line analysis

To test for the heterogeneity (homogeneity) of slopes β, it was determined whether the probit lines fitted for each site and years were parallel (with separate intercepts γi but common slope β equation 2). A model with separate intercepts for each site and year was fitted, the model included a common slope for accumulation of degree days and a set of parameters representing the deviation from sites and years from the common slope $\boldsymbol{x}'\beta$.

$$F^{-1}(\mathrm{p}) = probit(\mathrm{p}) = \alpha + \gamma \mathrm{i} + \boldsymbol{x}'\beta \quad (2)$$

where α is the overall intercept, γi is the parameter for the ith site representing the deviation of the site i intercept from the overall intercept, and β is the common slope on $\boldsymbol{x}'$ cumulative degree days.

Since the test for site and accumulation of degree day interaction (deviations of the site slopes γi and the common slope βX) in the above model was significant, this indicated that the deviations of the sites' slopes from the common slope were not all zero, meaning that some slopes were unequal. Then a nonparallel lines model with separate intercepts and slopes for the groups was fitted (equation 3):

$$F^{-1}(p) = probit(p) = \alpha + \gamma i + \boldsymbol{x}'\beta + \boldsymbol{x}'\delta i = \alpha + \gamma i + \boldsymbol{x}'(\beta + \delta i) \quad (3)$$

where α is the overall intercept, γi is the parameter for the ith site representing the deviation of the site i intercept from the overall intercept, β is the common slope on X δ_i is the deviation of the site i slope from the common slope, β.

The nonlinear mixed model procedure (PROC NLMIXED) in SAS 9.3 (SAS Institute 1999) was used to fit the non-parallel lines model (equation 3). Zero was used as the initial parameter for each variable estimate, similar to (Allison 1999). To compare the differences among the parameters estimated for each model the ESTIMATE statement was used. Contrasts comparing differences among sites and years were produced. This analysis was of particular importance to the study, because if there were no significant differences among years and sites then a general model, with the same parameters for every site, would be able to predict emergence for all sites and years.

3.4. Results

3.4.1. Temperature and Accumulation of Degree Days

Average daily temperatures in 2012 were higher than 2011 (Figure 3.2). Corresponding to the latitudinal gradient, average air temperatures for Cincinnati were the higher than the rest of the sites, and Midland had the lowest average air temperatures (Table 3.1). Columbus was the second warmest location, followed by Delaware, Wooster, and Toledo, OH (Figure 3.3, Table 3.1). The accumulation of degree patterns were correlated with the temperatures observed, and more degree days were accumulated in 2012 in all sites compared to 2011. Cincinnati, OH had the highest accumulation of degree days and Midland, MI the lowest. Columbus, OH had the second highest accumulation of degree days, followed by Toledo, Delaware and Wooster, OH.

3.4.2. Characterization of adult emergence phenology

In 2011 and 2012, emergence started earliest at the southernmost site in Cincinnati, OH (20 May, at 334 DD_{10} and 25 April, at 265 DD_{10}, respectively) and latest at the northernmost site in Midland, MI (17 June at 373 DD_{10} and 31 May at 367 DD_{10}, respectively) (Table 3.1). Adult emergence period was longer in Cincinnati, OH (80 days 2011 and 103 days 2012) than Midland, MI (44 days 2011 and 77 days 2012) (Table 3.1).

No clear pattern on the effect of latitude in the length, start and end of emergence period was observed for the rest of the sites. Emergence started in Delaware, OH in 2 June at 350 DD_{10}, Wooster, OH in 1 June at 341 DD_{10} and Toledo, OH in 2011 in May 30 at 221 $DD_{10.}$ End of emergence occurred in 7 August (at 1198 DD_{10}) in Wooster, OH, August 9 (at 1263 DD_{10}) in Delaware, OH, and 15 August in Toledo, OH (1510 DD_{10}). In 2011, the emergence period lasted 70 days in Delaware, OH, and 76 days in Wooster, OH and 77 days in Toledo, OH. In 2012, emergence started earlier in all sites compared to 2011, phenology was advanced 3 to 5 weeks in average across all sites. This earlier emergence phenology can be attributed to the warmer winter experienced in 2012 (Table 3.1).

Peak of emergence or 95 % of emergence occurred during the last week of June and the third week of July at 600 - 1000 DD_{10} in both years across all sites (excluding Delaware, OH 2012 and Midland, MI 2011). In 2011 peak of emergence was observed first in 26June (at 603 DD_{10}) in Wooster, OH and last in Toledo, OH and Delaware, OH in 11 July at 828 and 911 DD_{10} respectively. In 2012 95% of emergence occurred first in Columbus, OH in 18 June at 804 DD_{10} and last in Midland, MI in July at 946 DD_{10}.

Emergence finished at 1200 - 1500 degree days in most sites except Delaware, OH 2012 and Midland, MI 2011. End of emergence occurred the earliest in Columbus, OH in 15 July 2012 at 1253 DD_{10} and the latest in Delaware, OH in 10 September 2012 (at 1852 DD_{10}), these two locations also had the shortest and longest emergence period (66 and 138 days) respectively. Emergence in Cincinnati, OH ended 2 days earlier (6 August at 1508 DD_{10}) in 2012 than 2011 and 4 days earlier (11 August at 1496 DD_{10}) in Toledo, OH. Emergence in 2012 ended 20 days later in Wooster, OH (27 August at 1529 DD_{10}) and 13 days later in Midland, MI (10 August at 924 DD_{10}) compared to 2011 (Table 3.2).

Emerald ash borer emergence curve in all sites was characterized by a large peak of emergence that occurred during the first four to six weeks of emergence and accounted for up to 60% of the total emergence (Table 3.4). After this first large peak of emergence a smaller second peak of emergence accounting for 3-5% of the total emergence was observed, this second peak of emergence was observed in: Cincinnati, OH in 2011 and 2012, Columbus, OH 2012, Toledo, OH 2012, Wooster, OH 2011 and Midland, MI 2012. After the first peak and second peak (some locations) of emergence, the percentage of beetles emerged decreased significantly, especially during the final weeks of emergence where less than five beetles emerged on a single week.

3.4.3. Model Results

The probit link function provided the best overall fit for the model and therefore used for all the analyses. The models fitted individually for each site provide an accurate overall fit to the data (Tables 3.7 – 3.12) and all the adjusted R-squares were above 95% (Table 3.3). The fitted models provided accurate estimation of 10, 25, 50, 75, and 95% of emergence for all sites and years, where differences from observed emergence were in average 3- 4 days. The probit models fitted did not provide accurate estimations for first and end of emergence. Predicted fist emergence differed by ± 7 days and 50 GDD. Similarly, end of emergence differed by ± 7 days or in the most extreme case by 36 days in Wooster, OH 2012 (Table 3.10).

3.4.4. Parallel Line analysis

Comparison of differences of intercepts and slopes across sites and years, were found significant ($p<0.05$), meaning that parameters estimated for each site and year were significantly different from each other (Tables 3.4-3.6).

3.5. Discussion

3.5.1. Characterization of adult emergence phenology

The present study documents the effects of latitude and different climate regimes on EAB. Adult emergence phenology was accelerated in southern latitudes. Emergence started 3-5 weeks earlier in Cincinnati, OH than at the northern site in Midland, MI. The emergence period was also larger in southern latitudes, but no clear differences on the effect of latitude were observed for the rest of the locations (Toledo, Wooster, Delaware and Columbus, OH). Based on the emergence patterns observed it is possible that the actual dates for first emergence in Toledo, OH 2011, Midland, MI 2011 and Columbus, OH 2012 and Wooster, OH 2012 were not properly characterized. The number of insects observed for first emergence, in these four sites, is significantly higher compared to that observed in the rest of the sites.

The unusual length of emergence period observed in Delaware, OH in 2012, 138 days, can be attributed to a mistake on how the initial data was collected. This seems to be the case for the start of emergence, observed in 25 April at 182 DD base 10°C (328 DD base 50°F), represents an accumulation of degree days for first emergence lower than what was observed in the rest of the sites, as well as what has been reported in the literature (Cappaert et al 2005b and Brown-Rytlewsky and Wilson 2005). Then, it is possible that emergence in Delaware, OH in 2012 started later than what is being presented in the data, and the initial emergence holes observed in 25 April where old exit holes from the previous years.

Habitat differences from which the bolts where collected could have played an important role in the differences on the length of the emergence periods observed across sites. For example Columbus, OH 2012 bolts where obtained from street trees, this site had the shortest emergence period in 2012, and the highest accumulation of degree days, similar to the one observed in Cincinnati, OH, the southernmost site. It is possible that the microhabitat where the Columbus trees were growing was exposed to higher temperatures. Another factor that could have affected the phenology of the beetles was the level of infestation, even though the trees where selected based on their likelihood of being infested with EAB larvae, it is unknown what was the density and total number of larvae developing under the tree.

The first peak of emergence in the emergence curves of EAB (Figure 3.4) indicate that most EAB emergence occurs during the first four to six weeks from the start of emergence, indicating that diapause has a role in synchronizing EAB emergence, this results are similar to what has been observed in bronze birch borer (Herms and Muilenburg 2012). These results correlate with the percentages of diapause development observed in Chapter 2, where 60% of EAB larvae where able to complete their chilling requirements by mid-December, and are just waiting for favorable weather conditions to continue development (Denlinger 1972). This peak of emergence could also indicate that the larvae were closely related in age, meaning that the eggs that eventually developed into the adults observed were laid within a similar time frame.

It is unclear why the second smaller peak of emergence occurred, it seems unlikely that is just coincidence or a mistake on of how the data was collected (monitoring the logs on a weekly basis) since the same pattern was observed in different sites and years. Possibly the adults that emerged during this second peak needed a longer time to develop. These slow developers could be explained by differences in phenotypic plasticity among EAB larvae, probably some larvae might take a longer time to pupate than the rest. It is unlikely that the beetles that emerged during the smaller second peak are larvae that were overwintering from earlier instars, this would mean that EAB has a facultative diapause and that these larvae where able to develop into adults without going into diapause. Sober-Swant *et al.* (2012) and Bauer *et al.* (2004) have suggested that EAB has a facultative diapause, however, the question whether EAB has a facultative or not has not been addressed. To determine if EAB has a facultative diapause it would require rearing larvae without having the larvae enter into diapause, the authors are not aware of any method that successfully

3.5.2. Modeling emerald ash borer adult emergence

The probit regression models provided an accurate estimation of key phenological events, 10, 25, 50, 75 and 95% of emergence, which were accurately predicted within 3-4 days of the actual data. The poor prediction of first and last emergence can be explained by how degree days are accumulated, accumulation of degree days occurs faster as first emergence approaches, as temperatures get warmer; this rate of change in accumulation of degree days is larger than the slopes of the models fitted. Similarly as the end of emergence approaches, degree days are accumulated more rapidly.

Additionally, the poor prediction of first and last emergence by the models can be a problem on how the calculated probabilities are expressed (from zero to one). For example, Wooster, OH had the highest number of beetles that emerged in both years (619 in 2011 and 1221 in 2012), and observed emergence during the final weeks of emergence was often less than one or two beetles, less than 0.25% of the total emergence, and thus the statistical program rounded 0.999 to 1 when calculating the predicted probabilities.

Transforming degree days to a logarithmic scale helped to improve accuracy of first and end of emergence prediction. Niemczyk *et al.* (1992) did a similar logarithmic transformation to degree days and suggested that GDD accumulation has a Poisson distributed error (McCullagh and Nelder 1983). One way to address this problem of the end tails of emergence would be by using a different cumulative distribution function such as Weibull (Wagner *et al.* 1984).

In 2011, estimated slopes for all sites were steeper and estimated intercepts smaller compared with 2012, except for Toledo, OH. These lower intercepts and steeper slopes accounted the shorter emergence period observed in 2011. In contrast, the larger intercepts and smaller slopes in 2012 accounted for the more protracted emergence period. Toledo, OH had a lower intercept and a higher slope in 2012 compared with 2011 because the end of emergence occurred earlier in 2012 (4 days earlier) and less cumulative degree days were accumulated in 2012 (14 DD_{10} less).

There are elements of the proposed model that can be improved: the air temperatures of the stand (location of the trees where bolts were deployed) and the temperature under the bark were not estimated. There could be microhabitat differences in temperature compared with the weather stations used. Bolstad *et al.* (1997) found that the stand air and phloem temperatures experienced by the mountain pine beetle *(Dendroctonus ponderosae* Hopk.) were significantly different from the maximum and minimum air temperatures obtained from weather stations close to the stands.

Under the bark temperatures during winter can be warmer than air temperature, thus providing a buffer zone for insect cold tolerance. For instance, Vermunt *et al.* (2012b) found that ash trees minimum air and under-bark temperature can vary significantly, minimum under-bark temperatures can fluctuate by more than 4°C from minimum air temperatures on woodlot trees. Moreover, Vermunt *et al.* (2012a) tested a Newtonian cooling model to predict under the bark minimum temperatures in ash trees in Canada. Their model was able to correct variances of air temperatures within 1.31°C root mean squared error, compared with actual measurements of the temperature under the bark. The probit model presented in this study could be further improved and benefited from a similar Newtonian cooling model. The assumptions made by the degree day models in general are another limitation because it is well known that insect development is not linear (Logan *et al.* 1976, Sharpe and DeMichele 1977, Wagner *et al.* 1984a); thus the model would not make accurate predictions under temperature extremes.

Despite the limitations of the presented model, this study provides an accurate estimation of EAB emergence patterns during an entire season. Accuracy of EAB emergence model was comparable to the bronze birch borer model by Akers and Nielsen (1984). Fitting the model using 10°C as the base temperature and the same starting date 1 January for calculating degree days, provided similar results as Akers and Nielsen (1984) where they used the coefficient of variation method (Arnold 1959, Yang *et al.* 1995). Some advantages on using the same base temperature for all sites, instead of estimating a different base temperature for each site, is that it allows for easier comparisons of the model parameters among sites and years. Estimating the lower developmental threshold, LDT, from our field data is not possible. Estimating the actual LDT, would be experimentally difficult, it would require rearing EAB on laboratory conditions on an artificial diet and exposing it to different constant temperatures.

In summary, this study was designed to document the effect of different climate regions on EAB adult future emergence phenology and to develop a phenological model for predicting future emergence. We conclude that the predictive model presented in this study can be more precise and consistent to predict emergence than using the traditional 305 GDD ($Base_{10}$) scheduled first emergence; thus, the model can be further refined and calibrated using additional data sets and can the model can be used for time management decisions of EAB.

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ILLUSTRATIONS

Table 3.1 Average, average high, average low monthly temperatures for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH temperature available only for 2012

Site	Year	Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Cincinnati, OH	2011	Average High °C	2	8	12	20	23	28	33	31
		Average °C	-2	3	7	14	18	23	27	24
		Average Low °C	-7	-3	2	8	12	17	21	17
	2012	Average High °C	8	9	19	19	27	30	33	31
		Average °C	3	4	13	12	20	22	27	24
		Average Low °C	-3	-2	6	5	13	15	21	16
Columbus, OH	2012	Average High °C	5	8	18	18	28	30	33	31
		Average °C	1	3	12	12	21	23	27	24
		Average Low °C	-4	-2	6	5	14	16	21	17
Delaware, OH	2011	Average High °C	-2	2	8	16	22	27	31	27
		Average °C	-6	-2	3	11	17	22	25	22
		Average Low °C	-11	-7	-2	5	12	16	19	16
	2012	Average High °C	3	5	16	16	26	29	32	28
		Average °C	-1	1	10	10	19	22	25	21
		Average Low °C	-6	-4	4	3	12	14	18	14
Wooster, OH	2011	Average High °C	-2	3	8	16	22	26	31	28
		Average °C	-5	-1	3	10	16	21	24	21
		Average Low °C	-9	-6	-2	5	11	15	18	16
	2012	Average High °C	4	6	17	16	26	28	31	29
		Average °C	0	1	10	9	19	21	25	21
		Average Low °C	-4	-3	4	3	11	14	18	14
Toledo, OH	2011	Average High °C	-8	-12	1	7	11	22	29	24
		Average °C	-13	-17	-3	4	9	19	24	21
		Average Low °C	-18	-22	-7	-3	4	12	18	13
	2012	Average High °C	-7	1	-1	7	18	18	26	21
		Average °C	-12	-2	-3	4	12	14	22	18
		Average Low °C	-16	-8	-6	-1	5	9	14	12
Midland, MI	2011	Average High °C	-13	-8	-3	4	9	15	25	22
		Average °C	-17	-14	-8	2	7	13	19	18
		Average Low °C	-20	-20	-14	-3	3	8	13	11
	2012	Average High °C	-9	-6	15	7	23	27	22	18
		Average °C	-13	-11	9	4	17	21	19	17
		Average Low °C	-17	-16	3	-2	11	15	14	9

Table 3.2 Dates for first emergence, peak of emergence (95%), end of emergence, cumulative degree days using 10°C and 50°F number of beetles emerged and length of emergence of emerald ash borer (*Agrilus planipennis* Fairmaire).

Site	Coordinates	Year	First Emergence			Peak of Emergence			End of Emergence			Adults observed	Length of emergence (days)
			Date	DD 10°C	DD 50°F	Date	DD 10°C	DD 50°F	Date	DD 10°C	DD 50°F		
Cincinnati, OH	39°8'0"N, 84°21'29"W	2011	20-May	334	601	27-Jun	871	1568	8-Aug	1508	2714	80	80
		2012	25-Apr	265	477	5-Jul	1047	1885	6-Aug	1586	2855	567	103
Columbus, OH	40°0'41"N, 83°2'11"W	2011	--	--	--	--	--	--	--	--	--	--	--
		2012	10-May	365	657	18-Jun	804	1447	15-Jul	1253	2255	223	66
Delaware, OH	40°21'15"N, 83°3'52"W	2011	2-Jun	350	630	11-Jul	828	1490	9-Aug	1263	2273	32	70
		2012	25-Apr	182	328	27-Jul	1338	2408	10-Sep	1852	3334	323	138
Wooster, OH	40°46'45"N, 81°56'14"W	2011	1-Jun	341	614	26-Jun	603	1085	7-Aug	1198	2156	619	76
		2012	18-May	352	634	22-Jun	594	1069	26-Aug	1529	2752	1221	100
Toledo, OH	41°39'25"N, 83°33'9"W	2011	30-May	291	524	11-Jul	911	1640	15-Aug	1510	2718	472	77
		2012	12-May	287	517	22-Jun	738	1328	11-Aug	1496	2693	505	91
Midland, MI	43°37'28"N, 84°14'49"W	2011	17-Jun	372	670	1-Jul	519	934	29-Jul	924	1663	21	44
		2012	31-May	367	661	17-Jul	946	1703	10-Aug	1270	2286	120	71

Table 3.3 Parameter estimates for the probit regressions fitted to emerald ash borer emergence in 2011- 2012 for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH temperature available only for 2012

Site	Year	n	Parameter				
			Intercept	SE	AGDD	SE	R-Square
Cincinnati, OH	2011	220	-22.84	0.47	8.38	0.17	0.99
Cincinnati, OH	2012	219	-18.87	0.13	6.92	0.05	0.99
Delaware, OH	2011	221	-19.93	0.70	7.44	0.26	0.97
Delaware, OH	2012	274	-15.95	0.12	5.74	0.04	0.99
Columbus, OH	2012	197	-25.74	0.35	9.71	0.13	0.99
Wooster, OH	2011	219	-45.34	0.53	17.05	0.20	0.99
Wooster, OH	2012	239	-30.67	0.19	11.56	0.07	0.99
Toledo, OH	2011	227	-18.34	0.17	6.82	0.06	0.99
Toledo, OH	2012	224	-21.82	0.18	8.16	0.07	0.99
Midland, MI	2011	210	-36.56	2.07	13.85	0.78	0.98
Midland, MI	2012	223	-21.56	0.35	7.86	0.13	0.99

Table 3.4 Parallel line analysis of site and year estimates of probit regressions to model the proportion of emerald ash borer emergence (*Agrilus planipennis* Fairmaire) against accumulated degree days at six sites across a latitudinal gradient

Variable	Coefficient	SE	t Value	Pr > |t|
Site	-17.86	0.09	-198.90	<.0001
Year	-1.67	0.36	-11.93	<.0001

Table 3.5 Parallel line analysis of site slope parameters estimated by probit regression analyses to model the emergence phenology of emerald ash borer (*Agrilus planipennis* Fairmaire) six sites across a latitudinal gradient

Site	Cincinnati, OH	Columbus, OH	Delaware, OH	Wooster, OH	Toledo, OH	Midland, OH
Cincinnati, OH		2.06	-0.2356	5.8402	0.6771	1.9003
Columbus, OH	2.06		-3.12	-4.595	2.635	-1.2
Delaware, OH	-0.2356	-3.12		-0.2356	0.4415	1.6647
Wooster, OH	5.8402	-4.595	5.6046		5.1631	-3.9399
Toledo, OH	0.6771	2.635	0.4415	5.1631		0.6771
Midland, OH	1.9003	-1.2	1.6647	-3.9399	1.2232	

Table 3.6 Parallel line analysis of difference of slope estimates in 2011 and 2012 of probit regression analyses to model the emergence phenology of emerald ash borer (*Agrilus planipennis* Fairmaire) six sites across a latitudinal gradient

Site	Slope Difference	t Value	Pr > |t|
Cincinnati, OH	0.146	-9.120	<.0001
Delaware, OH	0.311	-20.100	<.0001
Wooster, OH	0.026	-2.600	0.0094
Toledo. OH	-0.058	5.930	<.0001
Midland, OH	0.368	-15.840	<.0001

Table 3.7 Comparison of observed cumulative degree days (Base$_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Cincinnati, OH 2011-2012

Year	Emergence Event		Cumulative GDD			Julian Date		
	Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
2011	First	6	334.72	280.43	54.29	140	132	8
	10	8	370.27	373.69	-3.42	143	144	-1
	25	20	463.04	441.53	21.52	151	150	1
	50	40	541.38	531.43	9.94	157	157	0
	75	60	641.10	639.65	1.45	164	164	0
	95	76	870.81	835.10	35.70	178	181	-3
	End	80	1508.32	1476.62	31.69	220	218	2
			Avg Diff/SE	21.60	19.04	Avg Diff/SE	1	3.21
2012	First	4	265.12	244.37	20.75	116	109	7
	10	57	385.38	346.35	39.03	130	127	3
	25	142	435.31	424.09	11.23	136	136	0
	50	284	502.55	531.02	-28.48	144	147	-3
	75	425	649.77	664.86	-15.08	158	160	-2
	95	539	1046.66	918.54	128.12	186	179	7
	End	567	1586.38	1491.66	94.72	219	213	6
			Avg Diff/SE	35.76	52.86	Avg Diff/SE	2.57	3.96

Table 3.8 Comparison of observed cumulative degree days (Base$_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Columbus, OH 2012

Emergence Event		Cumulative GDD			Julian Date		
Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
First	--	--	257.34	--	--	--	--
10	--	--	329.99	--	--	--	--
25	56	410.65	381.26	29.39	137	134	3
50	112	415.43	447.58	-32.15	138	141	-3
75	167	479.42	525.40	-45.99	144	147	-3
95	212	804.47	661.66	142.81	170	160	10
End	223	1253.21	1082.83	170.38	197	187	10
		Avg Diff/SE	52.89	88.83	Avg Diff/SE	3.40	5.82

Table 3.9 Comparison of observed cumulative degree days ($Base_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Delaware, OH 2011-2012

Year	Emergence Event		Cumulative GDD			Julian Date		
	Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
2011	First	3	349.91	230.79	119.12	151	143	8
	10	3	357.96	319.25	38.71	152	151	1
	25	8	433.25	385.42	47.83	159	156	3
	50	16	461.03	475.10	-14.07	159	163	-4
	75	24	488.53	585.58	-97.05	164	173	-9
	95	30.4	828.53	790.99	37.54	192	188	4
	End	32	1263.42	1237.85	25.56	221	219	2
			Avg Diff/SE	22.52	61.07	Avg Diff/SE	0.71	5.17
2012	First	3	182.47	235.47	-52.99	116	125	-9
	10	32	387.03	358.58	28.45	145	142	3
	25	81	512.12	457.73	54.39	157	150	7
	50	162	562.25	600.27	-38.02	162	166	-4
	75	242	691.90	787.10	-95.20	172	180	-8
	95	307	1337.81	1162.13	175.68	209	204	5
	End	323	1852.49	1811.30	41.19	274	263	11
			Avg Diff/SE	16.21	82.64	Avg Diff/SE	0.71	7.19

Table 3.10 Comparison of observed cumulative degree days (Base$_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Wooster, OH 2011-2012

Year	Emergence Event		Cumulative GDD			Julian Date		
	Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
2011	First	3	341.03	331.72	9.31	152	152	0
	10	62	402.52	382.12	20.39	158	156	2
	25	155	418.02	414.84	3.17	159	159	0
	50	310	457.47	454.49	2.98	162	162	0
	75	464	474.95	497.91	-22.96	165	168	-3
	95	588	603.64	567.75	35.90	177	173	4
	End	619	1198.39	841.34	357.04	219	196	23
			Avg Diff/SE	57.98	123.22	Avg Diff/SE	3.71	8.12
2012	First	--	--	281.7352777	--	139	--	--
	10	122	352.00	347.14	4.87	142	133	9
	25	305	375.85	391.87	-16.02	145	142	3
	50	611	419.76	448.36	-28.60	148	146	2
	75	916	500.14	512.96	-12.82	158	160	-2
	95	1160	594.76	622.53	-27.78	174	170	4
	End	1221	1528.75	1088.96	439.79	238	202	36
			Avg Diff/SE	59.91	170.25	Avg Diff/SE	8.67	12.64

Table 3.11 Comparison of observed cumulative degree days ($Base_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Toledo, OH 2011-2012

Year	Emergence Event		Cumulative GDD			Julian Date		
	Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
2011	First	--	--	221.46	--	--	143	--
	10	47	291.56	315.52	-23.96	150	152	-2
	25	118	392.40	387.49	4.91	157	157	0
	50	236	492.66	486.81	5.85	164	164	0
	75	354	577.95	611.51	-33.56	171	173	-2
	95	448	911.28	848.87	62.42	192	189	3
	End	472	1509.88	1383.78	126.10	227	218	9
			Avg Diff/SE	23.63	55.09	Avg Diff/SE	1.33	3.82
2012	First	30	286.66	243.42	43.24	133	126	7
	10	51	322.48	327.19	-4.70	139	140	-1
	25	126	403.59	388.48	15.12	146	145	1
	50	253	481.65	470.10	11.55	153	151	2
	75	379	543.72	568.81	-25.09	160	162	-2
	95	480	737.87	748.20	-10.33	174	175	-1
	End	505	1496.20	1343.90	152.30	224	213	11
			Avg Diff/SE	26.01	55.36	Avg Diff/SE	2.43	4.47

Table 3.12 Comparison of observed cumulative degree days (Base$_{10}$) and Julian Dates and predicted by probit regression of adult emergence of emerald ash borer (*Agrilus planipennis* Fairmaire) in Midland, MI 2011-2012

Year	Emergence Event		Cumulative GDD			Julian Date		
	Emergence %	Number Emerged	Observed	Predicted	Difference	Observed	Predicted	Difference
2011	First	--	--	294.89	--	166	159	7
	10	2	--	351.00	--	--	166	--
	25	5	--	388.37	--	--	170	--
	50	11	372.61	434.55	-61.94	168	173	-5
	75	16	450.39	486.21	-35.83	175	179	-4
	95	20	519.00	571.48	-52.48	182	186	-4
	End	21	924.27	807.09	117.18	210	202	8
			Avg Diff/SE	-8.27	73.03	Avg Diff/SE	0.40	5.82
2012	First	9	366.78	279.09	87.70	152	143	9
	10	12	400.72	379.41	21.31	158	155	3
	25	30	410.43	453.48	-43.04	159	162	-3
	50	60	496.19	552.80	-56.61	167	171	-4
	75	90	596.75	673.81	-77.06	174	181	-7
	95	114	945.89	895.75	50.14	198	196	2
	End	120	1270.33	1243.66	26.68	223	220	3
			Avg Diff/SE	1.30	56.51	Avg Diff/SE	0.43	5.01

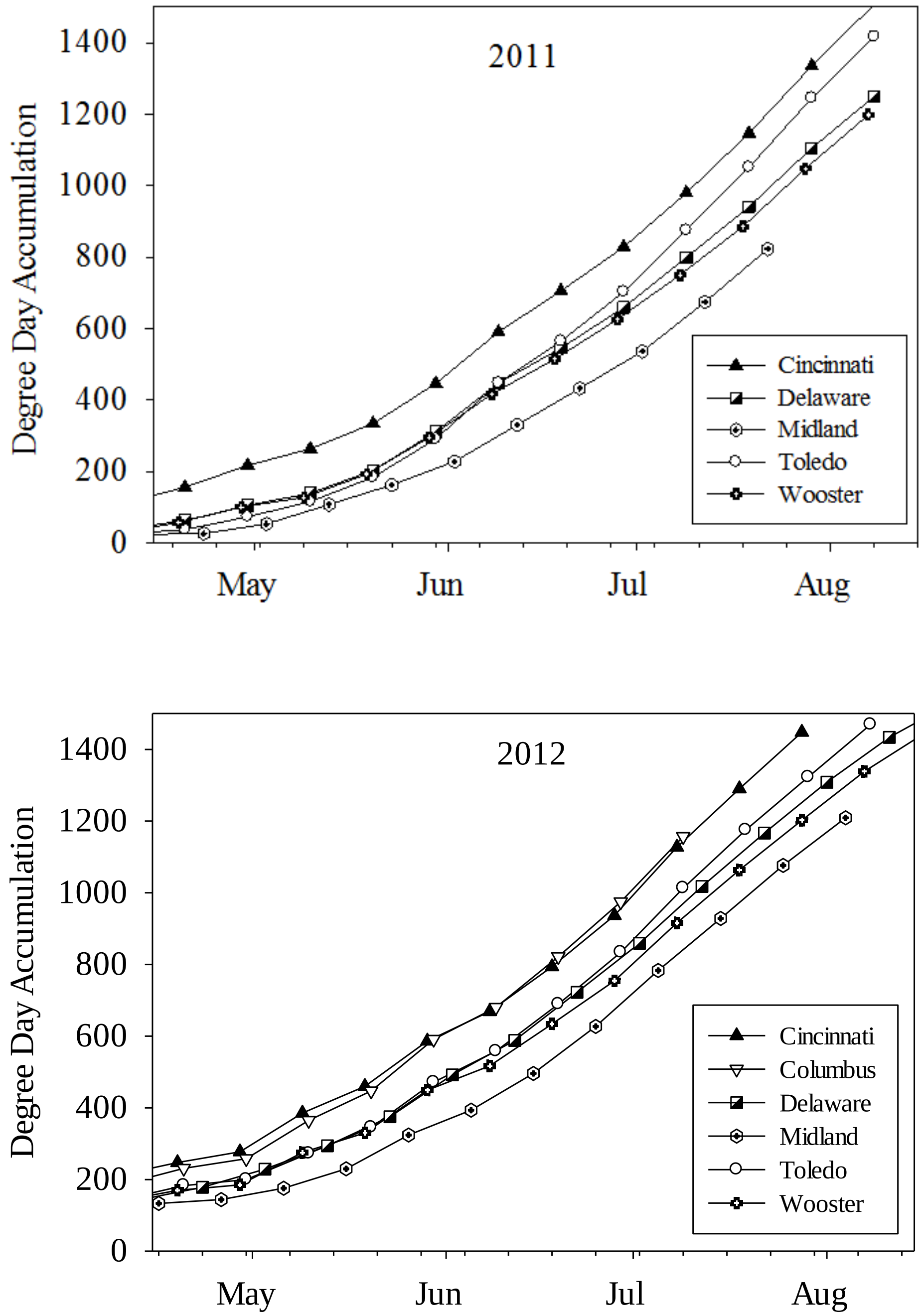


Figure 3.1. Growing degree day accumulation calculated using 1 January as starting date and 10°C base temperature for 2011-2012 across a latitudinal gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012.

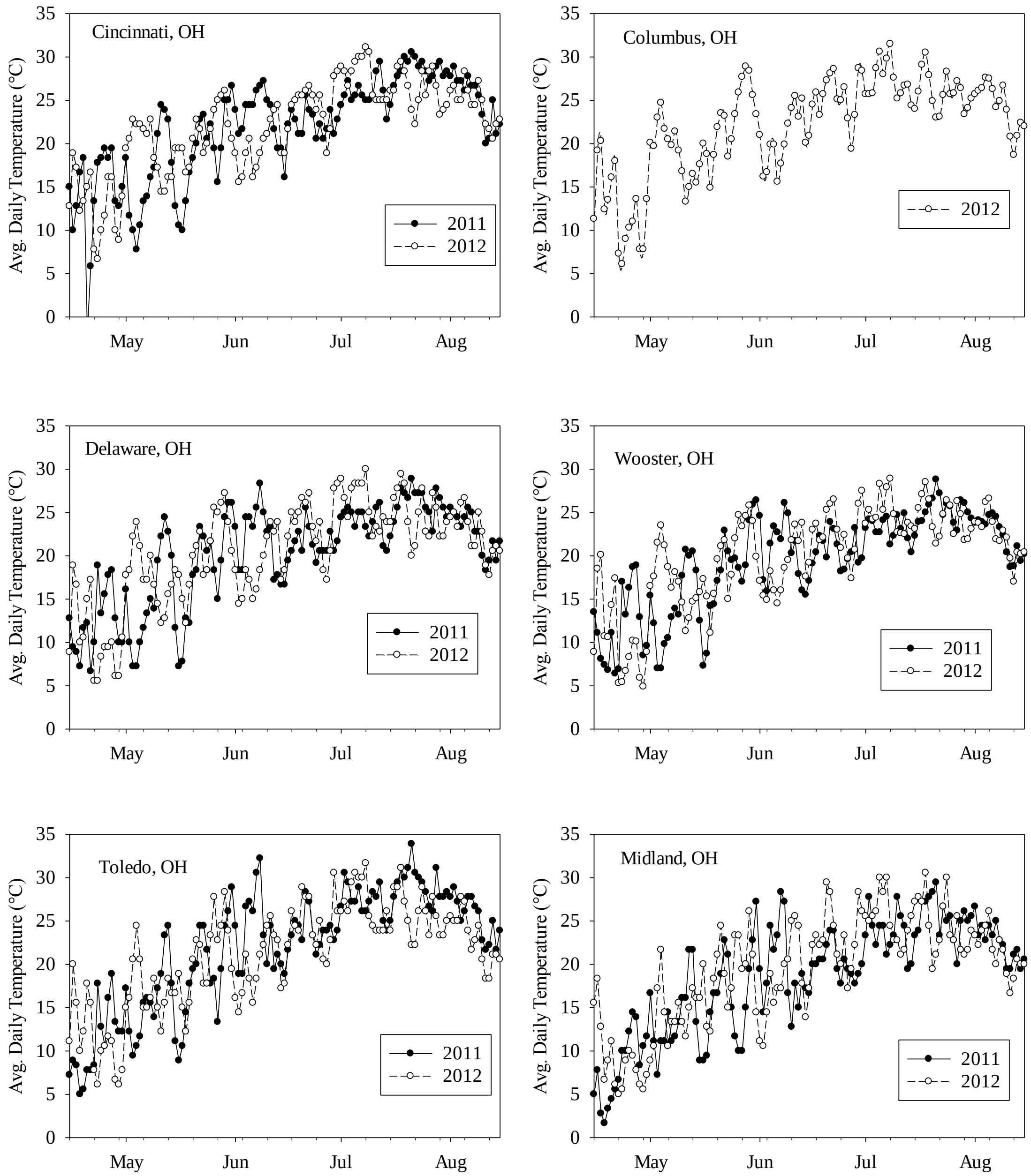
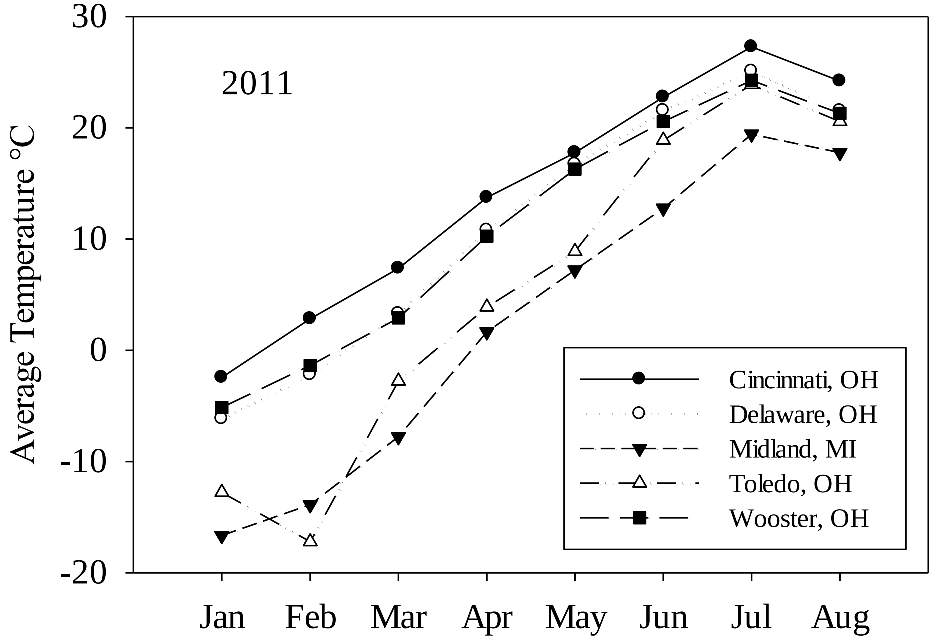


Figure 3.2. Average daily temperatures (°C) in 2011-2012 for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH temperature available only for 2012



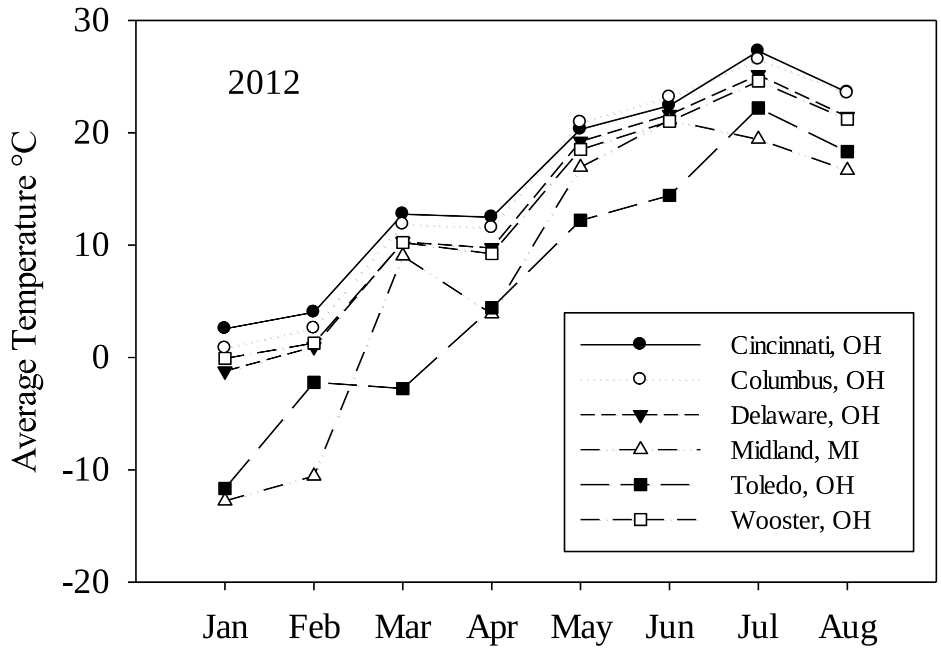
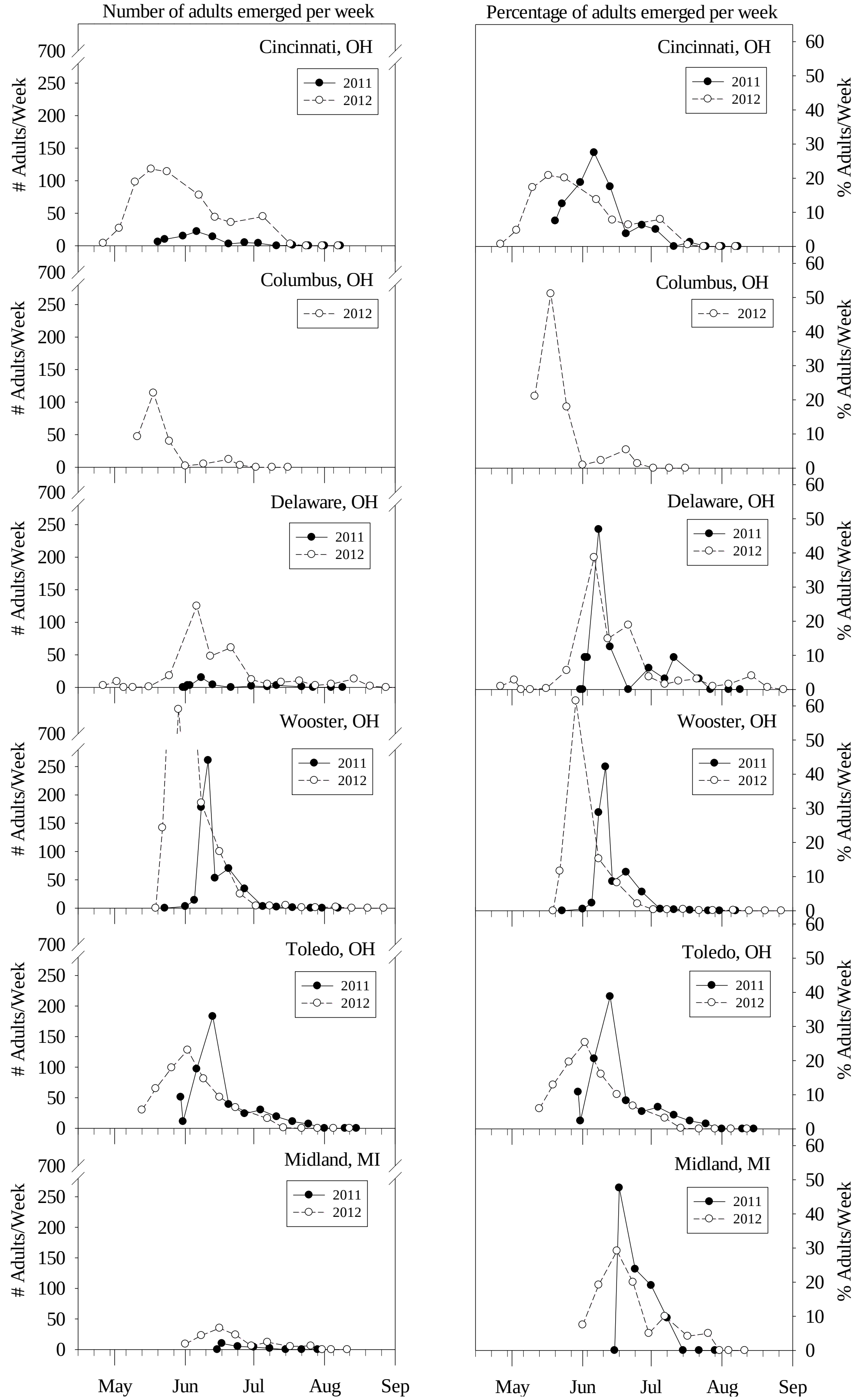


Figure 3.3. Monthly temperatures (°C) in 2011-2012 for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH temperature available only for 2012



Number of adults emerged per week
Percentage of adults emerged per week
Cincinnati, OH
Columbus, OH
Delaware, OH
Wooster, OH
Toledo, OH
Midland, MI
2011
2012
Adults/Week
% Adults/Week
May
Jun
Jul
Aug
Sep

Figure 3.4. Number of adults emerged per week and % of total emergence per week of emerald ash borer (*Agrilus planipennis* Fairmaire) for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012

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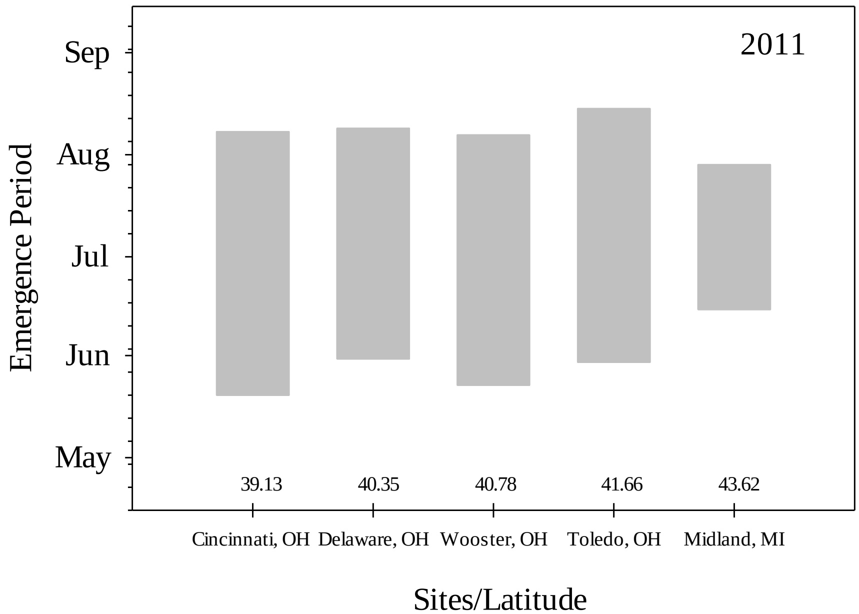


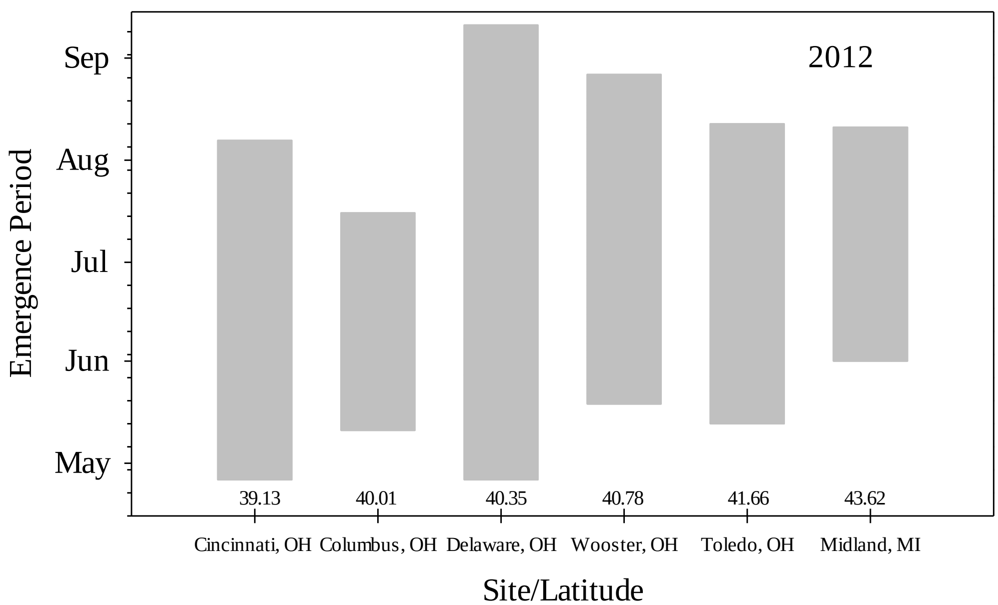


Figure 3.5. Length of emergence period (days) of emerald ash borer in 2011- 2012 for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012

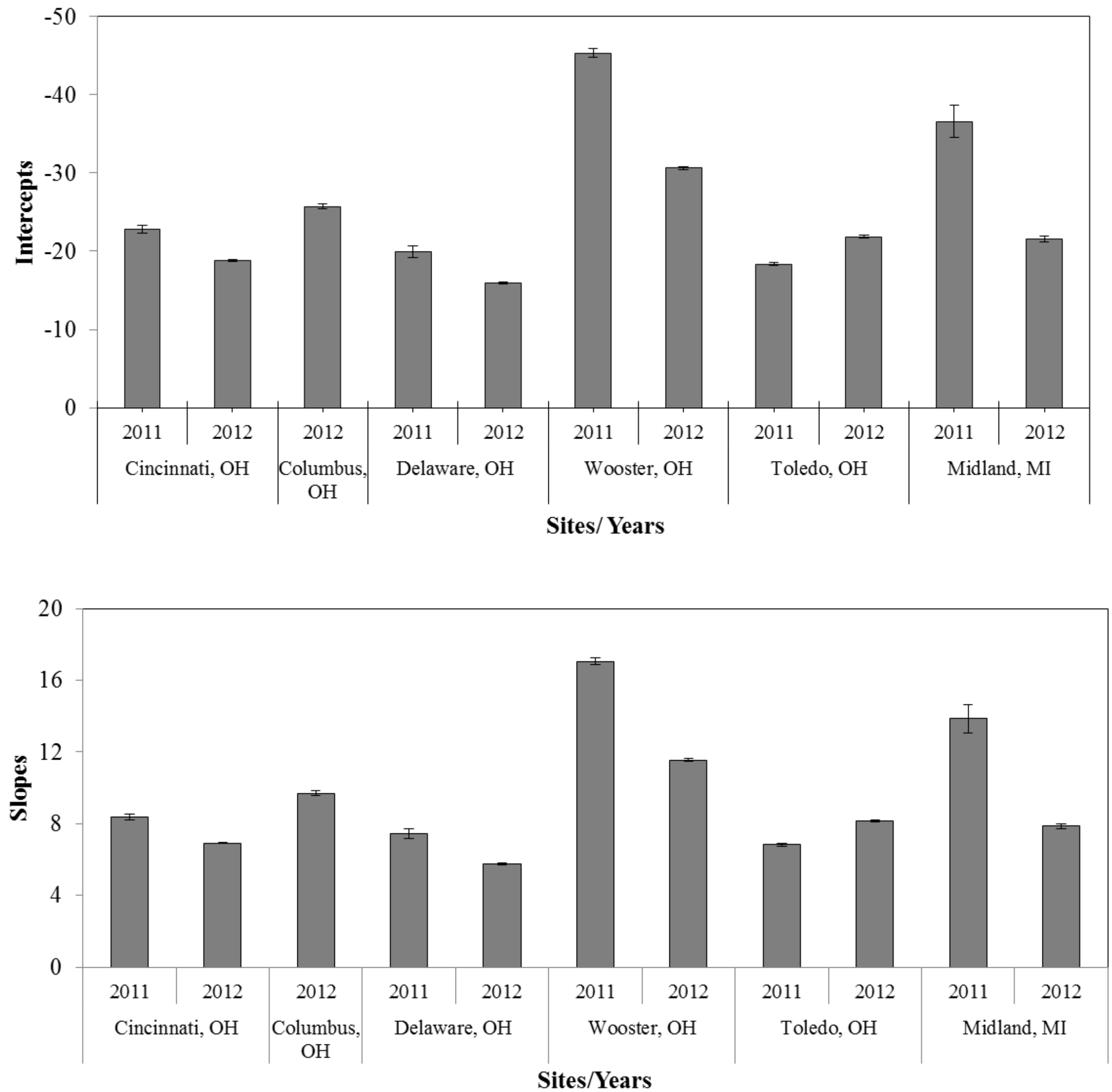
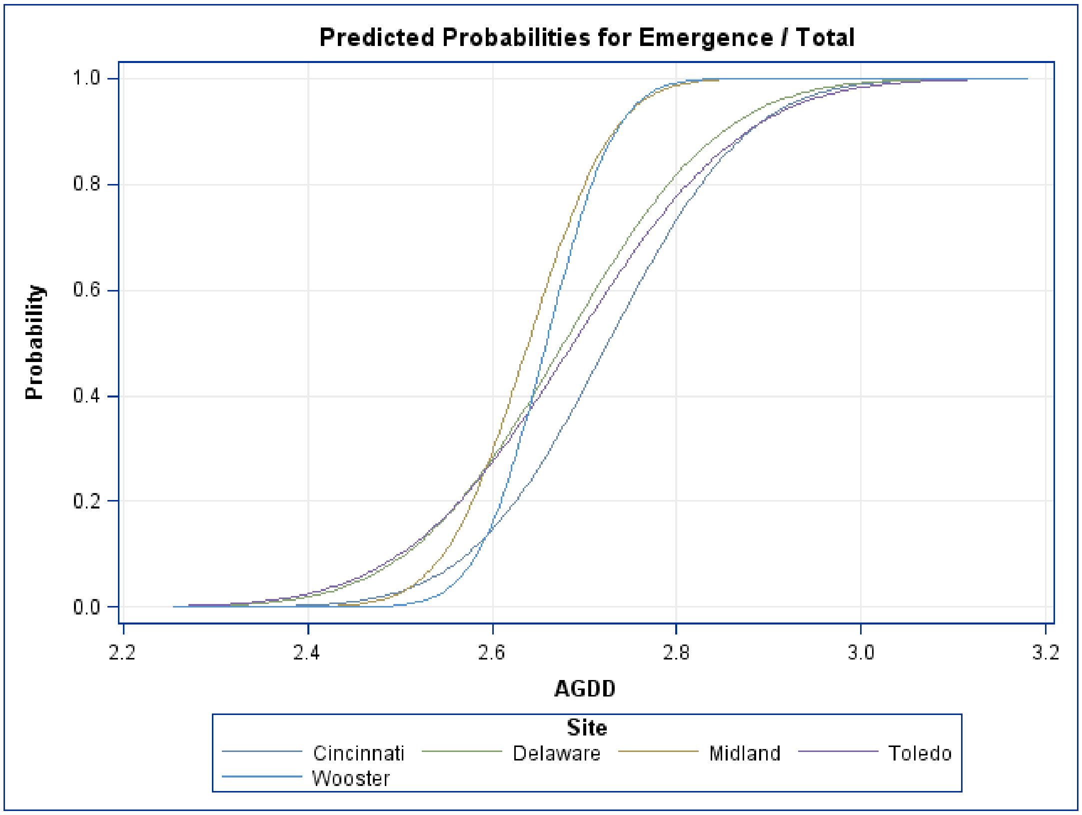


Figure 3.6. Slopes and intercepts estimated using probit regression to model the proportion of emerald ash borer emergence in 2011- 2012 for six sites across a south to north gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012


Predicted Probabilities for Emergence / Total
Probability
1.0
0.8
0.6
0.4
0.2
0.0
2.2
2.4
2.6
2.8
3.0
3.2
AGDD
Site
Cincinnati
Delaware
Midland
Toledo
Wooster

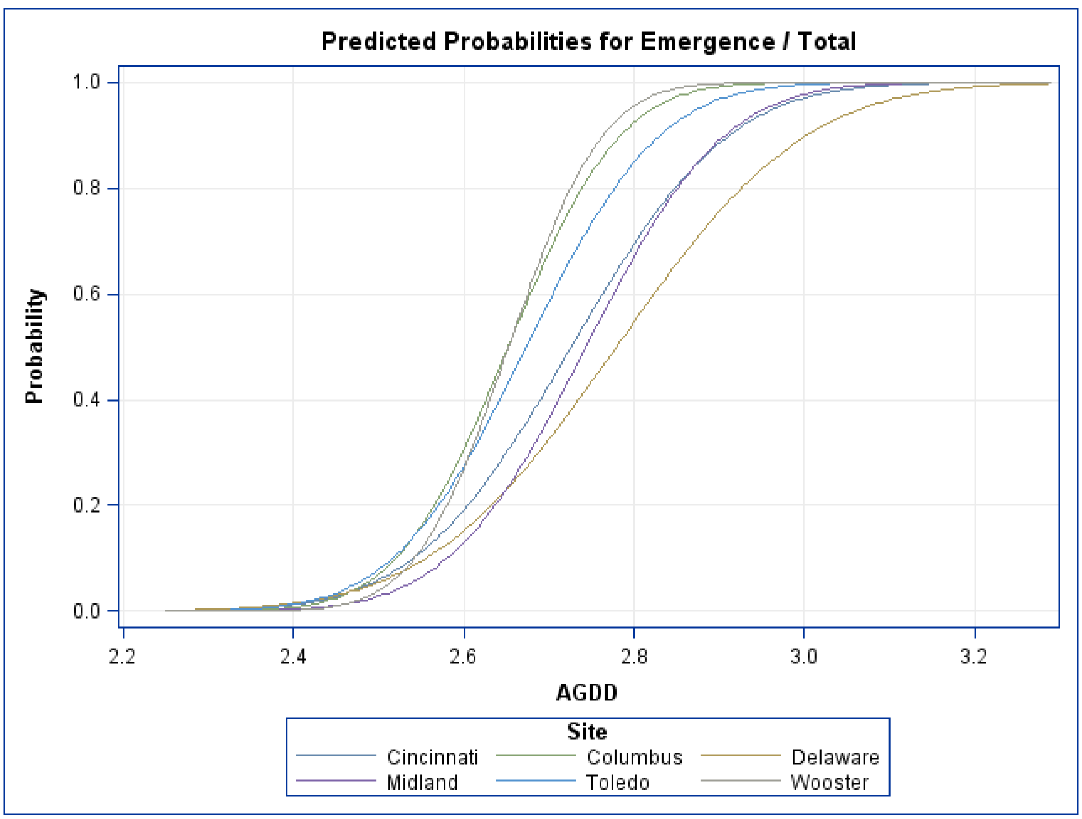

Predicted Probabilities for Emergence / Total
Probability
1.0
0.8
0.6
0.4
0.2
0.0
2.2
2.4
2.6
2.8
3.0
3.2
AGDD
Site
Cincinnati
Columbus
Delaware
Midland
Toledo
Wooster

Figure 3.7. Probit regressions of proportion of total emerald ash borer emergence (*Agrilus planipennis* Fairmaire) against accumulated degree days, AGDD ($Base_{10}$, $Log_{10}(n+1)$) across a north to south gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012.

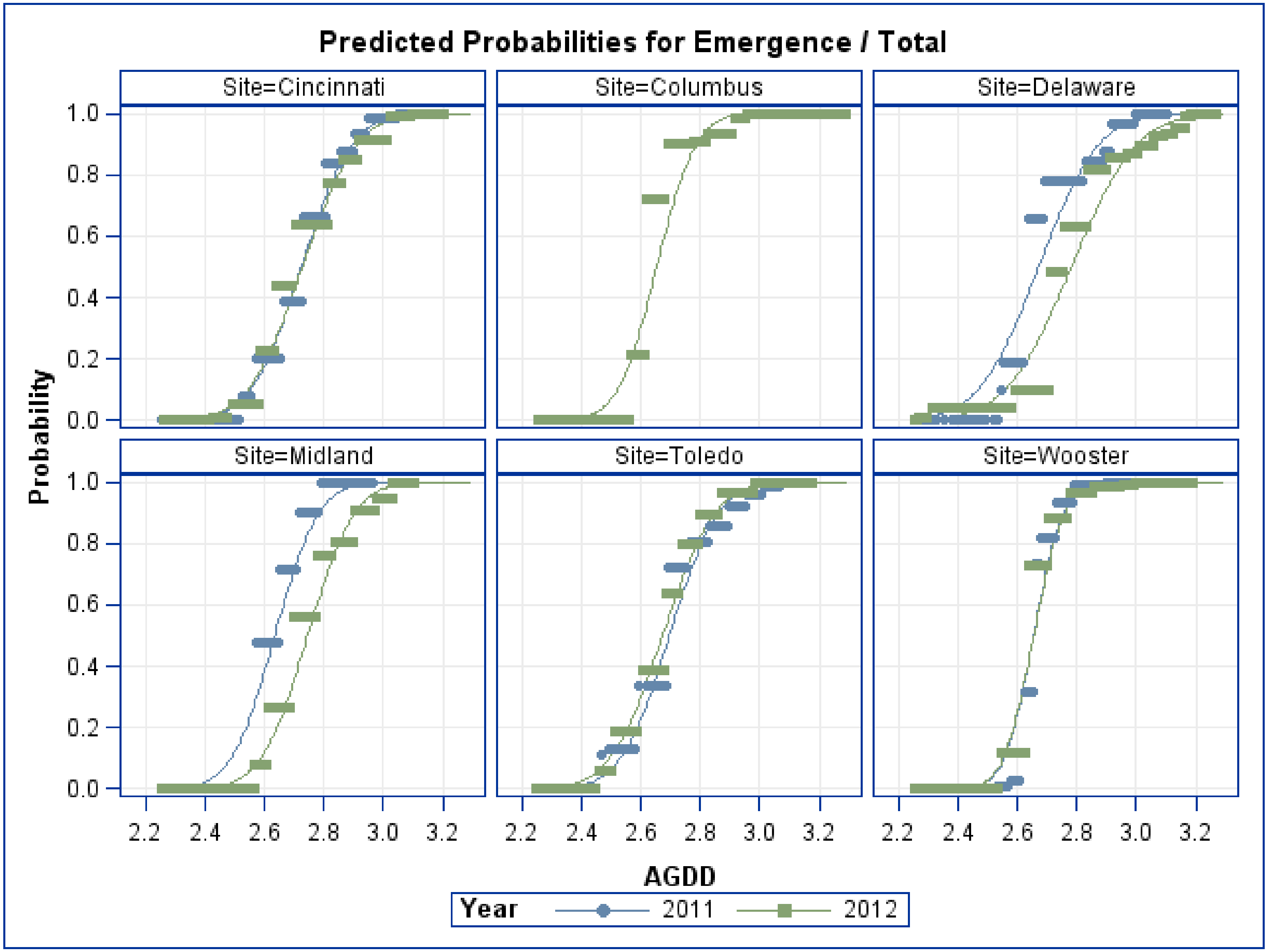

Predicted Probabilities for Emergence / Total
Site=Cincinnati
Site=Columbus
Site=Delaware
Site=Midland
Site=Toledo
Site=Wooster
Probability
0.0
0.2
0.4
0.6
0.8
1.0
2.2
2.4
2.6
2.8
3.0
3.2
AGDD
Year
2011
2012

Figure 3.8. Probit regressions fitted for individually for each site of proportion of total emerald ash borer emergence (*Agrilus planipennis* Fairmaire) against accumulated degree days, AGDD ($Base_{10}$, $Log_{10}(n+1)$) across a north to south gradient (500 km), Cincinnati, OH is the southernmost site and Midland, MI is the northernmost site. Columbus, OH data available only for 2012.

CHAPTER 4

OVERALL CONCLUSIONS AND FUTURE WORK

Conclusions

The present study documents the effects of temperature on diapause termination and the adult emergence phenology of emerald ash borer (EAB, *Agrilus planipennis* Fairmaire). These results are a significant contribution for understanding the seasonal phenology of EAB. Here is a summary of the key findings of this study:

Results from Chapter 2 provide evidence of the importance of temperature on diapause termination of EAB. The percentage of diapause development increased as the months advanced, the percentage of diapause termination increased from 7% in late October to 79% in mid-January. 60% of EAB larvae collected by the second week of December were able to break diapause, after being transferred to 18°C and 25°C constant temperatures, suggesting that the majority of EAB have completed their chilling requirements by mid-December. EAB larvae were able to break diapause after being transferred to 18°C and 25°C constant temperatures in all months they were collected them (from late October through late April). Furthermore, EAB pupa were found since late March, and all animals found by late April where pupae. These results also provide data for modeling purposes, it is possible to develop a time development model to model the proportion of insects in diapause by estimating some additional parameter to fit a rate equation to the data (Bentz *et al.* 1991).

Results from Chapter 2 provide insights on the EAB adult emergence patterns observed in Chapter 3. Diapause of EAB synchronizes its adult emergence phenology; this was observed in the adult emergence curves, where a peak of emergence accounting for up to 60% of the adult emergence occurred within the first four to six weeks for all sites.

In Chapter 3, the adult emergence phenology of EAB was characterized and using this emergence data a statistical model to predict future emergence was developed. EAB development is affected by latitude and temperature; in the latitudinal gradient used beetles emerged earlier in the southernmost site Cincinnati, OH, than in the northernmost site Midland, MI. Even though, Cincinnati and Midland are only 500 km apart, the emergence period was shorter by four to six weeks; and emergence started three to five weeks later in Midland, MI. There was not a clear pattern for the rest of the sites in the latitudinal gradient (Toledo, Wooster, Delaware, and Columbus OH); start and end of emergence varied in both years for these four sites, suggesting that the habitat where trees are growing, street trees or urban landscapes, affect the accumulation of degree days and the emergence patterns of EAB. The warmer temperatures in 2012 advanced EAB emergence and extended the length of the emergence period. The warmer year provided a longer season for larvae to develop and lay eggs thorough the summer.

The statistical model proposed in Chapter 3, provided an accurate representation of the EAB emergence period. Key phenological events, 10%, 25% 50%, 75% and 95%, were accurately predicted by the model. Prediction of the start and end of emergence events was less accurate; logarithmic transformation of the data improved accuracy of prediction, but the model can be further improved by changing the cumulative distribution function or validating the results using further data sets. Results from this model are comparable to those of a previous model developed for the closely related North American buprestid bronze birch borer (Akers and Nielsen 1984). Using the same starting date (1 January) and base temperature 10°C allows for easier comparisons across models, and did not affect the overall fit of the models. The parallel line analysis of the models fitted for each site showed that a general model cannot for be used for all sites, as slopes and intercept parameters estimated for all sites and years were different in both years and across sites.

The model proposed in Chapter 3 can be a valuable tool for predicting EAB adult emergence; the model accurately represents the adult emergence patterns of EAB. Therefore the model proposed provides a valuable tool for IPM and time management programs as well as a starting framework for simulating the adult emergence phenology of EAB.

Further research questions

This research leaves many questions unanswered about the effects of latitude on EAB development, voltinism and the simulation of its phenology. Further research is needed to understand the diapause of EAB, questions such as what other factors are affecting diapause termination? Is EAB diapause really facultative? Studying the hormonal regulation of diapause of EAB is another area of study worth pursuing, very little is known about the endocrine regulation of diapause in buprestids, similar studies to the preliminary study in Chapter 2 are necessary to understand the effects of hormones such as 20 hydroxyecdysone in diapause termination.

Studying the effect of latitudinal clines on EAB development is a fascinating question. In this study we documented the effects of latitude on adult phenology. However, this study can be further expanded to investigate the effects of latitude on the life history traits of EAB. Does latitude affect body size, fecundity or voltinism? It is very likely that EAB can only have one generation per year or every other year (univoltine or semivoltine). However, the extent to which EAB phenology is advanced, fecundity and body size are changed at lower latitudes are largely unknown. Also the question about what is the southernmost potential range of EAB in the US remains unanswered. EAB in China has a widespread distribution; it is also known that EAB in southern provinces (Laniujuan) of China have mostly an univoltine life cycle compared with northern regions that have a higher proportion of larvae showing a semivoltine life cycle (Liu *et al.* 2007).

Models that simulate EAB phenology and density distribution in each life stage can be developed, similar to models available to mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Bentz *et al.* 1991) and gypsy moth (*Lymantria dispar dispar*) (Gray 2004). The phenological model presented in this study can be utilized for making EAB population dynamics, time development rate and distribution models as well as increasing the accuracy vast number of EAB spread and dispersal models already available (BenDor and Metcalf 2006, Muirhead *et al.* 2006, Mercader *et al.* 2009, Sargent *et al.* 2010, Siegert *et al.* 2010).

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