EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON HOST-PARASITOID SYNCHRONY AND EVALUATION OF SAMPLING METHODS FOR *OOBIUS AGRILI*, AN INTRODUCED EGG PARASITOID OF EMERALD ASH BORER

By

Toby R. Petrice

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology—Doctor of Philosophy
Ecology, Evolutionary Biology and Behavior—Dual Major

2020

ABSTRACT

EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON HOST-PARASITOID SYNCHRONY AND EVALUATION OF SAMPLING METHODS FOR *OOBIUS AGRILI*, AN INTRODUCED EGG PARASITOID OF EMERALD ASH BORER

By

Toby R. Petrice

Emerald ash borer (EAB), *Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae) is one of the most destructive forest insect pests to be introduced into North America. *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) is an EAB egg parasitoid native to northeastern China. EAB has spread to most of eastern North America and *O. agrili* has been released throughout most of this distribution. Both species now occur well beyond their endemic climatic ranges. Furthermore, photoperiod modulates *O. agrili* diapause. Therefore, host-parasitoid synchrony could be affected in novel climatic distributions. Studies were conducted to determine the *O. agrili* life stages that respond to photoperiod and the critical day length for diapause induction. Results demonstrated that photoperiod induced diapause is modulated in the developing larvae and critical day length for diapause induction is between 14.25 and 14.5 hours of day light. Next. a temperature driven multiple cohort rate summation model was developed to simulate the phenology of *O. agrili* and EAB. Critical day length was integrated into the model to predict interactions of photoperiod and temperature regimes on host-parasitoid synchrony. The model was validated with *O agrili* and EAB trapping from Michigan sites. Model predictions compared with trapping data demonstrated that *O. agrili* has primarily two generations per year in south central and northwestern Michigan, and *O. agrili* enters diapause before critical day length occurs in south central Michigan. According to simulations, spatiotemporal variation in temperature regimes does not affect *O. agrili*-EAB synchrony, with the exception of some northern locations. However, the effect of spatial variation in day length

is still unclear and dependent on how *O. agrili* measures day length in the field. Finally, sampling methods, sample size and seasonal timing for detecting and monitoring *O. agrili* in the field were evaluated. Yellow pan traps and bark sifting for parasitized eggs were more effective at recovering *O. agrili* compared to sentinel EAB eggs in screened pouches and bark rearing for adults. A minimum of ten yellow pan trap or bark sifting samples should be taken from each site. Yellow pan trap sampling should be conducted between 400–1200 DD_{10}. Results of this project provide insight and tools for evaluating *O. agrili* phenology and spatiotemporal synchrony with EAB oviposition, determining optimal release times, and detecting and monitoring its efficacy across its current and potential distribution in North America.

Dedicated to my father, Bill Petrice, who taught me success cannot be had without hard work and perseverance.

ACKNOWLEDGEMENTS

First, I must express my gratitude to my USDA Forest Service supervisor, Therese Poland, and my previous supervisor, Robert Haack, for their inspiration, guidance, and flexibility that allowed me to pursue this degree. I would like to thank my advisor, Bill Ravlin, who has not only been a tremendous mentor and become a close friend, but has also gone beyond his responsibilities to provide resources and support that this work could not have been completed without. I thank my graduate committee, including Deb McCullough, Dave Smitley and Ken Raffa. for their guidance and input for this research, and especially Leah Bauer, who sparked my passion for EAB biocontrol and provided continuous encouragement throughout my studies. I would like to thank Debbie Miller for her research guidance and advice that she shared from the years of experience she accumulated while working with EAB biocontrol. I owe gratitude to all of the MSU student employees that helped complete the many tedious and unpleasant tasks that this project required over the last several years. I also thank John Stanovick for statistical advice and David Gray for guidance on phenology model development. Thanks to USDA Forest Service, Northern Research Station and Forest Health Protection, and Michigan State University for funding this work. Finally, I would like to thank my family, especially my wife Lauret, who endured countless days of my physical absence and even more of my mental absence while completing this endeavor.

TABLE OF CONTENTS

LIST OF TABLES viii

LIST OF FIGURES xi

KEY TO ABBREVIATIONS iv

CHAPTER 1: INTRODUCTION 1
EAB history and management 1
EAB classical biological control 3
Oobius agrili biology and knowledge gaps 5

CHAPTER 2: PHOTOPERIODIC MODULATION OF DIAPAUSE IN *OOBIUS AGRILI* ZHANG AND HUANG (HYMENOPTERA: ENCYRTIDAE) 10
Abstract 10
Introduction 11
Materials and Methods 15
Effects of maternal and progeny photoperiod exposures and maternal age on F_0-diapaused adults and their progeny. 18
Effects of grand-maternal, maternal, and progeny photoperiod exposures and maternal age on F_1-nondiapaused adults and their progeny. 19
Critical day length for diapause induction in *O. agrili*. 21
Critical age of developing parasitoids for photoperiod induced diapause in progeny of F_1-nondiapaused adults. 21
Effects of photoperiod and chill duration on diapause termination on *O. agrili* mature larvae. 22
Effects of photoperiod on diapause termination in diapausing *O. agrili* larvae not subjected to chill. 23
Comparison of day length and average degree day accumulation at *O. agrili* release sites. 24
Results 31
Effects of maternal and progeny photoperiod exposures and maternal age on F_0-diapaused adults and their progeny. 31
Effects of grand-maternal, maternal, and progeny photoperiod exposures and maternal age on F_1-diapaused adults and their progeny. 34
Critical day length for diapause induction in *O. agrili*. 38
Critical age of developing parasitoids for photoperiod induced diapause in progeny of F_1-nondiapaused adults. 40
Effects of photoperiod and chill duration on diapause termination on *O. agrili*. 42
Effects of photoperiod on diapause termination in diapausing *O. agrili* not subjected to chill. 43
Comparison of day length and average degree day accumulation at *O. agrili* release sites. 45

Discussion 46
Conclusion 54

CHAPTER 3: EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON *OOBIUS AGRILI* POPULATION DYNAMICS AND SYNCHRONY WITH EMERALD ASH BORER OVIPOSITION 55
Abstract 55
Introduction 56
Materials and Methods 60
Temperature assays for determining development times. 60
Rearing chambers 64
Developmental rate functions 65
Phenology model description and simulations 72
EAB and *O. agrili* field data 73
Comparison of simulations with field data. 75
Results 75
Model validation. 75
Spatiotemporal comparison of simulations 82
Discussion 84
Model evaluation 84
Spatiotemporal comparisons 87
Conclusion 93

CHAPTER 4: EVALUATION OF SAMPLING METHODS, INTENSITY, AND TIMING FOR RECOVERING AND MONITORING *OOBIUS AGRILI* IN THE FIELD. 95
Abstract 95
Introduction 96
Materials and methods 101
Sampling methods 101
Study sites 105
2016 Sampling 106
2017 and 2018 Sampling 107
Data analyses 108
Results 110
Comparison of sample methods 110
Estimation of sample size 114
Sample timing 122
Discussion 125
Conclusion 131

CHAPTER 5: CONCLUSION 133

REFERENCES 139

LIST OF TABLES

Table 2.1. Summary of experiments and treatments conducted to evaluate the effects of photoperiod and chill duration on diapause of *O. agrili*. All experiments were conducted in environmental chambers set at 25 ± 1 °C constant temperature and ~70% relative humidity. 25

Table 2.2. Statistical results for experiments examining the effects of photoperiod and chill on *O. agrili* diapause induction and termination, and parasitism. 28

Table 2.3. Least squared mean (LSM ± SE) percent of larval progeny entering diapause (percent diapause) from F_0-diapaused adults that were held at one of three photoperiods, and their progeny transferred to one of three photoperiods within 24hr of oviposition (Experiment 2.1). Data were combined for all progeny produced by F_0-diapaused adults when they were presented EAB eggs at 1, 2 and 3wk of age. Overall LSMs with the same letter were not significantly different ($P < 0.05$; Tukey's HSD; 16L:8D not included in the statistical analyses because a perfect correlation caused model separation). 32

Table 2.4. Least squared mean (LSM ± SE) percent of progeny that entered diapause (percent diapause) that were produced by F_0-diapaused adults that were 1wk, 2wk, or 3wk old when presented EAB eggs (Experiment 2.1). Within 24hr after oviposition, parasitoid progeny were transferred to 8L:16D or 12L:12D photoperiod treatments (16L:8D egg treatments were excluded because no progeny entered F_0-diapaused for this treatment). Data were combined for three different maternal photoperiod treatments to which F_0-diapaused adults were exposed (8L:16D, 12L:12D, and 16L:8D). LSMs for overall percent diapause of progeny among ages of F_0-diapaused adults when exposed to EAB eggs with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). 33

Table 2.5. Least squared mean (LSM ± SE) percent successful parasitism (i.e., progeny emerged or entered diapause) for *Oobius agrili* F_0-diapaused adults by photoperiod treatment and adult age (Experiment 2.1). EAB eggs were pooled from three progeny photoperiod treatments (8L:16D, 12L:12D, and 16L:8D). LSMs for overall percent parasitism with the same letter were not significantly different ($P < 0.05$; Tukey's HSD) among F_0-diapaused adult ages when exposed to EAB eggs. 34

Table 2.6. Least squared mean (LSM ± SE) for percent of progeny that entered diapaused (percent diapause) from *Oobius agrili* F_1-nondiapaused adults that were maintained for two generations at one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments (Experiment 2.2). Progeny of the F_1-nondiapaused adults were transferred to one of three photoperiod treatments within 24hr of oviposition for development to complete. Data were combined for all progeny produced by F_1-nondiapaused adults when they were presented EAB

eggs at 1, 2 and 3wk of age. LSMs with the same letter (16L:8D progeny treatment only) were not significantly different ($P < 0.05$; Tukey's HSD). 36

Table 2.7. Least squared mean (LSM ± SE) percent of progeny that entered diapause (percent diapause) from *Oobius agrili* F_1-nondiapaused adults that were exposed to one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments and presented EAB eggs when F_1-nondiapaused adults were 1, 2, and 3 wk old (Experiment 2.2). Eggs were transferred to 16L:8D photoperiod 24 hr after oviposition (progeny transferred to 8L:16D photoperiod were not included because 100% entered diapause; see Table 2.6). Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). 37

Table 2.8. Least squared mean (LSM ± SE) percent successful parasitism (i.e., progeny emerged or entered diapause) of EAB eggs by *Oobius agrili* F_1-nondiapaused adults that were exposed to one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments and were presented eggs when F_1-nondiapaused adults were 1, 2, and 3 wk old (Experiment 2.2). Data were pooled for 8L:16D and 16L:8D progeny treatments. Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). 38

Table 2.9. For five different locations where *Oobius agrili* has been released in the U.S., the mean calendar day for a 38-yr period (1978-2016) estimating the beginning of EAB adult emergence ($DD_{10} = 250$) and peak abundance ($DD_{10} = 560$), and hours and minutes of daylight on this date [1]Hours and minutes of daylight for each calendar day were obtained from the following website: http://aa.usno.navy.mil/data/docs/Dur_OneYear.php)..... 46

Table 3.1. Mean temperatures, rates, number of individuals, and replicates for *O. agrili* from diapausing larvae to adult, *O. agrili* from parasitoid egg to adult, and EAB from diapausing larvae to adult reared in growth chambers. 63

Table 3.2. Mean temperatures, oviposition longevity rates, eggs per female, and number of individuals and replicates for female EAB reared in growth chambers. 64

Table 3.3. Parameter estimates and adjusted R^2 values for development rates and cumulative probability for *O. agrili* and EAB adult emergence. 66

Table 3.4. Parameter estimates and adjusted R^2 for EAB oviposition..... 70

Table 3.5. Julian day comparisons of model predictions and trap captures for the beginning of *O. agrili* and EAB adult emergence with the first individuals of each captured in traps, 95% *O. agrili* F_1 emergence with the peak trap capture, 95% EAB adult emergence with the peak trap capture, and model prediction of 95% of EAB oviposition being completed and the last female captured in traps for three years at three sites in south central Michigan (Gratiot-Saginaw, Ithaca, MI; Harris Nature Center and Legg Park, Okemos, MI) and one site northwestern Michigan (Eastport, MI). Trapping was not done in Eastport in 2017 or 2018. 77

Table 4. 1. Data collected from Michigan study sites (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) in 2016 including: dominant ash species, mean tree diameter, site was lowland or upland, year that EAB was detected in the county in which site was located, year *Oobius agrili* was released, mean percent of canopy dieback of ash trees, percent of trees with fresh woodpecker feeding holes, and total EAB captured in green funnel traps (three traps at each site)..... 112

Table 4. 2. Total number of *O. agrili* recoveries and positive trees for four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; Sentinel eggs = sentinel EAB eggs; yellow pan traps) at four sites (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) for three years. Eastport was only sampled in 2016. Number of trees sampled at each site was 40 in 2016 and 10 in 2017 and 2018..... 113

Table 4. 3. Percent probability of *O. agrili* recover and 95% confidence interval as a function of number of trees that were bootstrap sampled from data that included all trees or only trees with fresh woodpecker feeding holes within plots for four different sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). 116

Table 4. 4. Correlation matrix showing p-value (*P*) and correlation coefficient (R) of the relationship between signs or symptoms of EAB attack (woodpecks = fresh woodpecker feeding holes; epicormics shoots; exits = EAB exit holes; EAB eggs= total number of EAB eggs collected from bark samples) and recoveries of *O. agrili* adults or EAB eggs (parasitized and total) using four different sampling methods (bark rearing= bark rearing for adult *O. agrili*; bark sifting = bark sifting for parasitized EAB eggs; sentinel eggs = sentinel EAB egg screened envelopes; and yellow pan traps)..... 121

Table 4. 5. Information provided, site visits required, and relative labor and technology required for each of four *O. agrili* sampling method. 125

LIST OF FIGURES

Figure 2.1. Graphical depiction of the estimated annual phenology of *O. agrili* generations and their synchrony with emerald ash borer oviposition. *Oobius agrili* synchrony with emerald ash borer oviposition has not been confirmed in the field. 16

Figure 2.2. Least-squared mean (LSM ± SE) percent of *O. agrili* progeny entering diapause (percent diapause) produced by F_1- and F_2-nondiapaused adults and allowed to develop under different photoperiods (Experiment 2.3). Means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). ^{1}N = total number of successfully parasitized EAB eggs (i.e., emerged as adults or became diapausing mature larvae). 40

Figure 2.3. Mean (± SE) percent of individuals that entered diapause (percent diapause) when developing parasitoids were transferred from 16:8 to 8:16 or 14:10 photoperiod at different progeny developmental ages (Experiment 2.4). Developmental ages with the same letter within each photoperiod transfer treatment are not significantly different ($P < 0.05$; Wald's χ^2 test; Table 2.2). ^{1}N = number of diapausing mature larvae that were transferred. 41

Figure 2.4. Least-squared mean (LSM ± SE) degree days base 10°C (DD_{10}) required for *O. agrili* to develop from diapausing mature larvae to adult emergence when exposed to different photoperiods at 25 ± 1°C after exposure to different chill periods at 4 ± 1°C (Experiment 2.5). LSMs with the same letter are not significantly different ($P < 0.05$; Tukey's HSD). ^{1}N = number of adults that developed and emerged from diapausing mature larvae. 43

Figure 2.5. Mean (± SE) percent of *O. agrili* that developed to adults from mature larvae that were in photoperiod induced diapause and were not subjected to a chill period before they were transferred to photoperiods with longer day lengths (Experiment 2.6). Means with the same letter were not significantly different ($P < 0.05$; Wald X^2 test). ^{1}N = number of diapausing mature larvae that were transferred and either developed and emerged as adults or remained as diapausing mature larvae. 44

Figure 3.1. Functions fitted to observed development rates (1/days) and normalized cumulative development for F_0 *O. agrili* (A. and B.), F_1 *O. agrili* (C. and D.), and EAB development (E. and F.) to adult. 67

Figure 3.2. Functions fitted to predicted EAB oviposition longevity rate (1/days); A.), cumulative oviposition (B.), EAB fecundity (C.), and cumulative oviposition survival (D.). 71

Figure 3.3. Map of eastern U.S. showing sites where *O. agrili* and EAB phenology were simulated and where adults of both species were trapped (red circles—Michigan only) for model validation. 74

Figure 3.4. Percent incidence of predicted adult emergence of F_0, F_1, and F_2 *O. agrili,* cumulative percentage of EAB oviposition, and *O. agrili* adults captured by Julian day over three years for one site in northwestern Michigan [Eastport (A = 2016, B = 2017, C = 2018)], and three sites in south central Michigan [Gratiot-Saginaw (D=2016, E = 2017, and F = 2018); Legg Park (G. = 2016, H = 2017, I = 2018); and Harris Nature Center (J = 2016, K = 2017, and L = 2018). N = the total number of *O. agrili* captured in traps for each site and year. See Fig. 2.3 for location of sites. 78

Figure 3.5. Percent incidence of predicted EAB adult emergence, cumulative percentage of EAB oviposition, and EAB adults captured in funnel traps by Julian day over three years for one site in northwestern Michigan [Eastport (A = 2016, B = 2017, C = 2018)], and three sites in south central Michigan [Gratiot-Saginaw (D=2016, E = 2017, and F = 2018); Legg Park (G. = 2016, H = 2017, I = 2018); and Harris Nature Center (J = 2016, K = 2017, and L = 2018). N = the total number of EAB captured in traps for each site and year. See Fig. 2.3 for location of sites. 80

Figure 3.6. Percent incidence of predicted adult emergence of F_0, F_1, and F_2 *O. agrili,* and cumulative percentage of predicted EAB oviposition for three years at four locations along a north south gradient where *O. agrili* has been released: Duluth, Minnesota (A = 2016, B = 2017, C = 2018); Indianapolis, Indiana (D=2016, E = 2017, and F = 2018); Knoxville, Tennessee(G. = 2016, H = 2017, I = 2018); and Shreveport, Louisiana (J = 2016, K = 2017, and L = 2018). See Fig. 2.3 for location of sites. 83

Figure 4. 1. Photos of different methods for sampling *O. agrili*: A) yellow pan trap; B) EAB eggs in screened envelopes (sentinel eggs); C) bark sheering (Photo by D.M. Miller); D.) sifting sheered bark for EAB eggs (bark sifting); sheered bark in rearing container (bark rearing). 105

Figure 4. 2. Mean number of recoveries (top row) and mean percentage of positive trees (bottom row) for *O. agrili* by sampling method (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps) for three sample years. Means with different letters within each year and row are significantly different ($P \leq 0.05$; Tukey-Kramer means separation). 114

Figure 4. 3. Four-parameter Weibull probability function fitted to percentage of bootstrapping iterations with *O. agrili* recoveries for samples of 1–40 trees at four different sites [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI, 3) Harris Nature Center and 4) Legg Park: Okemos, MI] for each of four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all trees sampled at each site and right column includes only trees that had fresh woodpecker feeding holes. 117

Figure 4. 4. Coefficient of variation of mean *O. agrili* recoveries for bootstrapped samples of 1-40 trees each iterated 2000 times for 4 sites in Michigan [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI, 3) Harris Nature Center and 4) Legg Park: Okemos, MI] and for 4 different sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for

parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all 40 trees within sample plots at each site and right column includes only trees within each plot that had fresh woodpecker feeding holes. CVs are only shown for sites and methods that recovered *O. agrili* (see Fig. 4.3 and Table 4.1). 119

Figure 4. 5. Cumulative percent capture of *O. agrili* adults in yellow pan traps by growing degree days (base =10°C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a three-year period. Samples collected once every two weeks in 2016 and once every week in 2017 and 2018. Eastport was only sampled in 2016. 123

Figure 4. 6. Cumulative percent parasitism of sentinel eggs by growing degree days (base =10°C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a two-year period. Samples collected once every two weeks in 2016 and once every week in 2017. Eastport was only sampled in 201 124

KEY TO ABBREVIATIONS

Bark rearing – rearing adult *O. agrili* from bark sheered from ash trees

Bark sifting – examining for parasitized eggs in sifted bark that was sheered from ash trees

EAB – emerald ash borer

EAB Biocontrol Guidelines – Emerald Ash Borer Biological Control Release and Recovery Guidelines

Eastport – Eastport, MI study site

Gratiot-Saginaw – Gratiot –Saginaw study site (Ithaca, MI)

Harris Nature Center – Harris Nature Center study site (Okemos, MI)

Legg Park - Legg Park study site (Okemos, MI)

R^2 – adjusted R-squared value

Sentinel eggs –emerald ash borer eggs on filter papers that were placed in screened envelopes

CHAPTER 1: INTRODUCTION

EAB history and management

Emerald ash borer (EAB), *Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae), is one of the most destructive invasive forest insect pests to be introduced into North America. Since its introduction in the early 1990's (Siegert et al., 2014), and subsequent detection in 2002 near Detroit, Michigan and Windsor, Ontario (Haack et al., 2002), EAB has spread to most eastern U.S. states and Canadian provinces (Emerald Ash Borer Information Network, 2020). EAB has killed hundreds of millions of ash (*Fraxinus* spp.) trees and threatens to functionally extirpate many of the native ash species in North America. EAB continues to spread and its North American range appears to be limited primarily by the distribution of ash trees (Sobek-Swant et al., 2012a, 2012b).

Soon after the discovery of EAB in North America, resources and research were directed toward detection and eradication of EAB populations. However, eradication was quickly abandoned after the extent of EAB's distribution was realized. Eradication failure was also attributed to lack of sensitive detection methods and limited effective treatment tools; both of these are crucial for successful eradication of exotic forest pests (Liebhold et al., 2016; Tobin et al., 2014). Efforts then shifted to slowing EAB spread and long-term management.

Research on insecticides for EAB management has primarily focused on systemic compounds, given that EAB spends much of its life in the cambial region of trees, limiting its exposure to topical insecticides. Among the products tested, emamectin benzoate applied via trunk injection was very effective for protecting ash trees and allowing them to recover from EAB damage if they were treated before extensive damage had occurred (Bick et al., 2018; McCullough et al., 2019, 2011; Smitley et al., 2010). A single treatment application of

emamectin benzoate provides trees with three years of nearly complete EAB protection and emamectin benzoate treatments have conserved thousands of ash trees that would have otherwise perished. However, emamectin benzoate treatments often are not practical in forested or other natural systems due to the logistical challenge of accessing all trees, costs and labor associated with treatments, and hazards of applying insecticides in sensitive areas such as wetlands and waterways. Use of lethal trap trees may mitigate some of these constraints (McCullough et al., 2016). Lethal trap trees are created by treating trees with emamectin benzoate and then girdling the stem to attract EAB adults that perish when feeding on treated foliage, or alternatively their larval progeny die as they tunnel in treated cambial tissue (McCullough et al., 2016).

Sl.ow A.sh M.ortality (SLAM) is a landscape-scale EAB management strategy, first initiated as a pilot project in 2010 in Michigan (Poland and McCullough, 2010). The SLAM objective is to slow EAB population growth and ash mortality, thus prolonging ash ecosystem services and allowing managers additional time to develop and implement long term management plans for ash resources. SLAM tactics are site-specific and may include: selective removal of infested ash trees, using girdled trees as population sinks that are subsequently destroyed, and emamectin benzoate treatments (McCullough et al., 2019, 2011; Poland and McCullough, 2010). Model simulations suggest that ash mortality and EAB population growth can be reduced with SLAM (McCullough et al., 2016, 2015; McCullough and Mercader, 2012; Mercader et al., 2011). Also, incorporating lethal trap trees as a tactic may improve SLAM efficacy.

Tree resistance to EAB attack is another management approach that is currently being explored. Scions from green ash (*F. pennsylvanica* Marshall) and white (*F. americana* L.) ash tree genotypes surviving in areas of high EAB-caused ash mortality are collected and grafted

onto ash root stock, and once they become sapling-size, are evaluated for resistance to EAB. Greenhouse trials have found reduced EAB leaf feeding and higher EAB larval mortalities on some of these genotypes compared to susceptible genotypes (Koch et al., 2015). In addition, North American and Asian species of ash are being crossed in search of EAB-resistant ash varieties (Koch et al., 2011). It will require many years of testing to determine if these genotypes possess, and ontogenetically retain, strong enough resistance to tolerate EAB-attack.

Classical biological control is another landscape scale management strategy for EAB and is the only self-sustaining strategy that is currently being implemented. Foreign exploration of EAB natural enemies began in 2003 after preliminary surveys found very low attack rates on EAB from North American natural enemies, with the exception of woodpeckers (Cappaert et al., 2005; Liu et al., 2003). Releases of EAB parasitoids began in the U.S. in 2007 and in Canada in 2014 (Bauer et al., 2015a). To date, four Asian parasitoid species have been released in North America (MapBioControl, 2020). Three species are well established and show promise for reducing EAB's impact on ash trees (Bauer et al., 2015a; Duan et al., 2018; Kashian et al., 2018; Margulies et al., 2017).

EAB classical biological control

Soon after EAB was discovered in North America, surveys for North American natural enemies identified several hymenopteran parasitoid species attacking EAB larvae (Liu et al., 2003). However, overall parasitism rates were very low. Among the most common included a complex of *Atanycolus* (Hymenoptera: Braconidae) species and the recently described species, *A. cappaerti*, was the most prevalent of this complex with EAB larval parasitism as high as 71% at one site (Cappaert and McCullough, 2009; Marsh et al., 2009) However, parasitism rates by *A. cappaerti* vary considerably among study sites and sample years (Cappaert and McCullough,

2009; Duan et al., 2015, 2010). Other hymenopteran species that frequently attack EAB in North America include *Phasgonophora sulcata* Westwood (Chalcididae), a native larval parasitoid of several North American *Agrilus* species; and *Balcha indica* Mani and Kaul (Eupelmidae), an adventive exotic larval parasitoid of woodborers that has been established in the U.S. for several decades (Liu et al., 2003). Although there are several North American egg parasitoids that attack native *Agrilus* spp., none have been recovered from EAB eggs (Triapitsyn 2015; Taylor 2012).

In anticipation of long-term needs for EAB management due to lack of innate tree resistance to EAB and limited control from North American natural enemies, exploration for EAB natural enemies in Asia began in 2003 (Liu et al., 2003). Three hymenopteran species were identified from northeastern China as classical biological control candidates: *Oobius agrili* Zhang and Huang (Encyrtidae), an egg parasitoid*; Spathius agrili* Yang (Braconidae), a larval ectoparasitoid*;* and *Tetrastichus planipennisi* Yang (Eulophidae), a larval endoparasitoid. (Liu et al. 2003, 2007, Zhang et al. 2005, Yang et al. 2006) After host specificity testing, applications were submitted to NAPPO (North American Plant Protection Organization) for release of these species in the U.S. (Lelito et al. 2013). A FONSI (Finding of No Significant Impact) was issued in 2007 by USDA APHIS PPQ (U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine) after a) the proposed releases were posted on the federal register and public comments were considered, b) a cost risk benefit analysis was conducted, and c) concurrence was obtained from individual states (Bauer et al., 2015a; Duan et al., 2018; Federal Register, 2007). Small scale releases of these three species occurred during 2007–2008, and in 2009 a formal EAB classical biological program was initiated, including the development of a parasitoid rearing facility in Brighton, MI (Bauer et al., 2015a). A third larval parasitoid, *S. galinae* Belokobylskij & Strazanac was collected in South Korea in 2008 and

Russian Far East in 2009 (Belokobylskij et al., 2012), and approved for release in the U.S. 2015 (Federal Register, 2015). In 2011, a new EAB egg parasitoid species, *O. primorskyensis* Yao & Duan, was discovered in the Russian Far East (Yao et al., 2016) but has not been approved for release to date (Duan et al., 2018).

Tetrastichus planipennisi is established in several states and provinces and has proven very effective at protecting ash saplings from EAB mortality (Duan et al., 2017); however, its short ovipositor limits its effectiveness at protecting larger diameter ash trees (Abell et al., 2012). *Oobius agrili* has been recovered in 14 U.S. states and 3 Canadian provinces, and Abell et al. (2014) reported parasitism rates as high as 40% at original release sites in Michigan. *Spathius galinae* is established in three U.S. states and parasitism rates as high as 49 % have been reported from Connecticut (Duan et al., 2019) and 23 % in Michigan (Pers. observation); however, data on the impact of this parasitoid on EAB because of its relatively recent introduction in 2015. To date, establishment of *S. agrili* has not been confirmed despite multiple releases in several U.S. states.

Oobius agrili biology and knowledge gaps

Oobius agrili was formally described in 2005 from specimens collected near Beijing, China in 2003 and Changchun, Jilin Province, China in 2004 (Zhang et al., 2005). Wang *et al.*, (2016) also collected individuals from Liaoning Province and Heilongjiang Provinces in China . The original description of *O. agrili* included males but this remains the only documented report of male *O. agrili* (Zhang et al., 2005). All *O. agrili* populations in North America are thelytokous parthenogenetic, meaning that females produce diploid females without mating. The *O. agrili* being released in North America are genetically identical, originating from Changchun, Jilin Province, China where they parasitize up to 32–42% of EAB eggs (Liu et al. 2007). Almost

two million *O. agrili* have been released in North America, and as of 2019, it has been recovered at release sites in 13 U.S. states and 2 Canadian provinces (MapBioControl, 2020). Parasitism rates as high as 40% have been reported at some of the earliest release sites in Michigan (Abell et al., 2014). To date, releases have been made as far north as Quebec, south to Louisiana, east to Maryland and west to Colorado (MapBioControl, 2020). This is well beyond the known climatic distribution of *O. agrili* in China (Wang et al., 2015).

Oobius agrili is a solitary egg parasitoid that overwinters as a mature diapausing larva inside the EAB egg. After the pupation is complete in the spring or summer, adults chew a round hole on EAB egg surface to exit through (Abell et al., 2016; Liu et al., 2007; Wang et al., 2010). *Oobius agrili* can parasitize EAB eggs the same day they eclose (Pers. observation), and live for several weeks in the laboratory (Hoban et al. 2016, Larson and Duan 2016). Larson and Duan (2016) determined *O. agrili* mean lifetime fecundity was 52–71 eggs depending on diapause history of the mothers. EAB eggs parasitized by *O. agrili* usually darken and eventually become almost black; however, some parasitized eggs will remain light brown. Other signs and symptoms of *O. agrili* parasitism include: swollen appearance, distorted aeropyles on the outer surface, and protruding parasitoid egg stalks. Development from parasitoid egg to emerged adult requires approximately 21 days at 24°C in the laboratory (Bauer et al., 2015b).

Diapause of *O. agrili* can be induced by rearing individuals under short day photoperiod (8L:16D) (Hoban et al. 2016, Larson and Duan 2016, Petrice et al. 2019). Diapause history determines if *O. agrili* adults are one of two phenotypes: 1) adults that developed from diapausing larvae that had undergone a chill period (diapaused adults); and 2) adults that developed directly from larvae that did not diapause (nondiapaused adults). When diapaused adults and their progeny are held in long-day photoperiod (16L:8D), progeny do not (or rarely)

enter diapause and develop directly to adults. Under short-day photoperiod, a small percentage of diapaused-adult progeny enter diapause and the remainder develop directly to adults (Hoban et al., 2016; Larson and Duan, 2016; Petrice et al., 2019). In contrast, a small percentage of progeny produced by nondiapaused adults enter diapause when reared under long-day photoperiod (the modulation of this diapause is unclear), while the remaining individuals will emerge as adults. When reared under short-day photoperiod, 100% of the progeny of nondiapaused adults enter diapause. Although this *O. agrili* diapause pattern has been demonstrated is several studies, the critical day length [i.e., day length at which 50% of the population enters facultative diapause (Tauber et al., 1986)] has never been determined for *O. agrili*. Also, the life stage in which photoperiod modulates diapause is not known.

Oobius agrili is a multivoltine species and Yao et al. (2016) reared eleven consecutive nondiapaused *O. agrili* generations under long-day photoperiod in the laboratory. In Jilin Province, China, *O. agrili* has at least two generations per year. However, the number of *O. agrili* annual generations where it is established in North America is not known. It is conceivable that similar climates should effect *O. agrili* phenology similarly. However, given that *O. agrili*'s native range is much smaller compared to EAB (Chamorro et al., 2015; Wang et al., 2015), it is not known how host-parasitoid synchrony will be affected in novel climates. For example, Duan et al. (2014) found EAB can survive higher temperatures compared to *O. agrili* and anticipated that *O. agrili* may be less fit for more southern U.S. climates compared to EAB. Furthermore, day length varies significantly among latitudes which could affect *O. agrili* diapause patterns, depending on the critical day length that induces *O. agrili* diapause.

Detecting and monitoring *O. agrili* in the field is crucial for determining establishment and evaluating efficacy at different geographic locations. *Oobius agrili*'s small size (~ 1-mm

long) and EAB's habit of ovipositing eggs in cracks or between layers of bark (Liu et al., 2007; Wang et al., 2010) make recovery and monitoring challenging. Nevertheless, several methods have recovered *O. agrili* in the field. One method is yellow pan traps attached to ash trees to collect *O. agrili* adults (Parisio et al., 2017). Another method is visual inspection of tree bark for parasitized EAB eggs, or alternatively sheering off the outer layer of bark and sifting and sorting for EAB eggs in the laboratory (Abell et al., 2014; Jennings et al., 2018). Also, *O. agrili* has been reared in the laboratory from bark or cut logs collected from EAB-infested trees (Abell et al., 2014; Parisio et al., 2017). Finally, sentinel EAB eggs on small ash bolts or within screened containers have recovered *O. agrili* in the field (Abell et al., 2016; Duan et al., 2012; Jennings et al., 2014). Each sampling method provides different information regarding *O. agrili* presence and activity, and each has different technical and logistical requirements. Also, the appropriate sampling size for each method to confidently monitor *O. agrili* established has not been determined. Furthermore, sampling times for methods that target active *O. agrili* adults are unclear.

The objectives of this project are to address important knowledge gaps discussed above related to *O. agrili*'s establishment and efficacy in North America. The first objective is to determine the critical day length and life stage that responds to photoperiod for *O. agrili* diapause induction. The second objective is to evaluate the effect of different temperature regimes on synchrony, voltinism, and diapause patterns of *O. agrili* in respect to EAB oviposition. This will be accomplished by developing a multiple cohort temperature driven rate summation model simulating *O. agrili* and EAB development and validating the model using field collected data. Findings from the first objective will be integrated into the model to examine the interaction of photoperiod and temperature regime on host-parasitoid synchrony.

The third objective is to evaluate sampling methods and determine appropriate sample size and timing for detecting and monitoring *O. agrili* in the field. Results of this project will provide an understanding of how geographic location and temperature regime affect *O. agrili*-EAB synchrony. This research will also develop tools to predict *O. agrili*'s potential range and the optimal release times for each phenotype, and effective sampling protocols to monitor *O. agrili*'s establishment and efficacy.

CHAPTER 2: PHOTOPERIODIC MODULATION OF DIAPAUSE IN *OOBIUS AGRILI* ZHANG AND HUANG (HYMENOPTERA: ENCYRTIDAE)

Petrice et al. 2019. Biol. Control. 138: 1–13.

Abstract

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid from China being introduced into North America as a biological control agent of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an extremely invasive and destructive pest of ash trees (*Fraxinus* spp.). *Oobius agrili* has been released over a broad geographic range in North America and photoperiod varies considerably throughout this distribution during the season of parasitoid-host activity. Laboratory studies were conducted to determine 1) if photoperiod-induced diapause is modulated maternally, grand-maternally, or directly in immature parasitoids; 2) interactions of maternal age and photoperiod exposure on diapause induction; 3) the critical day length for diapause induction; 4) the critical age at which photoperiod-induced diapause is modulated in developing larvae; 5) the effects of photoperiod and length of chill on diapause termination; and, 6) the effects of photoperiod on *O. agrili* biology across a latitudinal gradient. Photoperiod exposure of *O. agrili* larvae developing inside host eggs directly induced diapause, and maternal or grand-maternal photoperiod treatments did not affect diapause induction in their progeny. Diapause response to photoperiod declined dramatically after larvae were 6–7 days old. The critical day length for diapause induction was between 14.25 and 14.5 hours of daylight (at 25°C). Photoperiod and duration of chill affected diapause termination of *O. agrili* mature larvae. The cumulative number of degree days (base 10°C) required for adult emergence was highest for the combination of 12 hours light :12 hours dark (12L:12D) photoperiod combined with the shortest chill period, and lowest for 14.5L:9.5D

and 16L:8D combined with the longest chill period. The effects of photoperiod on parasitoid-host synchrony, population dynamics, and fitness of *O. agrili* across the geographical area where it is being released are discussed.

Introduction

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), from northeastern China. Emerald ash borer was inadvertently introduced into North America in the early 1990s from China, and as of December 2018, has spread to 34 U.S. states and 5 Canadian provinces where it has killed hundreds of millions of ash trees (*Fraxinus* spp.) (Bray et al., 2011; Haack et al., 2002; Herms and McCullough, 2014; Siegert et al., 2014). EAB threatens many or all North American ash species, given that all North American ash species it has encountered to date are susceptible and ash tree mortality > 99% has been documented at some locations (Herms and McCullough, 2014; Klooster et al., 2014; Knight et al., 2013). To suppress EAB populations and reduce ash mortality, a classical biological control program was initiated in the U.S. in 2003, with approved pilot releases beginning in 2007, and mass rearing and large scale releases beginning in 2009 (Bauer et al., 2015a). In addition, Canada began releasing classical biological control agents of EAB in 2014 (Bauer et al., 2015a). *Oobius agrili* is among the parasitoid species approved for release, and as of 2019, almost 1.6 million *O. agrili* have been reared and shipped for release among 27 states and the District of Columbia in the U.S., and 5 provinces in Canada. To date, recovery of *O. agrili* has been confirmed at release sites in 13 U.S. states and 2 Canadian provinces (MapBioControl, 2020), while parasitism rates as high as 40% have been recorded where it was released in Michigan (Abell et al., 2014). *Oobius agrili* continues to be released in areas newly invaded by EAB in the U.S. and Canada.

Oobius agrili is a solitary, multivoltine parasitoid endemic to northeastern China (Bauer et al., 2015a; Liu et al., 2007; Wang et al., 2015; Zhang et al., 2005). The species reproduces asexually by thelytokous parthenogenesis, in which females produce female progeny from unfertilized eggs. Males were reported from populations in China (Zhang et al. 2005); however, they have not been documented in laboratory or field populations in North America to date. During the winter, *Oobius agrili* diapauses as a mature larva inside the EAB egg (Larson and Duan, 2016). Development is completed in the spring, and adult females emerge and are active in spring and summer during EAB oviposition (Abell et al., 2016; Liu et al., 2007; Wang et al., 2015). *Oobius agrili* can develop from egg to adult in approximately 320 degree days (base 10°C; DD_{10}), and at least 11 consecutive nondiapaused generations have been produced under a long day photoperiod [16 hours light: 8 hours dark (16L:8D)] in the laboratory (Yao et al., 2016). In its native range, at least two generations are predicted to occur during the period of EAB oviposition (Liu et al. 2007; Fig. 2.1).

Oobius agrili can be divided into two distinct phenotypes based on their diapause history: 1) adults that developed from diapausing *O. agrili* larvae that experienced a chill period (i.e., diapaused), and 2) adults that developed from larvae that did not enter diapause (i.e., nondiapaused). Overall, studies have found that most progeny produced by diapaused *O. agrili* adults did not enter diapause and developed directly to adults if they were exposed to long day photoperiod (16L:8D), while a proportion entered diapause as mature larvae when exposed to short day photoperiod (8L:16D) with this proportion increasing over time (Hoban et al., 2016; Larson and Duan, 2016). In contrast, a small percentage of progeny produced by nondiapaused *O. agrili* adults entered diapause as mature larvae when exposed to long day, while 100%

entered diapause as mature larvae when exposed to short day photoperiods (Hoban et al., 2016; Larson and Duan, 2016).

Although a diapause response to a short day photoperiod has been documented in laboratory studies for *O. agrili* (Hoban et al., 2016; Larson and Duan, 2016), the critical day length that induces diapause has not been determined. Tauber et al. (1986) defined "critical day length" for diapause induction as the length of daylight resulting in 50% of the insect population entering diapause and noted that the critical day length can vary considerably among insect species. For example, critical day lengths were found to be 14–15hr for *Ooencyrtus ennomophagus* Yoshimoto (Hymenoptera: Encyrtidae) (Anderson and Kaya, 1974), 14–16hr for *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae) (Jackson, 1963), and 12–16hr for *Trichogramma embryophagum* Htg. (Hymenoptera: Trichogrammatidae) (Reznik et al., 2011). The critical day length for egg parasitoids is influenced by the species' overwintering life stage, and the seasonal occurrence of it and its host. Critical day length is also a function of the geographical location of the parasitoid and host populations, considering that day length on a given calendar day varies along a latitudinal gradient. Therefore, critical day lengths may vary intraspecifically for photoperiod sensitive species that occur over broad latitudinal areas (Tauber et al., 1986).

Oobius agrili diapause as mature larvae within EAB eggs; however, the life stage(s) responsive to photoperiod has not been determined. The life stage that is sensitive to photoperiod and modulates diapause induction differs among egg parasitoid species (Beck, 1980; Boivin, 1994). For example, in many *Trichogramma* spp. the photoperiod response is maternally mediated, meaning the photoperiod experienced by ovipositing females modulates the production of diapausing progeny (Boivin, 1994; Pizzol and Pintureau, 2008). Furthermore, this

maternal effect can be conserved through multiple generations, such that diapausing progeny may be a product of their grand-maternal or even their great-grand-maternal photoperiod exposure (Reznik et al., 2012). In other egg parasitoids, such as *O. ennomophagus*, maternal photoperiod exposure also determines diapause of their progeny, but the window of sensitivity to photoperiod occurs when the mothers are pupae or pharate adults within the hosts' eggs (Anderson and Kaya, 1974). Photoperiod may also directly affect the life stage that undergoes diapause such as in larvae of *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae) (Jackson, 1963) and *T. embryophagum* (Reznik et al., 2011).

Photoperiod can also affect diapause termination in insects (Beck, 1980). In the laboratory, *O. agrili* diapause is terminated by exposing diapausing mature larvae to several months of chill (e.g., 4°C), and then exposing them to ≥25°C at a 16L:8D photoperiod (Pers. observation). However, it has not been determined if day length after chill (e.g., shorter day lengths) can affect diapause termination of *O. agrili*. Examples of photoperiod affecting diapause termination in egg parasitoids are rare in the literature. One example is the egg-larval parasitoid, *Holcothorax testaceipes* (Ratzeburg) (Hymenoptera: Encyrtidae), that was found to reach 50% of total emergence earlier when diapaused larvae were held at 16L:8D compared to 12L:12D photoperiod (Wang and Laing, 1989). Photoperiod can also interact with overwintering period (i.e., length of chill) to affect diapause termination in insects (Beck, 1980; Tauber et al., 1986).

Oobius agrili is currently being released over a broad geographical range in efforts to manage expanding EAB populations (Bauer et al., 2015a; Duan et al., 2018; USDA–APHIS/ARS/FS, 2019). Given that there is significant temporal photoperiod variation throughout this range during the ovipositional period of EAB, it is essential to determine the

critical day length for diapause induction, and possibly diapause termination, as well as the life stages that respond to photoperiod in order to adequately study the efficacy of *O. agrili* as a biological control agent of EAB. To determine how photoperiod modulates diapause induction and termination in *O. agrili*, laboratory studies were conducted in 2016–2017 with specific objectives to determine 1) if photoperiod induced diapause is modulated maternally, grand-maternally, or directly in immature parasitoids; 2) interactions of maternal age and photoperiod exposure on diapause induction; 3) the critical day length for diapause induction; 4) the critical age at which photoperiod induced diapause is modulated in developing larvae; 5) the effects of photoperiod and length of chill on diapause termination; and, 6) the effects of photoperiod on *O. agrili* biology and population dynamics across a latitudinal gradient. Information from this study can provide insight into the likely geographical range across which *O. agrili* can establish and will be integrated with temperature-based phenology models to predict the degree to which temporal synchrony may affect establishment success of *O. agrili* at different geographic locations.

Materials and Methods

Oobius agrili used in this study were from a colony of parthenogenetic females maintained for 36 generations at the USDA Forest Service Northern Research Station laboratory in East Lansing, Michigan. This colony originated from parasitized EAB eggs collected from infested ash (*Fraxinus* spp.) trees near Changchun, Jilin Province, China (43.8666 lat., 125.3500 long.) in 2004–2005 during foreign exploration for EAB biocontrol agents (Bauer et al., 2015a). Subcultures of *O. agrili* from this colony were provided to the USDA APHIS EAB Biocontrol Rearing Facility in Brighton, MI from 2009–2014 for mass-rearing and releasing.

Three consecutive generations of *O. agrili* adults were included in this study (Fig. 2.1). These generations are defined as: 1) adults that developed from mature larvae that had entered diapause, which will hereinafter be referred to as "F_0-diapaused adults"; 2) adults that developed from nondiapaused mature larvae that were progeny of F_0-diapaused adults, which will hereinafter be referred to as "F_1-nondiapaused adults"; and, 3) adults that developed from nondiapaused mature larvae that were progeny of F_1-nondiapaused adults, which will hereinafter be referred to as "F_2-nondiapaused adults."

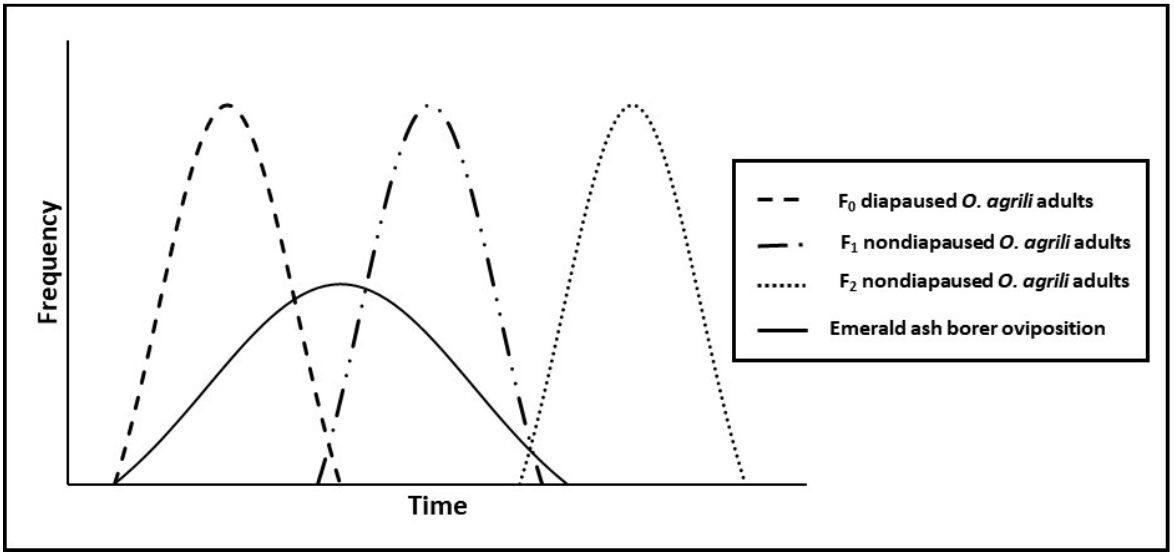

Figure 2.1. Graphical depiction of the estimated annual phenology of *O. agrili* generations and their synchrony with emerald ash borer oviposition. *Oobius agrili* synchrony with emerald ash borer oviposition has not been confirmed in the field.

The experiments were conducted in environmental chambers (Percival Scientific Inc., Perry, IA) set at 25 ± 1 °C constant temperature and ~70% relative humidity. HOBO Pro temperature data recorders (Onset, Pocasset, MA) were used to measure temperature every 30 minutes in each of the environmental chambers. Temperature data were used to calculate DD_{10}

required for emergence (discussed below). An EXTECH HD450 light meter (www.extech.com) was used to measure light intensity in chambers, which ranged between 1450 and 1850 lux. This variation in light intensities was considered unimportant for the current study given that photoperiod responses of egg parasitoids have been shown to be induced by changes in light intensity rather than the absolute light intensity to which they are exposed (Boivin, 1994). Laboratory-reared EAB eggs were used to rear *O. agrili* for this study. EAB eggs were produced by adults that emerged in the laboratory from naturally infested ash logs that were cut during the winter. Logs were stored in a cold room at 5 ± 1 °C for up to 10 months until they were transferred to cardboard rearing tubes and held in constant light at room temperature (~25 °C) to allow overwintering EAB mature larvae to complete development and emerge as adults. Emerging EAB adults were collected daily from tubes and held at 25 ± 1 °C and 16L:8D photoperiod. Pairs of female and male EAB were held in 950-mL plastic cups (Fabri-Kal Corp., Piedmont, SC, Item No. PK32T) and fed fresh, mature greenhouse-grown *Fraxinus uhdei* (Wenz.) foliage with petioles inserted in vials of water. The foliage was replaced and the cups cleaned every 2–3 days. The top of each cup was covered with a piece of plastic window screening that was overlaid with coffee filter paper. EAB females inserted their ovipositors through the screen mesh and deposited eggs directly onto the filter paper. Filter papers with eggs were removed every 2–3 days, and held at 25 ± 1°C for an additional 24hr, to allow embryogenesis to occur in eggs that were laid within the last 24hr. Eggs were then transferred to 10 ± 1°C to retard further development until they were exposed to *O. agrili*. EAB eggs were stored for no more than 3 days at 10°C for all experiments described below, although previous experiments found that EAB eggs can be held at 10°C for up to 5 days without negative effects on *O. agrili* oviposition or development (Pers. observation). Only EAB eggs that were tan or

brown were used for experiments, which is evidence of successful fertilization and embryogenesis (Rutledge and Keena, 2012). EAB eggs were randomly assigned to photoperiod treatments to reduce bias due to egg quality or age. *Oobius agrili* were reared and assayed in 50 × 9 mm Falcon® polystyrene dishes (Corning Incorporated, Corning, NY; Item No. 351006) with friction-fitting lids streaked with honey to provide food for parasitoids.

Effects of maternal and progeny photoperiod exposures and maternal age on F_0-diapaused adults and their progeny.

This experiment examined the effects of photoperiod on F_0-diapaused adults (i.e., maternal effects) and their progeny in two separate replicates (Table 2.1). For the first replicate, conducted October–December 2016, 30 naïve F_0-diapaused adults (10 adults per photoperiod treatment) were selected that had developed from diapausing larvae that were transferred to 25 °C and 16L:8D, 12L:12D, or 8L:16D photoperiod after they were subjected to 10 °C for 1 month followed by 4 °C for 8.5 months. The second replicate was conducted October–December 2017 and consisted of 27 naïve F_0-diapaused adults (9 per photoperiod treatment) that had developed from diapausing mature larvae that were transferred to 25 °C and 16L:8D, 12L:12D, or 8L:16D photoperiod after they were subjected to 10 °C for 1 month followed by 4 °C for 9.0 months. For each replicate, the F_0-diapaused adults were held throughout the experiment in the respective photoperiod that they had developed in. Each adult was presented 9 EAB eggs for a 24-hr period once a week for 3 consecutive weeks. Maternal age for 1wk, 2wk and 3wk was 1-5, 9-12 and 16-19 days respectively. After each 24hr exposure, the EAB eggs were removed from females and randomly assigned to 16L:8D, 12L:12D, or 8L:16D progeny photoperiod treatments (3 eggs per progeny photoperiod treatment), where they were held for 8wks (Table 2.1). After 8wks in

their respective progeny photoperiod treatments, each EAB egg was inspected under a dissecting microscope to determine if it had been parasitized and the fate of the parasitoid progeny: a) parasitoid developed to adult and emerged from EAB egg; b) diapausing parasitoid larvae remained inside the EAB egg; c) parasitoid died inside EAB egg; or, d) EAB egg not parasitized. Percent successful parasitism of EAB eggs was calculated by dividing the number of successfully parasitized eggs (i.e., parasitized EAB eggs that produced *O. agrili* adults or diapausing mature larvae) by the total number of eggs exposed and then multiplying by 100. The percent of progeny that entered diapause (i.e., percent diapause) was calculated by dividing the number of diapausing mature larvae by the total number of successfully parasitized eggs and then multiplying by 100. F_0-diapaused adults that died or did not successfully parasitize any EAB eggs during the experiment were omitted from analyses. A generalized linear mixed model (PROC GLIMMIX; SAS Institute 2012)) was used with a repeated measures design and a binomial distribution to compare the percentage of successful parasitism and the percentage of diapaused progeny among maternal and progeny photoperiod treatments, maternal age (i.e., weeks) when presented with EAB eggs, as well as interactions between these variables. Least squared means (LSM) that were significantly different ($P < 0.05$) were then compared using Tukey's Honestly Significant (HSD) test.

Effects of grand-maternal, maternal, and progeny photoperiod exposures and maternal age on F_1-nondiapaused adults and their progeny.

This experiment examined grand-maternal (i.e., photoperiod exposure of F_0-diapaused adults), maternal (i.e., photoperiod exposure of F_1-nondiapaused adults) and progeny, as well as maternal age of F_1-nondiapaused adults on parasitism and diapause (Table 2.1). Adults for this

experiment were the progeny of the F_0-diapaused adults from the previous section. The photoperiod exposures of F_0 and F_1 adults included: a) F_0-diapaused adults developed and held at 8L:16D and F_1-nondiapaused adults developed and held at 8L:16D ; b) F_0-diapaused adults developed and held at 16L:8D and F_1-nondiapaused adults developed and held at 8L:16D; c) F_0-diapaused adults developed and held at 16L:8D and F_1-nondiapaused adults developed and held at 16L:8D. The 12L:12D photoperiod treatments were not included in this study because results for progeny of F_0-diapaused adults in did not differ significantly between 8L:16D and 12L:12D photoperiods, and a limited number of EAB eggs were available during this phase of the study. All adults were naïve at the beginning of this experiment and 12–20 adults were selected for each of the 3 treatments. The F_1-nondiapaused adults were held at the respective photoperiod treatments in which they developed and emerged throughout this experiment. This experiment was conducted from November 2016 to February 2017.

To assess the effects of photoperiod and maternal age on diapause induction in the progeny of these F_1-nondiapaused adults, each adult was presented with 10 EAB eggs for a 24-hr period once a week for 3 consecutive weeks while being held under its respective maternal photoperiod. Maternal age for 1wk, 2wk and 3wk was 1–5, 9–12 and 16–19 days, respectively (Table 2.1). After each 24-hr exposure period, EAB eggs were removed and randomly assigned to 16L:8D or 8L:16D progeny photoperiod treatments (5 eggs per photoperiod treatment) where they remained for 8 wk; the fate of the progeny from each treatment was determined, and percent parasitism and percent diapause calculated. F_1-nondiapaused adults that died or did not parasitize any EAB eggs during the experiment were omitted from analyses. A generalized linear mixed model (PROC GLIMMIX) with a repeated measures design and a binomial distribution were used to compare percent successful parasitism and percent of diapausing

progeny produced by each adult among photoperiod treatments, maternal ages (i.e., weeks) when presented EAB eggs, and interactions between these variables. LSMs that were significantly different ($P<0.05$) were compared using Tukey's HSD test.

Critical day length for diapause induction in *O. agrili*.

To estimate the critical day length for diapause induction in the larval progeny of F_1- and F_2-nondiapaused adults, 20–40 naïve adults were randomly selected that had developed and emerged in 16L:8D photoperiod and ranged in age from 1–7 days (Table 2.1). This experiment was conducted during December 2016–March 2017. Individual F_1- or F_2-nondiapaused adults were provided with 20 EAB eggs and randomly assigned to one of the following photoperiods: 12L:12D, 12.5L:11.5D, 13L:11D, 13.5L:10.5D, 14L:10D, 14.25L:9.75D, 14.5L:9.5D, 15L:9D, 15.5L:8.5D, or 16L:8D. The adults remained with the EAB eggs for a minimum of 10 days (Table 2.1). Eight weeks after the photoperiod exposures began, the eggs were examined to determine fate of the progeny and the percentage of progeny that entered diapause per dish of 20 EAB eggs was calculated. Dishes with no successful parasitism were excluded from analyses. A generalized linear mixed model (PROC GLIMMIX) with binomial distribution was used to compare the percent of progeny that entered diapause among photoperiod treatments, between progeny from F_1- and F_2-nondiapaused adult generations, and interactions between treatments and generation. LSMs that were significantly different were compared using Tukey's HSD test.

Critical age of developing parasitoids for photoperiod induced diapause in progeny of F_1-nondiapaused adults.

During March–May 2017 the developmental age of immature *O. agrili* (inside EAB eggs) was examined to determine when diapause response to photoperiod occurred for the

progeny of F_1-nondiapaused adults (Table 2.1). Approximately 110 EAB eggs were presented to 15 naïve, 1–10 day-old F_1-nondiapaused adults for a 24-hr period in 16L:8D photoperiod. Two days later 110 EAB eggs were presented to the same adults for a 24-hr period in 16L:8D photoperiod. On days 1, 3–8, 10, 12, and 14 after adult exposure, ca. 20 parasitized eggs (10 per exposure interval) were randomly transferred from 16L:8D and divided between 8L:16D or 14L:10D photoperiods. Ten parasitized eggs from each of the two exposures remained in 16L:8D to serve as controls. About 8wk after transfer to their respective photoperiod treatments, each parasitized egg was inspected to determine if the progeny developed to an adult or entered diapause. Parasitoids that died within the EAB egg before completing development were excluded from analyses. Parasitized eggs from both exposures were pooled for statistical analyses. Logistic regression (Proc LOGISTICS; SAS 2008) was used to compare the likelihood of entering diapause (vs. emerging as an adult) among transfer days for each of the photoperiod treatments. Means that were significantly different ($P < 0.05$) were individually compared using Wald X2.

Effects of photoperiod and chill duration on diapause termination on *O. agrili* mature larvae.

In this experiment, the effects of photoperiod on diapause termination in diapausing larvae subjected to different chill durations were examined (Table 2.1). This experiment was conducted during October 2016 –June 2017 using the progeny of F_1- and F_2-nondiapaused adults held at 25 ± 1°C that had been exposed to 8L:16D photoperiod to induce diapause. Six- to eight-weeks after parasitism, the diapausing mature larvae were held for 30 days at 10°C, and then randomly assigned to a chill period of 152, 181 or 258 days at 4±1°C. After chill, the diapausing

larvae were transferred to 25 ± 1°C and randomly assigned to one of four photoperiod treatments: 8L:16D, 12L:12D, 14.5L:9.5D, or 16L:8D (N=25–35 diapausing larvae per photoperiod treatment from each chill period); the emergence of adult parasitoids was monitored daily for 10 wks. A generalized linear mixed model (PROC GLIMMIX) with a Poisson distribution was used to compare the mean number of degree days (base 10°C; DD10) required for adult emergence among photoperiod treatments, chill periods, and interactions between these variables. LSMs that were significantly different were compared using Tukey's HSD test.

Effects of photoperiod on diapause termination in diapausing *O. agrili* larvae not subjected to chill.

The effects of photoperiod on diapause termination in diapausing larvae not exposed to a period of chill were examined (Table 2.1). Diapausing larvae used for this experiment were the progeny of F_1-nondiapaused adults that had entered diapause when exposed to photoperiods above and below the critical day length of 14.25–14.5 hours of day light as determined in subsection 2.3. This experiment was conducted from March to June 2017. Diapausing larvae were held for 3–4 months after parasitism in their original photoperiod treatments of 12L:12D, 14L:10D, 14.25L:9.75D, or 14.5L:9.5D at 25±1°C until they were randomly transferred to different photoperiods with day lengths that were longer than their original treatment. Transfer treatments included: 14L:10D, 14.25L:9.75D, 14.5L:9.5D, 15L:9D, or 16L:8D. Each transfer treatment received 40–65 diapausing larvae. Some individuals were also randomly selected to remain in their original photoperiod treatment (approximately 35–80 for each treatment) to serve as controls. Adult emergence was evaluated after 10 wks to determine the percent that emerged as adults or remained as diapausing larvae. Immature parasitoids that died were excluded from

the analyses. Logistic regression (PROC LOGISTIC; SAS Institute 2012) was used to compare the likelihood of emerging from diapause among transfer treatments independently for each of the originating photoperiods. Means that were significantly different among transfer treatments were individually compared for each originating photoperiod using Wald X^2.

Comparison of day length and average degree day accumulation at *O. agrili* release sites.

To predict how critical day length for *O. agrili* diapause induction may affect its establishment and population dynamics in North America, the mean calendar day that EAB adult emergence was estimated to begin (i.e., 250 DD_{10}) and peak (i.e., 560 DD_{10}) was calculated at five locations along a north-south gradient where *O. agrili* has been released (Brown-Rytlewski and Wilson 2004). Mean calendar days for each location were calculated over a 38-yr period (1978–2016) using North American Regional Reanalysis data (https://www.ncdc.noaa.gov/data-access/model-data/model-datasets/north-american-regional-reanalysis-narr). The day length for the mean calendar day for the beginning and peak EAB emergence for each location was determined using the following: http://aa.usno.navy.mil/data/docs/Dur_OneYear.php.

Table 2.1. Summary of experiments and treatments conducted to evaluate the effects of photoperiod and chill duration on diapause of *O. agrili*. All experiments were conducted in environmental chambers set at 25 ± 1 °C constant temperature and ~70% relative humidity.

Subsection/Experiment	No. ovipositing adults	Maternal photoperiod treatments	Maternal age when given eggs	No. EAB eggs per adult	Developing progeny photoperiod	No. parasitized eggs per progeny photoperiod
2.1. *Effects of maternal and progeny photoperiod exposures and maternal age F_0-diapaused adults and their progeny.*	Rep 1 = 10 F_0 adults per maternal photoperiod Rep 2 = 9 F_0 adults per maternal photoperiod	1) 16L:8D 2) 12L:12D 3) 8L:12D	1) 1–5 days 2) 9–12 days 3) 16–19 days	9 eggs for each adult exposure	1) 16L:8D 2) 12L:12D 3) 8L:16D	3 eggs from each adult exposure
2.2. *Effect of grandmaternal, maternal, and progeny photoperiod exposures and maternal age on F_1-diapaused*	12–20 F_1 adults per maternal and grand-maternal photoperiod	1) F_1 16L:8D and F_0 grand-maternal 16L:8D 2) F_1 16L:8D and	1) 1–5 days 2) 9–12 days 3) 16–19 days	10 eggs for each adult exposure	1) 16L:8D 2) 8L:16D	5 eggs from each adult exposure

Table 2.1. (cont'd)

and their progeny.		F_0 grand-maternal 8L:16D 3) F_1 8L:16D and F_0 grand-maternal 8L:16D				
2.3. *Critical day length length for diapause induction.*	20-40 F_1 adults per progeny photoperiod	Same as progeny photoperiod during oviposition	1–7 days	20 eggs per adult	1)12L:12D, 2) 12.5L:11.5D, 3)13L:11D,4)13.5L:10.5D, 5)14L:10D, 6)14.25L:9.75D 7)14.5L9.5D,8)15L:9D 9)15.5L:8.5D,10)16L:8D	20 eggs per photoperiod replicate
2.4. *Critical age of developing parasitoids for photoperiod induced diapause in progeny of F1-diapaused adults.*	Exposure 1 = 15 F_1 adults pooled Exposure 2 = Same 15 F_1 adults pooled	16L:8D	1–10 days	110 eggs for each exposure	Transfer from 16L:8D to 8L:16D or 14L:10D on day 1,3,4,5,6,7,8, 10,12,14,or no transfer	20 eggs (10 from each replicate) per day and transfer treatment

Table 2.1. (cont'd)

2.5. *Effects of photoperiod and chill duration on diapause termination on mature larvae.*	NA	8L:16D	Diapausing mature larvae in 4°C for 1)152, 2)181, or 3)258 days. Then to 25°C at 1)8L:16D, 2)12L:12D, 3)14.5L:9.5D, or 4)16L:8D	25–35
2.6. *Effects of photoperiod on diapause termination in diapausing mature larvae not subjected to chill.*	NA	1)12L:12D, 2)14L:10D, 3)14.25L:9.75D, 4)14.5L:9.5D,	Diapausing mature larvae not chilled and transferred to 1)14L:10D, 2)14.25L:9.75D, 3)14.5L:9.5D, 4)15L:9D, 5)16L:8D	21–79

Table 2.2. Statistical results for experiments examining the effects of photoperiod and chill on *O. agrili* diapause induction and termination, and parasitism.

Subsection/Experiment	Dependent variable	Treatments	DF	Test Statistic[1]	*P* value
3.1. *Effects of maternal and progeny photoperiod exposures and maternal age* F_0*-diapaused adults and their progeny.*	Percent diapause	Maternal photoperiod[2]	2, 78	$F = 0.62$	0.5397
		Maternal age[3]	2, 114	$F = 38.25$	<0.0001
		Progeny's photoperiod[4] (8L:16D vs. 12L:12D only)	1, 78	$F = 0.02$	0.8780
		Progeny's photoperiod (8L:16D vs. 12L:12D only) × Maternal age[3]	2, 114	$F = 1.15$	0.3183
		Progeny's photoperiod[4] × Maternal photoperiod[2]	2, 78	$F = 1.69$	0.1893
	Percent parasitism	Maternal photoperiod[2]	2, 41	$F = 0.78$	0.4627
		Maternal age[3]	2, 82	$F = 11.25$	<0.0001
		Maternal photoperiod[2] × Maternal age[3]	4, 82	$F = 1.50$	0.2129
3.2. *Effect of grandmaternal, maternal, and progeny photoperiod exposures*	Percent diapause	Maternal and grand-maternal photoperiod[5]	2, 38	$F = 0.57$	0.5723
		Maternal age	2, 47	$F = 1.59$	0.2159

Table 2.2. (cont'd)

and their progeny.		Maternal and grand-maternal photoperiod[5] × Maternal age[3]	4, 47	$F = 0.22$	0.9267
	Percent parasitism	Maternal and grand-maternal photoperiod[5]	2, 37	$F = 0.40$	0.6743
		Maternal age[3]	2, 74	$F = 11.69$	<0.0001
		Maternal and grand-maternal Photoperiod[5] × Maternal age[3]	4, 74	$F = 0.64$	0.6368
3.3 *Critical day length*	Percent diapause	Progeny's photoperiod[6]	1, 118	$F = 22.97$	<0.0001
length for diapause		Generation post diapause[7]	5, 118	$F = 89.31$	<0.0001
induction.		Progeny's photoperiod[6] × generation post diapause[7]	5,118	$F = 2.69$	0.0245
3.4. *Critical age of*	Percent diapause	8L:16D photoperiod	8	$\chi^2 = 28.6523$	<0.0004
developing parasitoids for photoperiod induced diapause in progeny of F1-diapaused adults.		14L:10D photoperiod	7	$\chi^2 = 31.6258$	<0.0001
3.5. *Effects of photoperiod*	Development	Post diapause photoperiod[8]	3, 332	$F = 932.60$	<0.0001

Table 2.2. (cont'd)

and chill duration on	time (DD_{10})	Chill period[9]	2, 332	$F = 1089.03$	<0.0001
diapause termination of		Post diapause photoperiod[8] ×			
mature larvae.		chill period[9]	6, 332	$F = 137.04$	<0.0001
3.6. *Effects of photoperiod*	Percent	12L:12D photoperiod	5	$\chi^2 = 48.6862$	<0.0001
on diapause termination	emerged	14L:10D photoperiod	4	$\chi^2 = 67.6677$	<0.0001
in diapausing mature larvae		14.25L:9.75D photoperiod	3	$\chi^2 = 39.8459$	<0.0001
not subjected to a chill.		14.5L:9.5D photoperiod	2	$\chi^2 = 1.2367$	0.5388

[1]*F* values given for data analyzed by general linear mixed models (Proc GLIMMIX) followed by Tukey's HSD (honestly significant difference) test, χ^2 values given for data analyzed by logistic regression (Proc LOGISTICIS) followed by Wald's Chi-squared test. [2] F_0-diapaused adult maternal photoperiod treatments were 8L:16D, 12L:12D, or 16L:8D. [3]Maternal age treatments were 1wk, 2wk, or 3wk old. [4]Only 8L:16D and 12L:12D progeny photoperiod compared because 100% progeny diapaused in 16L:8D which caused quasi-complete separation of the model. [5]Maternal-grand-maternal treatments of F_1-nondiapaused adults were 8L:16D grand-maternal and 8L:16D maternal; 16L:8D grand-maternal and 8L:16D maternal; or 16L:8D grand-maternal and 16L:8D maternal. [6]Progeny photoperiod treatments included 14L:10D, 14.25L:9.75D, 14.5L:9.5D, 15L:9D, 15.5L:9.5D, and 16L:8D. [7] Progeny of F_1-and F_1-nondiapaused adults were compared. [8]Post diapause photoperiod treatments included 8L:16D, 12L:12D, 14.5L:9.5D, 16L:8D. [9]Chill periods compared included 5.0, 6.0, and 8.5 months.

Results

Effects of maternal and progeny photoperiod exposures and maternal age on F_0-diapaused adults and their progeny.

Photoperiod had a significant effect on diapause induction (i.e., percent diapause) of developing progeny (inside EAB eggs) produced by the F_0-diapaused *O. agrili* adults (Table 1.2). Specifically, no developing progeny entered diapause if exposed to the 16L:8D progeny-photoperiod treatment regardless of maternal-adult-photoperiod treatment (Table 2.3). Of developing progeny exposed to 8L:16D and 12L:12D progeny photoperiod treatments, 10.5 ± 3.0% and 9.8 ± 3.6% entered diapause, respectively, for all three maternal photoperiods combined (Table 2.3). Because no developing progeny entered diapause for the 16L:8D progeny treatment, quasi-complete separation occurred for the generalized linear mixed model comparing percent diapause among all photoperiod treatments and maternal ages. Therefore, the 16L:8D progeny photoperiod treatment was excluded from the model to allow statistical comparison of the remaining treatments and no significant difference in percent diapause was found between 8L:16D and 12L:12D progeny-photoperiod treatments ($F = 0.02$; $df = 1, 78$; $P < 0.8780$; Table 2.2). Also, no significant differences were found among maternal photoperiod treatments or interactions between maternal and progeny photoperiod treatments (Table 2.2).

The maternal age of F_0-diapaused adults when presented EAB eggs had a significant effect on the percent of their progeny that entered diapause (i.e., percent diapause) for the 8L:16D or 12L:12D progeny photoperiod treatments ($F = 38.25$, $df = 2, 114$, $P < 0.0001$; Table 2.2). Progeny produced by 3-wk-old F_0-diapaused adults had a significantly higher rate of diapause (53.1 ± 5.3%) compared to progeny of 1-wk- (2.2 ± 1.3%) or 2-wk- (5.3 ± 1.8%) old adults (Table 2.4). Interactions between maternal age and progeny photoperiod (i.e., 8L:16D and

12L:12D only) treatments did not significantly affect the percentage of progeny that entered diapause ($F = 1.15$; $df = 2, 114$; $P < 0.3183$; Table 2.2). In addition, maternal age when presented EAB eggs significantly affected percent parasitism ($F = 11.25$; $df = 2, 82$; $P < 0.0001$; Table 2.2) being highest for 2wk old adults (66.5 ± 4.8%) compared to 1wk (52.9 ± 4.5%) or 3wk old (41.4 ± 5.2%) adults (Table 2.5).

Table 2.3. Least squared mean (LSM ± SE) percent of larval progeny entering diapause (percent diapause) from F_0-diapaused adults that were held at one of three photoperiods, and their progeny transferred to one of three photoperiods within 24hr of oviposition (Experiment 2.1). Data were combined for all progeny produced by F_0-diapaused adults when they were presented EAB eggs at 1, 2 and 3wk of age. Overall LSMs with the same letter were not significantly different ($P < 0.05$; Tukey's HSD; 16L:8D not included in the statistical analyses because a perfect correlation caused model separation).

Maternal photoperiod treatment of F_0-diapaused adults	Percent diapause (LSM±SE) of larval progeny by photoperiod treatment		
	8L:16D	12L:12D	16L:8D
8L:16D	14.3% ± 4.8(N[1]=51)	10.0 ± 4.5(51)	0 ± 0(51)
12L:12D	11.6 ± 4.4(42)	5.6 ± 3.1(42)	0 ± 0(42)
16L:8D	6.9 ± 3.8(39)	16.1 ± 7.5(39)	0 ± 0(39)
Overall	10.5 ± 3.0(132)A	9.8 ± 3.6 (132)A	0 ± 0(132)

[1]N = number of replicates with successful parasitism. Each replicate received 3 eggs.

Table 2.4. Least squared mean (LSM ± SE) percent of progeny that entered diapause (percent diapause) that were produced by F_0-diapaused adults that were 1wk, 2wk, or 3wk old when presented EAB eggs (Experiment 2.1). Within 24hr after oviposition, parasitoid progeny were transferred to 8L:16D or 12L:12D photoperiod treatments (16L:8D egg treatments were excluded because no progeny entered F_0-diapaused for this treatment). Data were combined for three different maternal photoperiod treatments to which F_0-diapaused adults were exposed (8L:16D, 12L:12D, and 16L:8D). LSMs for overall percent diapause of progeny among ages of F_0-diapaused adults when exposed to EAB eggs with the same letter were not significantly different ($P < 0.05$; Tukey's HSD).

Photoperiod treatment of progeny that were produced by F_0-diapaused adults	Percent diapause (LSM±SE) of progeny by maternal adult age		
	1 wk old	2 wk old	3 wk old
8L:16D	4.3% ± 2.6(N[1]=44)	4.1 ± 2.2(44)	45.6 ± 7.2(44)
12L:12D	1.2 ± 1.1(44)	6.8 ± 2.9(44)	60.4 ± 7.4(44)
Overall	2.2 ± 1.3(88)B	5.3 ± 1.8(88)B	53.1 ± 5.3(88)A

[1]N = number of replicates with successful parasitism. Each replicate received 3 eggs.

Table 2.5. Least squared mean (LSM ± SE) percent successful parasitism (i.e., progeny emerged or entered diapause) for *Oobius agrili* F_0-diapaused adults by photoperiod treatment and adult age (Experiment 2.1). EAB eggs were pooled from three progeny photoperiod treatments (8L:16D, 12L:12D, and 16L:8D). LSMs for overall percent parasitism with the same letter were not significantly different ($P < 0.05$; Tukey's HSD) among F_0-diapaused adult ages when exposed to EAB eggs.

Photoperiod treatment of F_0-diapaused adults	Percent parasitism (LSM±SE) by maternal age		
	1 wk old	2 wk old	3 wk old
8L:16D	48.4% ± 7.2(N[1]=17)	68.7 ± 7.6(17)	52.9 ± 8.0(17)
12L:12D	54.0 ± 7.9(14)	70.6 ± 8.2(14)	46.8 ± 8.9(14)
16L:8D	56.4 ± 8.1(13)	59.8 ± 9.2(13)	25.6 ± 8.0(13)
Overall	52.9 ± 4.5(44)B	66.5 ± 4.8(44)A	41.4 ± 5.2(44)B

[1]N = number of replicates with successful parasitism. Each replicate received 9 eggs.

Effects of grand-maternal, maternal, and progeny photoperiod exposures and maternal age on F_1-diapaused adults and their progeny.

All developing progeny of F_1-nondiapaused adults exposed to the 8L:16D progeny photoperiod treatment entered diapause, regardless of grand-maternal (F_0 adult treatment) or maternal (F_1 adult treatment) photoperiod treatments (Tables 2.6). As with subsection 3.1, this perfect correlation caused quasi-complete separation of the generalized linear mixed model. Therefore, the 8L:16D progeny-photoperiod treatment was excluded from the model. Percent

diapause for developing progeny exposed to 16L:8D photoperiod ranged from 6.3–8.1% among the three grand-maternal and maternal photoperiod treatments and did not differ significantly ($F = 0.57$; $df = 2, 38$; $P < 0.5723$; Table 2.2, 2.6). Maternal age did not significantly affect the percentage of progeny in 16L:8D photoperiod that diapaused reared ($F = 1.59$; $df = 2, 47$; $P < 0.2159$; Tables 2.2, 2.7). However, percentage diapaused for progeny produced by 1-wk-old mothers was consistently lower compared to 2wk- and 3-wk-old mothers within each maternal and grand-maternal photoperiod treatment, suggesting some effect of maternal age. Quasi-complete separation also prevented statistical comparison of interactions between adult photoperiod treatment and maternal age on percentage diapaused. Maternal age did significantly affect percent parasitism ($F = 11.69$; $df = 2, 74$; $P < 0.0001$, Table 2.2). In addition, 1wk- and 2wk- old-adults had higher parasitism percentages compared to 3-wk-old adults (Table 2.8). Parasitism percentages were not significantly affected by grand-maternal and maternal photoperiod treatments (Table 2.2). Also, no interaction was found between maternal age and grand-maternal and maternal treatments for percentage parasitized (Table 2.2).

Table 2.6. Least squared mean (LSM ± SE) for percent of progeny that entered diapaused (percent diapause) from *Oobius agrili* F_1-nondiapaused adults that were maintained for two generations at one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments (Experiment 2.2). Progeny of the F_1-nondiapaused adults were transferred to one of three photoperiod treatments within 24hr of oviposition for development to complete. Data were combined for all progeny produced by F_1-nondiapaused adults when they were presented EAB eggs at 1, 2 and 3wk of age. LSMs with the same letter (16L:8D progeny treatment only) were not significantly different ($P < 0.05$; Tukey's HSD).

F_0 and F_1 adult photoperiod treatments	Percent diapause (LSM±SE) of larval progeny by photoperiod treatment	
	8L:16D	16L:8D
8L:16D/8L:16D	100% ± 0(N^1=15)	6.3 ± 2.9(15)A
16L:8D/8L:16D	100 ± 0(23)	8.1 ± 2.4(23)A
16L:8D/16L:8D	100 ± 0(13)	6.3 ± 2.9(13)A

N^1 = number of replicates with successful parasitism. Each replicate received 5 eggs.

Table 2.7. Least squared mean (LSM ± SE) percent of progeny that entered diapause (percent diapause) from *Oobius agrili* F_1-nondiapaused adults that were exposed to one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments and presented EAB eggs when F_1-nondiapaused adults were 1, 2, and 3 wk old (Experiment 2.2). Eggs were transferred to 16L:8D photoperiod 24 hr after oviposition (progeny transferred to 8L:16D photoperiod were not included because 100% entered diapause; see Table 2.6). Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD).

F_0 and F_1 adult photoperiod treatment	Percent diapause (LSM±SE) for eggs placed in 16L:8D light treatments by maternal age		
	1 wk old	2 wk old	3 wk old
8L:16D/8L:16D	2.8% ± 2.2(N^1=8)	6.5 ± 4.6(4)	4.8 ± 3.8(4)
16L:8D/8L:16D	4.3 ± 2.5(12)	8.2 ± 4.2(8)	14.4 ± 4.8(3)
16L:8D/16L:8D	3.3 ± 2.7(6)	9.3 ± 5.3(6)	7.9 ± 6.3(2)
Overall	3.4 ± 1.4(26)A	14.1 ± 3.8(17)A	8.3 ± 3.2(9)A

N^1 = number of replicates with successful parasitism. Each replicate received 5 eggs.

Table 2.8. Least squared mean (LSM ± SE) percent successful parasitism (i.e., progeny emerged or entered diapause) of EAB eggs by *Oobius agrili* F_1-nondiapaused adults that were exposed to one of three F_0 (grand-maternal) and F_1 (maternal) photoperiod treatments and were presented eggs when F_1-nondiapaused adults were 1, 2, and 3 wk old (Experiment 2.2). Data were pooled for 8L:16D and 16L:8D progeny treatments. Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD).

F_0 and F_1 adult photoperiod treatment	Percent parasitism (LSM±SE) by maternal age of F_1 adult		
	1 wk old	2 wk old	3 wk old
8L:16D/8L:16D	47.7% ± 5.2(N^1=13)	49.2 ± 8.2(13)	31.5 ± 8.0(13)
16L:8D/8L:16D	55.9 ± 4.5(17)	58.2 ± 7.1(17)	32.9 ± 7.0(17)
16L:8D/16L:8D	54.0 ± 5.9(10)	65.0 ± 9.0(10)	24.0 ± 8.4(10)
Overall	52.5 ± 3.0(40)A	57.6 ± 4.8(40)A	29.3 ± 4.6(40)B

N^1=number of replicates with successful parasitism. Each replicate received 10 eggs.

Critical day length for diapause induction in *O. agrili*.

All progeny produced by F_1- and F_2-nondiapaused adults entered diapause when they developed in the following photoperiods: 12L:12D, 12.5L:11.5D, 13L:11D, or 13.5L:10.5D. For photoperiods with ≥14hr daylight, progeny photoperiod ($F = 22.97$; df = 1, 118; $P < 0.0001$), generation ($F = 89.31$; df = 5, 118; $P < 0.0001$), and the interaction between these treatments ($F = 2.69$; df = 5, 118; $P = 0.0245$) had significant effects on the percentage of progeny that entered

diapause (Table 2.2). Also for photoperiods ≥14hr daylight, the highest diapause rates were found for larval progeny from both F_1- and F_2- nondiapaused adults held at 14L:10D and for larval progeny from F_2-nondiapaused adults held at 14.25L:9.75D (Fig. 2.2). Diapause rates were lowest for progeny from both generations held at day lengths ≥14.5 hours, with the exception of progeny from F_2-nondiapaused adults held at 14.5L:9.5D and 16L:8D (Fig. 2.2). Over 50% of progeny from F_1 and F_2 adults entered diapause at day lengths ≤14.25 hours and less than 50% entered diapause at photoperiods with ≥ 14.5 hours of day length. Therefore, the critical day length at which 50% of the progeny entered diapause was between 14.25 and 14.5 hours of daylight (Fig. 2.2).

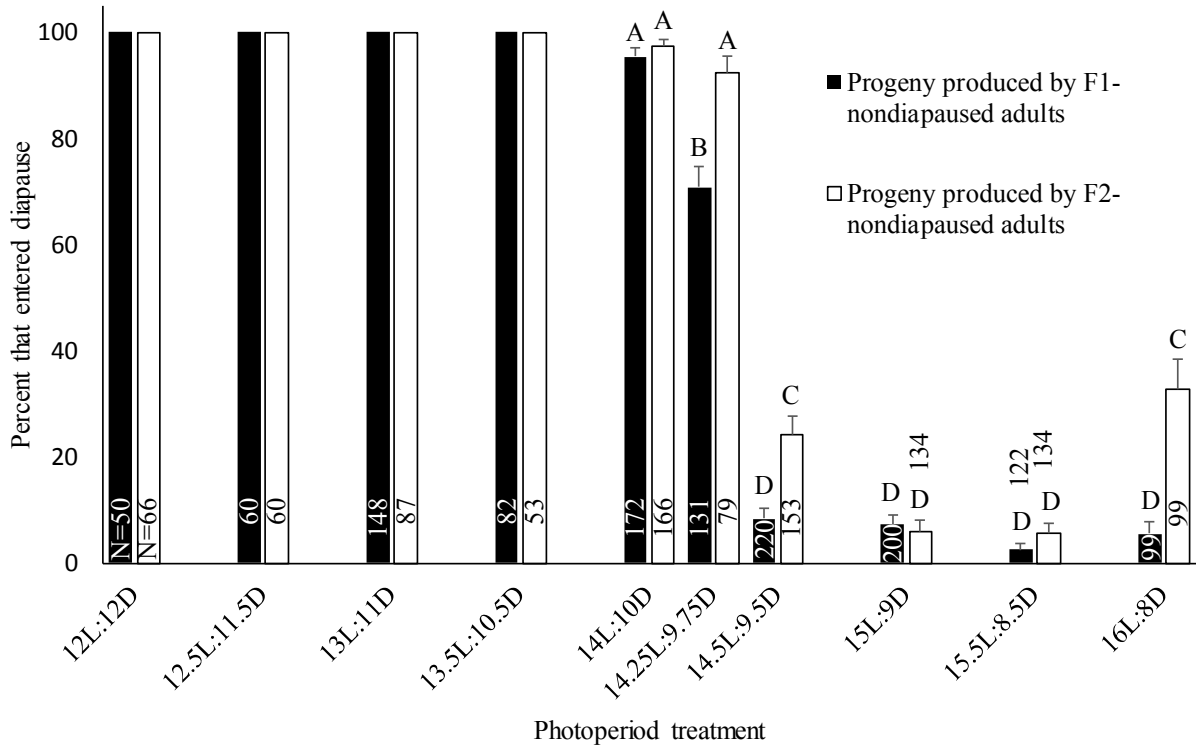

Figure 2.2. Least-squared mean (LSM ± SE) percent of *O. agrili* progeny entering diapause (percent diapause) produced by F_1- and F_2-nondiapaused adults and allowed to develop under different photoperiods (Experiment 2.3). Means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). [1]N = total number of successfully parasitized EAB eggs (i.e., emerged as adults or became diapausing mature larvae).

Critical age of developing parasitoids for photoperiod induced diapause in progeny of F_1-nondiapaused adults.

The age of developing *O. agrili* progeny produced by F_1-nondiapaused adults significantly affected the likelihood of progeny entering diapause when they were transferred to 8L:16D ($\chi 2 = 28.6523$; df = 8; $P < 0.0001$) or 14L:10D light ($\chi 2 = 31.6258$; df = 7; $P < 0.0001$; Table 2.2). All developing progeny that were ≤4 days old or younger when transferred to 14L:10D entered

diapause, and all developing progeny that were ≤5 days old or younger when transferred to 8L:16D entered diapause (Fig. 2.3). Diapause rates for developing progeny transferred to 14L:10D were significantly different for 5-day-old progeny compared to those that were ≥6 days old when transferred or the progeny that were not transferred (i.e., remained in 16L:8D; Fig. 2.3). Progeny transferred to 8L:16D differed significantly for 6- and 7-day-old progeny while those that were ≥8 days old did not differ significantly from progeny that were not transferred (Fig. 2.3).

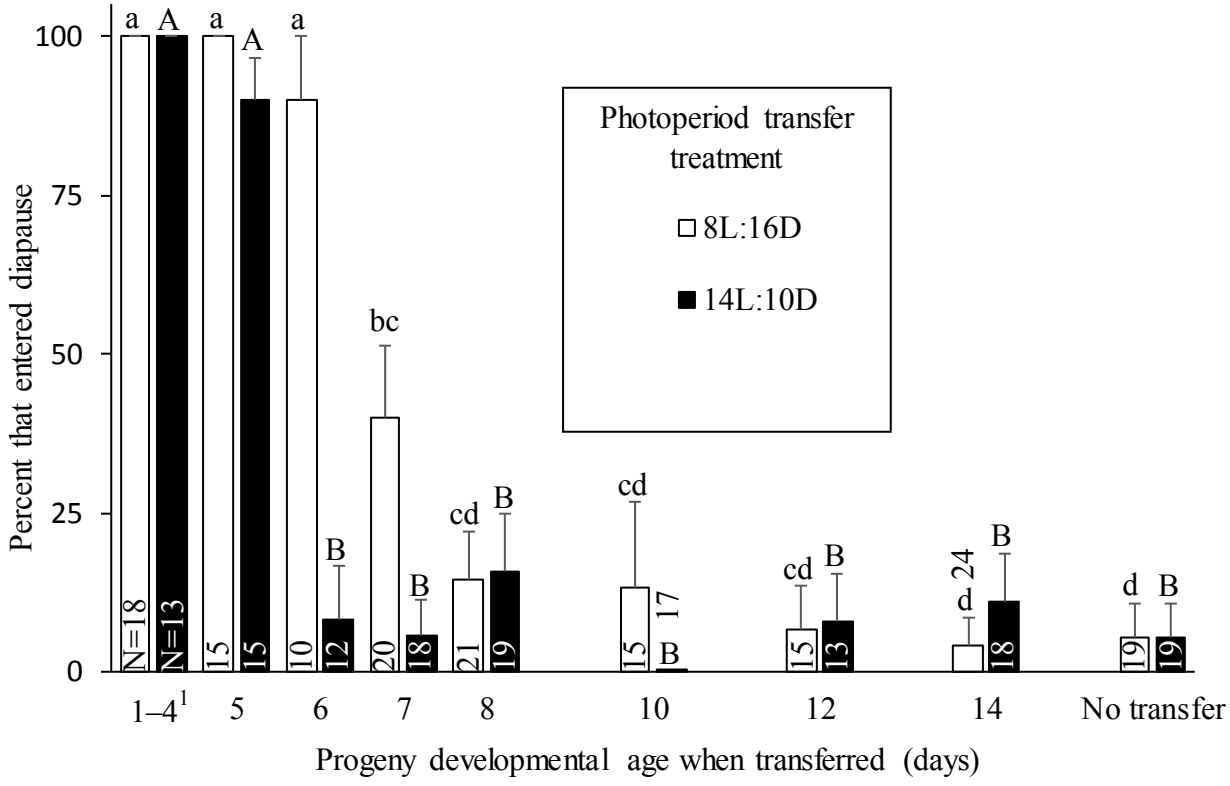

Figure 2.3. Mean (± SE) percent of individuals that entered diapause (percent diapause) when developing parasitoids were transferred from 16:8 to 8:16 or 14:10 photoperiod at different progeny developmental ages (Experiment 2.4). Developmental ages with the same letter within

Figure 2.3. (cont'd)

each photoperiod transfer treatment are not significantly different ($P < 0.05$; Wald's χ^2 test; Table 2.2). [1]N = number of diapausing mature larvae that were transferred.

Effects of photoperiod and chill duration on diapause termination on *O. agrili*.

The mean development time (DD_{10}) required for adult emergence from F_0-diapausing mature larvae subjected to a cold period varied significantly among photoperiods ($F = 932.6$; $df = 3, 332$; $P < 0.0001$), length of chill ($F = 1089$; $df = 2, 332$; $P < 0.0001$). Mean DD_{10} for adult emergence was lowest for 16L:8D and 14.5L:9.5D, followed by 8L:16D and 12L:12D (Fig. 2.4). Mean DD_{10} for emergence also varied significantly among chill periods, with DD_{10} increasing as chill times decreased (Fig. 2.4). There was also a significant interaction between photoperiod and length of chill ($F = 137$; $df = 6, 332$; $P < 0.0001$; Table 2.2). Diapausing *O. agrili* mature larvae subjected to the shortest chill period required much higher mean DD_{10} to emerge when reared in 12L:12D photoperiod than when reared at longer or shorter day lengths. Chill period had the least effect on DD_{10} required for adult emergence from diapausing mature larvae held at 16L:8D and 14.5L:9.5D photoperiods for which emergence times were similar among the three different chill durations (Fig. 2.4).

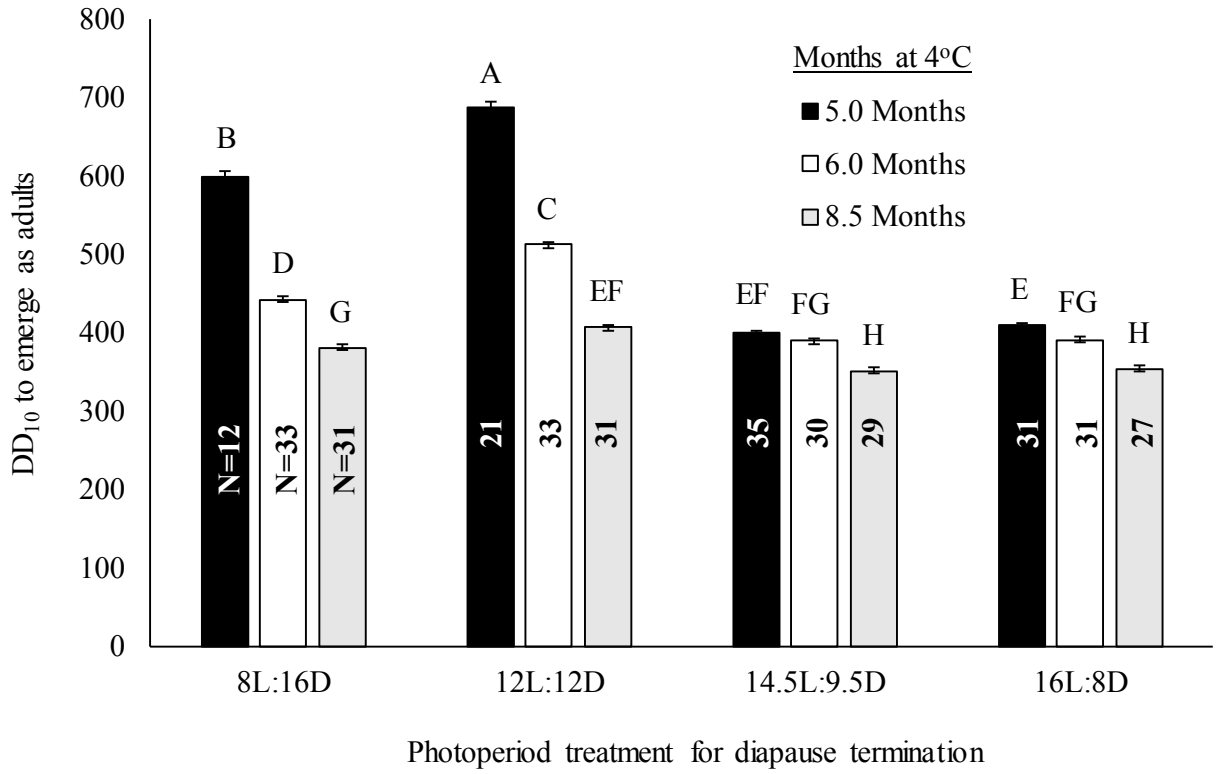

Figure 2.4. Least-squared mean (LSM ± SE) degree days base 10°C (DD_{10}) required for *O. agrili* to develop from diapausing mature larvae to adult emergence when exposed to different photoperiods at 25 ± 1°C after exposure to different chill periods at 4 ± 1°C (Experiment 2.5). LSMs with the same letter are not significantly different ($P < 0.05$; Tukey's HSD). [1]N = number of adults that developed and emerged from diapausing mature larvae.

Effects of photoperiod on diapause termination in diapausing *O. agrili* not subjected to chill.

Almost all diapausing mature larvae that originated in 12L:12D or 14L:10D photoperiods and were not subjected to a chill period, terminated diapause and emerged as adults after they were transferred to photoperiods with day lengths that were ≥14.5hr (Fig. 2.5). Significantly fewer terminated diapause and emerged when transferred from 12L:12D ($\chi 2 = 48.6862$; df = 5; P

< 0.0001) or from 14L:10D ($\chi 2 = 67.6677$; df = 4; P < 0.0001) to 14.25L:9.75D (Table 2.2, Fig. 2.5). No diapausing mature larvae that originated in 12L:12D, and remained in 12L:12D (i.e., control) or that were transferred to 14L:10D terminated diapause during this period (Fig. 2.5). One individual that originated and remained in 14L:10D (control) emerged as did a few individuals that originated and remained in 14.25L:9.75D (i.e., controls). Also, a few individuals that originated in 14.5L:9.5D and remained in 14.5L:9.5D or were transferred to 15L:9D or 16L:8D terminated diapause (Fig. 2.5).

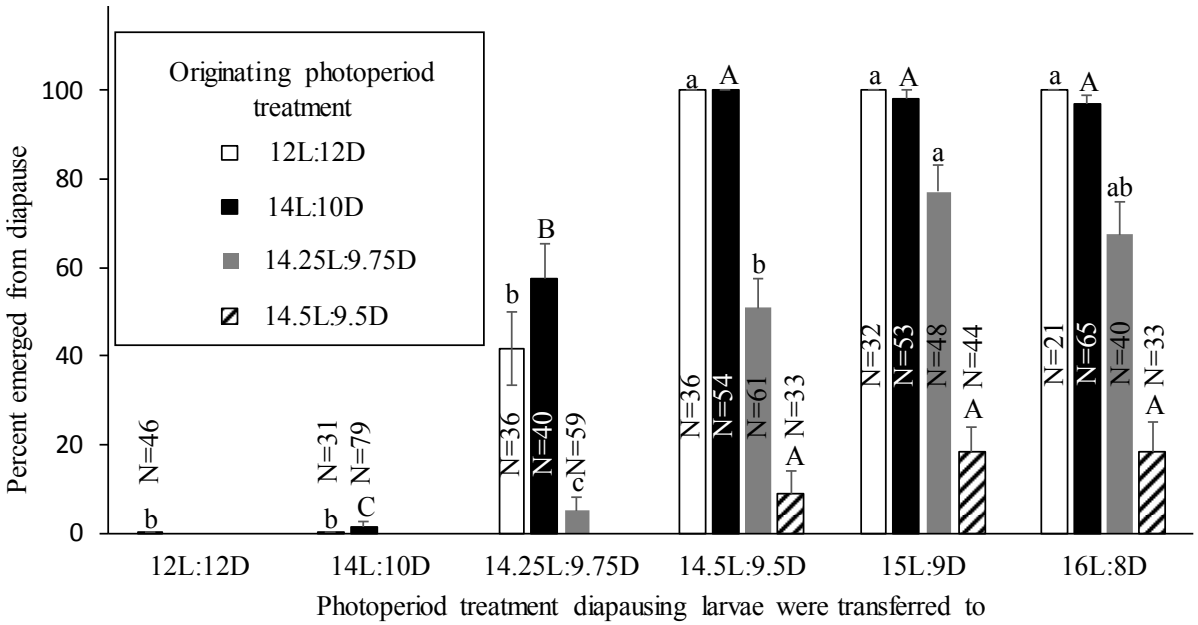

Figure 2.5. Mean (± SE) percent of *O. agrili* that developed to adults from mature larvae that were in photoperiod induced diapause and were not subjected to a chill period before they were transferred to photoperiods with longer day lengths (Experiment 2.6). Means with the same letter were not significantly different ($P < 0.05$; Wald X^2 test). [1]N = number of diapausing mature larvae that were transferred and either developed and emerged as adults or remained as diapausing mature larvae.

Comparison of day length and average degree day accumulation at *O. agrili* release sites.

The calendar dates that corresponded to the beginning of EAB adult emergence (DD_{10} = 250) and peak EAB adult emergence (DD_{10} = 560) varied across the 5 selected sites where *O. agrili* has been released (Table 2.9). At the most northern of the 5 sites, EAB emergence began in early July (ca., calendar day 190); whereas EAB emergence began in late January (ca., calendar day 20) at the most southern site. EAB peak emergence at the most northern site occurred in early-August (ca., calendar day 219) and in late-February (ca. calendar day 50) at the most southern site (Table 2.9). The hours of daylight varied across the five sites at the estimated dates for both EAB initial- and peak emergence (Table 2.9). At the beginning of EAB emergence there would be 15:55 hours of daylight at the most northern site and 10:24 hours of daylight at the most southern site. During the peak of EAB emergence there would be 14:40 hours of daylight at the most northern site and 11:10 at the most southern site (Table 2.9).

Table 2.9. For five different locations where *Oobius agrili* has been released in the U.S., the mean calendar day for a 38-yr period (1978-2016) estimating the beginning of EAB adult emergence (DD_{10} = 250) and peak abundance (DD_{10} = 560), and hours and minutes of daylight on this date [1]Hours and minutes of daylight for each calendar day were obtained from the following website: http://aa.usno.navy.mil/data/docs/Dur_OneYear.php).

			EAB adults begin emergence (250 DD_{10})		EAB peak adult abundance (560 DD_{10})	
Site no.	Latitude	Longitude	Mean Calendar day	Hours of daylight	Calendar day	Hours of daylight
1	47.2253	-88.4578	190	15:55[1]	219	14:40
2	43.5330	-84.5050	170	15:25	201	14:58
3	39.8015	-94.3763	51	10:57	126	14:04
4	36.2341	-85.7905	51	11:05	123	13:44
5	32.6625	-93.3693	22	10:24	50	11:10

Discussion

Photoperiodism modulates diapause in many insects, and the critical day length that elicits a response can vary considerably among species depending on their life history and geographic location (Beck, 1980). Photoperiod provides a consistent and accurate stimulus for signaling seasonal shifts when environmental conditions suitable for insect activity are beginning

or ending. Multivoltine species, as well as those that utilize ephemeral resources, such as egg parasitoids, often rely on photoperiod to regulate life history events (Beck, 1980; Boivin, 1994; Tauber et al., 1986). Therefore, it is not surprising that photoperiodism modulates diapause in *O. agrili*; a multivoltine egg parasitoid. The critical day length for *O. agrili* diapause induction occurred within a narrow range (c.a., 15min between 14.25 and 14.5 hours of daylight at 25° C) and diapause induction is directly determined by the photoperiod to which developing progeny are exposed prior to larval maturity. Adult emergence was also influenced by photoperiod and its interaction with the length of chill.

The photoperiodic-diapause response for *O. agrili* was weakest for larval progeny produced by F_0-diapaused adults, for which diapause was induced in some larvae exposed to shorter day lengths (i.e., 8L:16D and 12L:12D) while none entered diapause when exposed to long day length (i.e., 16L:8D). Diapause response to shorter day lengths was much higher when F_0-diapaused adults were 3wk old, while parasitism rates declined with maternal age. This suggests an interaction between maternal age and progeny photoperiod (i.e., 16L:8D vs. 8L:16D and 12L:12D) for induction of diapause, even though this interaction could not be statistically compared due to complete separation in the model as a result of the 16L:8D perfectly predicting the percent of progeny that entered diapause (i.e., no progeny placed in 16L:8D entered diapause). In a different study of *O. agrili* in which both the adult parasitoids and their developing progeny were exposed to 8L:16D or 16L:8D photoperiods, a significant interaction between maternal age and photoperiod on diapause induction was also found, as well as a similar decline in parasitism with increasing maternal age (Hoban et al., 2016).

The increase in photoperiod-induced diapause rates for progeny of F_0-diapaused adults as maternal age increased is likely attributed to an "interval timer" (Dixon, 1972; Lees, 1960;

Tauber et al., 1986). An interval timer is a species (or sub-species) specific post-diapause-period of time that is required for an insect to regain or reinstate its sensitivity to diapause inducing stimuli (e.g., photoperiod). This temporary restraint prevents early season progeny from entering diapause even though thresholds of diapause-inducing stimuli may be occurring in the current environment, thus allowing one or more generations to be completed before the majority of the population enters diapause. Sensitivity to stimuli is regained after an interval of time has occurred for the current generation (e.g., approximately 3wk for *O. agrili*), or in subsequent post-diapause generations.

The diapause response of *O. agrili* to short-day photoperiods is fully reinstated in the progeny of F_1- and F_2-nondiapaused adults, a finding reported in other *O. agrili* studies in which both adult parasitoids and their developing progeny were reared at the same photoperiods (Hoban et al., 2016; Larson and Duan, 2016). In this study, 100% of progeny produced by F_1- and F_2-nondiapaused adults entered diapause when they developed in photoperiods with day lengths that were ≤13.5hr long. Percent diapause was also very high for 14hr and 14.25hr day lengths, and transitioned to much lower rates for ≥14.5hr day lengths. As with progeny of F_0-diapaused adults, the photoperiod-diapause response occurs in the immature stages of progeny produced by F_1-nondiapaused adults and maternal or grand-maternal photoperiod treatments did not affect diapause rates (Tables 2.2, 2.6, 2.7). Response to photoperiod-induced diapause declined dramatically when developing *O. agrili* progeny where >5 days old (at 25°C). The larval stage of *O. agrili* lasts approximately 10–11 days at 25°C, after which meconium is excreted and pupation begins (Pers. observation). Therefore, diapause induction as a response to photoperiod occurred well before larvae reached maturity.

There was also a significant interaction between photoperiod and the nondiapaused generation of *O. agrili*. Increasing diapause rates with increasing post-diapause generation has been reported in *O. ennomophagus* (Anderson and Kaya, 1974). When developing larvae that were progeny of F_2-nondiapaused adults were exposed to longer days (14.25L:9.75D, 14.5L:9.5D, and 16L:8D), a significantly higher percent of progeny entered diapause compared to the progeny from F_1-nondiapaused adults. Low overall adult emergence (i.e., high diapause rates) for 14L:10D treatments may have inhibited the detection of the same significant interaction for this treatment, but it is unclear why differences among these generations were not found for 15L:9D or 15.5L:8.5D photoperiods. This interaction between photoperiod and nondiapaused generation could be evidence that the critical day length for the progeny of F_2-nondiapaused adults is slightly longer than for the progeny of F_1-nondiapaused adults. It is also possible that excessively long day lengths (i.e., 16L:8D) may induce diapause or be so unnatural they disrupt the pathway for photoperiodism in *O. agrili*, with this response being stronger for progeny from F_2-nondiapaused adults. The molecular mechanisms and pathways for determining day length in insects are not fully understood, however, it is generally agreed they are linked in some manner with circadian clock genes or analogues thereof (Ikeno et al., 2010; Meuti and Denlinger, 2013).

Study results indicate that the critical day length for *O. agrili* progeny from F_1- and F_2-nondiapaused adults, defined as the day length at which 50% of the population enters diapause (Tauber et al., 1986), was between 14.25–14.5hr day length. The critical day length response should presumably function to synchronize *O. agrili* diapause patterns with EAB's oviposition period within their endemic range (Fig. 2.1). Day length may vary above or below the critical day length during EAB peak emergence period (i.e., oviposition period) depending on

geographical location of the populations and this could have significant impacts on *O. agrili* population dynamics. For example, in some northern latitudes where *O. agrili* has been released (Table 2.9), the progeny of F_1-nondiapaused adults would experience day lengths longer than the critical day length for inducing diapause during peak EAB activity and therefore could have a higher percentage of progeny emerge as F_2-nondiapaused adults (i.e., two generations of nondiapaused adults would emerge in one season). This could result in higher parasitism rates because more *O. agrili* adults would be present to parasitize EAB eggs. However, if F_2-nondiapaused adults emerge too late, they may have difficulty finding suitable eggs to parasitize because the EAB oviposition period would be near completion for the season. In more southern latitudes, higher diapause rates could be expected for progeny from older F_0-diapaused adults as day lengths would be shorter than the critical day length for inducing diapause during EAB peak activity, and all progeny produced by F_1-nondiapaused adults should enter diapause, thus limiting *O. agrili* to a maximum of two generations. Fewer generations may result in lower parasitism rates but could increase the number of overwintering progeny and result in more F_0 adults available to perpetuate the population the following season. The effects of these possible scenarios on *O. agrili* fitness, along with EAB oviposition phenology, in more southerly regions of its current and potential ranges require further investigation. If photoperiod length in some regions results in parasitoid-host asynchrony, new strains of *O. agrili* with different critical day lengths may need to be acquired and evaluated for suitability.

The response of *O. agrili* to photoperiod may differ in natural settings compared to that determined under controlled laboratory conditions. For example, the day length perceived by *O. agrili* may differ from ambient day length considering that EAB often deposits its eggs in bark cracks and crevices (Wang et al., 2010). Depending on the size and orientation of the bark

cracks and crevices, day length may be reduced or undetectable for developing *O. agrili* larvae. Tree-bark texture, canopy cover, and topography may also affect diapause rates by reducing perceived day lengths. Additionally, *Oobius agrili* may be sensitive to day length into the twilight periods (i.e., civil, nautical, and astronomical), which could lengthen the perceived day length period. For example, Jackson (1963) found evidence that *C. cinctus* was sensitive to photoperiod into the astronomical twilight period.

Environmental conditions may also affect the critical day length of *O. agrili*. The most common interaction is that higher temperatures can increase critical day lengths for diapause induction as was found for *Microplitis mediator* Haliday (Hymenoptera: Braconidae) (Li et al. 2008). Also, Hoban et al. (2006) found that when F_0-diapaused *O. agrili* adults and their larval progeny were held in 8L:16D, more progeny entered diapause at 20°C compared to 30°C, while no difference was found between temperatures when adults and progeny were held at 16L:8D. Fluctuating humidity has also been found to affect diapause rates of *O. agrili* (Wetherington et al., 2017).

Emergence time of F_0 adults from diapausing mature larvae that underwent a chill period was significantly affected by photoperiod and length of chill, with significant interaction between these variables. Differences among treatments were small for all but the shortest chill period. However, significant differences were detected due to the highly synchronized emergence of *O. agrili* (i.e., most individuals emerged within a few days of each other for each treatment) that led to little variation in emergence times within treatments. The difference in mean DD_{10} between the latest emergence (at 12L:12D) and the earliest emergence (at 14.5L:9.5D) was only 55 DD_{10} for the longest chill period. This variable should be considered

when modelling *O. agrili* emergence and host synchrony in the field, given the window of susceptibility of EAB eggs to *O. agrili* parasitism is relatively short (Duan et al., 2014).
It is possible the delayed emergence of *O. agrili* reared under shorter day lengths (8L:16D and 12L:12D) was a result of an initial delay in morphogenesis due to photoperiods below the critical day length after mature larvae were exposed to 25°C post-diapause chill. However, the developmental rate of post diapause stages was not monitored. Wang and Laing (1989) found morphogenesis was delayed in 12L:12D compared to 16L:8D for *Holcothorax testaceipes Ratzeburg* (Hymenoptera: Encyrtidae), resulting in longer emergence times. But if this phenomenon did occur, then temperatures above the developmental threshold following diapause chill, appear to override the lack of critical day length and allow morphogenesis to occur. Photoperiod may have also directly affected development rates of *O. agrili*, as has been reported in several insect species (Beck, 1980; Tauber et al., 1986). Emergence differences among photoperiods may have also been attributed to a circadian basis for adult eclosion (Fantinou et al., 1998). For example, adults may only create exit holes and emerge from EAB eggs during daylight periods, and this could have delayed emergence for shorter day length treatments. However, it is unclear why emergence of adults in the 12L:12D photoperiod was slower compared to 8L:16D. From the results, it appears that *O. agrili* emergence from diapause follows a Type III photoperiod response curve as described by Beck (1980), where response to photoperiod occurs during short day lengths and long day lengths but not at intermediate day lengths.

Differences in emergence times for *O. agrili* among photoperiods were most significant for the shortest chill periods. This response has been documented in other insect species with the effect of photoperiod on emergence time tending to become less significant as chill periods

lengthen (Tauber et al. 1986). However, it is not clear if increasing the chill period hastens diapause completion in *O. agrili*, or if a time interval is required for diapause completion regardless of temperature during diapause. It seems reasonably intuitive that the interaction of chill period and photoperiod may function to prevent premature diapause termination in *O. agrili* when winter temperatures increase above the minimum developmental threshold and day length is below the critical day length. It is also likely that a chill period helps synchronize diapause development and ultimately spring emergence of F_0 nondiapaused adults.

Photoperiod-induced diapause can be terminated for most individuals by increasing the day length to ≥14.5hr without subjecting diapausing mature larvae to a chill period. A moderate number of individuals also terminated diapause when transferred to 14.25hr day length, while only one individual developed to adult that originated- and remained in 14.0hr day length (i.e., controls). Therefore, *O. agrili* does not require a cold period to terminate photoperiod-induced diapause. This may be important for *O. agrili*'s success as it is released and becomes established in southern latitudes in North America. However, survival of diapausing *O. agrili* larvae may be negatively affected if subjected to long periods at temperatures above minimum developmental thresholds due to any increased respiration which may deplete energy resources needed for development to adult and subsequent egg production. In addition, *O. agrili* individuals that enter photoperiod induced diapause prior to the summer solstice at some of the more southern latitudes, when day lengths are still increasing, may prematurely terminate this diapause if day length increases above the critical day length and remains above this threshold for a long enough period.

Conclusion

Results demonstrate that photoperiod affected both induction and termination of diapause in *O. agrili*. This likely has important implications in the population dynamics and fitness of this parasitoid at different latitudes. The critical day length for diapause induction was estimated to be between 14.25–14.5 hours of day length for progeny of F_1- and F_2-nondiapaused adults. Sensitivity to photoperiod occurs only during the larval stage of *O. agrili* progeny, and the likelihood of photoperiodic induced diapause declines abruptly after the first five to six days of *O. agrili* progeny development. During the course of this study, no evidence that adult (maternal or grand-maternal) photoperiod history affected diapause induction in progeny was found. However, the age of F_0-diapaused adults does play a role in sensitivity of their progeny to photoperiod and is likely attributed to a time interval that is required for sensitivity to photoperiod to be regained. Length of chill and its interaction with photoperiod also significantly affected the DD_{10} required for *O. agrili* adult emergence. Results from this study will be useful for predicting and evaluating the effectiveness of *O. agrili* in regulating EAB populations as it continues to be released within the expanding range of EAB in North America.

CHAPTER 3: EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON *OOBIUS AGRILI* POPULATION DYNAMICS AND SYNCHRONY WITH EMERALD ASH BORER OVIPOSITION

Abstract

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). In North America, EAB has spread and *O. agrili* has been released well beyond their endemic climatic ranges in Northeast China. A temperature driven multiple cohort rate summation model was developed to simulate *O. agrili* adult emergence from diapause (F_0 generation), two generations of nondiapaused adult emergence (F_1 and F_2), and EAB oviposition. The model was used to examine spatiotemporal host-parasitoid synchrony across a north-south gradient from Duluth, MN (lat. 46.8369, long. -92.1833) to Shreveport, LA (lat. 32.4469, long. -93.8242). Temporal occurrences of critical day length for *O. agrili* diapause induction were integrated into the model to examine spatiotemporal interactions between temperature regimes and photoperiods. *Oobius agrili* and EAB trapping data from south central and northwestern Lower Michigan were used for model validation. Model simulations demonstrated that F_0 adult emergence remained temporally synchronized with initiation of EAB oviposition across the north-south gradient. Emergence of F_1 adults was most synchronized with peak EAB oviposition compared to other *O. agrili* generations across all locations. However, EAB oviposition was almost complete when F_1 adults emerged at Duluth, MN during one year due to cooler summer temperatures. EAB oviposition was near completion or had completed when F_2 adult emergence was predicted at all locations. Comparison of *O. agrili* trap captures with model simulations demonstrated that primarily two adult *O. agrili* generations (F_0 and F_1) emerge per year in Michigan. Diapause was apparently induced in almost all F_2 larvae despite day lengths longer than critical day length in

south central Michigan. The critical day length for *O. agrili* diapause induction varied temporally across the north-south gradient during emergence of *O. agrili* generations. Determining day lengths perceived by *O. agrili* larvae in the field should improve model realism for examining spatiotemporal effects of photoperiod and temperature interactions on *O. agrili* population dynamics and its efficacy as an EAB biological control agent.

Introduction

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid introduced into North America as part of a classical biological control program for emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)(Bauer et al., 2015a). EAB is an invasive pest of ash trees and threatens to functionally extirpate many North American ash species (Herms and McCullough, 2014). Both EAB and *O. agrili* are native to China, with *O. agrili* more or less restricted to northeastern China (Liu et al., 2007; Wang et al., 2015; Yao et al., 2016) and EAB having a considerably broader geographic distribution (Bray et al., 2011; Chamorro et al., 2015; Haack et al., 2002).

Since the discovery of EAB in Michigan, U.S., and Ontario, Canada, in 2002 (Haack et al., 2002), it has spread south to Texas, U.S., north to Manitoba, Canada, east to Nova Scotia, Canada, and west to Colorado, USA. EAB occurs in all eastern U.S. states except for Mississippi and Florida and all eastern Canadian provinces except for Newfoundland (Emerald Ash Borer Information Network, 2020). *Oobius agrili*, a solitary, parthenogenetic egg parasitoid of EAB, was approved for release in the U.S. in 2007 and in Canada in 2013 (Bauer et al., 2015a; Duan et al., 2018). *Oobius agrili* has been released throughout most of EAB's distribution in North America and establishment has been confirmed in 13 U.S. states and 2 Canadian provinces

(MapBioControl, 2020). However, it is not known how well *O. agrili* will perform in climates that differ from those in its native distribution.

Oobius agrili is a multivoltine species that overwinters as mature diapausing larvae within host eggs. In its endemic range, *O. agrili* has at least two generations per year (Liu et al., 2007); however, it has not been determined how many generations occur throughout its distribution in North America. In the laboratory, *O. agrili* is capable of producing multiple generations that do not require diapause when reared under a 16 hours light:8 hours dark (16L:8D) photoperiod (Yao et al., 2016). Diapause of *O. agrili* is induced when developing *O. agrili* larvae, within EAB eggs, are subjected to shorter day lengths (<14.25 hr) (Hoban et al., 2016; Larson and Duan, 2016; Petrice et al., 2019). *Oobius agrili* adults can be separated into two distinct phenotypes based on their diapause history which determines their progeny's diapause response to photoperiod. These phenotypes are: 1) adults that developed from diapausing larvae (diapaused adults) and 2) adults that developed from nondiapaused larvae (nondiapaused adults) (Hoban et al., 2016; Petrice et al., 2019). When developing *O. agrili* larvae are reared at day lengths that are <14 hr, a small percentage (ca., 10%) of progeny produced by diapaused adults enter diapause, while 100% of progeny produced by nondiapaused adults entering diapause. The critical day length when 50% of individuals enters diapause falls between 14.25 and 14.5 hours of daylight (Petrice et al., 2019).

EAB is primarily univoltine, but can be semivoltine. Semivoltinism is most common for EAB larvae that develop in cooler climates or when relatively higher host resistance may occur (Cappaert et al., 2005; Wang et al., 2010; Wei et al., 2007). EAB overwinters as mature larvae folded in a J-shape within pupation chambers in the outer-bark or outer-sapwood of trees, or as immature larvae in the cambial region (Cappaert et al., 2005; Chamorro et al., 2015; Duan et al.,

2010). Overwintered immature larvae continue feeding the following spring and summer, and after they are mature, create pupal cells where they overwinter as J-larvae. Mature EAB larvae must experience a chill period before they pupate and develop to adults (Duan et al., 2013). EAB adults emerge in spring or summer and feed on host foliage for several days before they become sexually mature and continue to feed on host foliage throughout their life. Oviposition begins after EAB females are sexually mature and have mated, and females can oviposit for several weeks in the laboratory (Rutledge and Keena, 2012). EAB's phenology appears to be regulated predominately by temperature, with the exception of feeding larvae that may be influenced by host-plant resistance (Cappaert et al., 2005).

Temperature plays a significant role on the population dynamics and temporal synchrony of EAB and *O. agrili* (Duan et al., 2014; Wetherington et al., 2017). For instance, *O. agrili* adult emergence from diapause and emergence of subsequent generations must be synchronized with EAB oviposition. However, it is not clear how the interaction of photoperiod and temperature will affect *O. agrili* voltinism and diapause. Ideally, *O. agrili* diapause induction should be synchronized with the termination of EAB oviposition to prevent *O. agrili* adults from emerging after EAB oviposition has ended. The effects of temperature and photoperiod on the temporal synchrony of *O. agrili* with EAB oviposition is important for understanding their population dynamics (Petrice et al., 2019; Wetherington et al., 2017).

A degree day model is a common method for estimating the effects of temperature on insect development rates. With this method, the development time of ectothermic organisms is correlated with the number of degree days that have accumulated. Degree days are calculated as the number of degrees the temperature is above a pre-determined threshold. Brown-Rytlewski and Wilson (2004) developed a degree day model for EAB emergence that is commonly used for

making predictions of EAB adult emergence. More recently, Duarte (2013) developed a probit model based on degree day accumulation to predict EAB emergence. Currently, there is no model (degree day or otherwise) to predict EAB sexual maturation, oviposition, or *O. agrili* phenology.

The simplicity of degree day models makes them convenient for forecasting discrete phenological events of insects; however, their assumption that development rates are linear functions of temperatures can compromise their accuracy (Gray, 2012; Wagner et al., 1991, 1984). Damos and Savopoulou-Soultani (2012) cited numerous studies that demonstrate that insect development rates near their upper and lower developmental thresholds are nonlinear, which can reduce the accuracy of linear-based phenology models. Another approach to modeling insect development is to determine the development rates of insects at a range of temperatures including those near their upper and lower developmental thresholds. Nonlinear functions can then be fitted to rates and used to calculate the accumulated development over time as a function of temperature. This method is often referred to as "rate summation" (Gray, 2012; Régnière et al., 2012).

Realism of phenology models can also be improved by quantifying the variability of development rates within insect populations (Régnière, 1984; Wagner et al., 1984). Depending on the genetic diversity within a population and interactions with environmental factors, there can be considerable variation in development rates among individuals within populations. A cumulative probability function is frequently used to describe this variability (Wagner et al., 1984). Developmental variability can be integrated into phenology models allowing independent cohorts to be tracked as they pass through subsequent life stages, giving more realistic estimates of the populations' response to temperature (Gray, 2012; Régnière, 1984).

The objectives of this study were to evaluate the temporal synchrony of *O. agrili* adult emergence with EAB oviposition and its interaction with *O. agrili* photoperiod-induced diapause across their geographic distributions in North America. A temperature driven multiple cohort rate summation model was developed to simulate temporal synchrony of *O. agrili* with EAB oviposition. The accuracy of the model was assessed by comparing simulations with field trap captures of EAB and *O. agrili* from four sites sampled over a three-year period in Michigan. Model simulations were used to compare synchrony of *O. agrili* generations with EAB oviposition and the temporal occurrence of critical day length for *O. agrili* diapause induction at different geographic locations.

Materials and Methods

Temperature assays for determining development times.

Development time for *O. agrili* and EAB adult emergence.

Oobius agrili used in this study originated from Changchun, Jilin Province, China (43.8666 lat., 125.3500 long.) and were maintained in the USDA Forest Service Northern Research Laboratory (Michigan State University, East Lansing, MI) for 36 generations. Diapausing *O. agrili* larvae (F_0 individuals) used were progeny of nondiapaused females. Diapausing larvae were forced into diapause by holding them in a short day photoperiod (8L:16D) at 25°C immediately after parasitoid oviposition. After 30 days, F_0 *O. agrili* larvae were held at 10°C for 30 days, and then moved to 4°C for 7–9 months (chill period). After F_0 larvae had undergone chill periods, they were reared at: 10, 15, 20, 25, 30, 34, or 39°C constant temperatures and adult emergence was monitored daily. Temperature treatments were replicated 2–3 times (Table 3.1). Individuals held in the 10°C treatment were transferred to 25°C after 60

days. Rates for transferred individuals were calculated using the following equation from Gray (2009):

$$ {}_iR_T(t) = [1 - ({}_it_{25} / {}_it_{c25})] / t_T \quad (1) $$

where ${}_it_{25}$ is the median time in days that individuals from the ith replicate were held at 25°C, ${}_it_{c25}$ is the median time required for individuals held at 25°C to develop and emerge as adults for the ith replicate (determined from the 25°C temperature treatment); t_T is the time in days that individuals for the ith replicate were held at the experimental temperature T (i.e., 10°C); and ${}_iR_T(t)$ is the development rate for the ith replicate.

Developmental rates of first generation nondiapaused *O. agrili* progeny (F_1 individuals) were determined by holding EAB eggs parasitized by F_0 adults at 10, 15, 20, 25, 30, 34, or 39°C constant temperatures and monitoring adult emergence daily. Parasitized eggs were obtained by observing parasitism of EAB eggs by F_0 adults in petri dishes. Within three hours of parasitism, EAB eggs were placed in one of the temperature treatments. Individuals held in 10°C were transferred to 25°C after 60 days for development to be completed. Rates for transferred individuals were calculated using Eq. 1 and the median development time for the F_1 25°C-constant-temperature treatment. Each temperature treatment was replicated 1–3 times (Table 3.1). Developmental rates for the F_2 *O. agrili* generation were assumed to be the same as the F_1 generation.

EAB adult emergence rates were determined by rearing EAB adults from white (*F. americana* L.) and green ash (*F. pennsylvanica* Marshall) logs that contained diapausing J-larvae. Logs were cut from naturally infested ash trees during the month of January at Doake Research Forest, Indiana (41.368855 lat., −87.253270 long.), in 2016; West Lafayette, Indiana (40.504359, −86.901039), Okemos, Michigan (42.693989, −84.382596), and Ithaca, Michigan

(43.233431, −84.446431), in 2017; and West Lafayette, Indiana, in 2018. Logs were stored at 4°C for a maximum of 120 days before transferring them to one of the temperature treatments. A subset of logs was dissected for each replicate to determine which EAB larval stages were present in logs prior to rearing. Of the 69 larvae dissected from logs combined for all sites and years, 94% were diapausing J-larvae, 3% were prepupae, and 3% were immature larvae.

EAB infested logs were placed in growth chambers at 10, 15, 20, 25, 30, 34, or 39°C constant temperatures. Logs held at 10°C and 15°C remained there for 90 days before transferring them to 25°C. Logs held at 15°C were also transferred because preliminary assays found EAB could develop from J-larvae to adults at 15°C, but adults died before emerging. Development rates for transferred individuals were calculated using Eq. 1. EAB adult emergence from logs at each temperature was replicated 2–4 times (Table 3.1), with adult emergence recorded daily.

Table 3.1. Mean temperatures, rates, number of individuals, and replicates for *O. agrili* from diapausing larvae to adult, *O. agrili* from parasitoid egg to adult, and EAB from diapausing larvae to adult reared in growth chambers.

Mean (± se) temperature	Mean (± se) rate	No. individuals	No. replicates
O. agrili adult emergence from diapausing larvae.			
10.0 ± 0.1	0.00118 ± 0.00005	106	2
15.2 ± 0.1	0.00581 ± 0.00005	107	3
20.3 ± 0.3	0.02390 ± 0.00034	117	3
24.4 ± 0.6	0.03890 ± 0.00019	112	3
29.7 ± 0.0	0.05370 ± 0.00042	124	3
33.7 ± 0.1	0.04370 ± 0.00059	64	3
39.1 ± 0.1	0	0	2
O. agrili adult emergence from parasitoid eggs.			
10.1 ± 0.	0	0	2
14.9 ± 0.2	0.00786 ± 0.00009	49	3
20.4 ± 0.2	0.03000 ± 0.00015	57	2
24.9 ± 0.4	0.04720 ± 0.0040	67	3
29.8 ± 0.2	0.06980 ± 0.00043	60	3
33.7	0.05790 ± 0.00094	27	1
39.1	0	0	1
EAB emergence from diapausing larvae.			
10.0 ± 0.1	0 0	0	2
15.3 ± 0.3	0.00632 ± 0.00009	126	3
19.6 ± 0.1	0.0210 ± 0.00021	149	4
24.8 ± 0.3	0.03560 ± 0.00028	164	4
30.2 ± 0.2	0.05610 ± 0.00035	137	4
34.0 ± 0.4	0.05780 ± 0.00064	30	3
39.0 ± 0.1	0 0	0	2

Development time for EAB sexual maturation, oviposition period, and fecundity.

EAB sexual maturation and oviposition were determined by placing one male and one female in individual rearing containers within one day of emergence from logs held at room temperature (ca. 25°C). EAB adults were reared following the procedure described in Rutledge and Keena (2012). Each beetle pair was given fresh *F. uhdei* (Wenzig) Lingelsh. foliage while

held in 15, 20, 25, 30, 34, or 39°C constant temperature. Foliage was changed every other day for 30, 34, and 39°C; every 2–3 days for 25°C; and every 3–4 days for 15 and 20°C. Eggs were removed daily and placed in 25°C for 24–48 hours to allow embryogenesis to occur. Afterwards, eggs were inspected to determine if they were fertile (chorion brown and unbroken), infertile (yellow with the chorion unbroken), or defective (chorion broken, not completely formed, or damaged during handling) (Rutledge and Keena, 2012). Males were randomly reassigned among females within their respective rearing temperatures each week. Adults were maintained in their respective temperatures until they died. Three different EAB cohorts (i.e., replicates) that emerged in August 2017, May 2018, and May 2019 were used to determine EAB oviposition rates and fecundity.

Table 3.2. Mean temperatures, oviposition longevity rates, eggs per female, and number of individuals and replicates for female EAB reared in growth chambers.

Mean (± se) temperature	Mean (± se) rate $\times 10^{-2}$	Mean (± se) eggs per female	No. individuals	No. replicates
15.0 ± 0.2	0 ± 0	0 ± 0	34	2
19.6 ± 0.1	2.12 ± 0.16	10.5 ± 3.3	19	3
24.8 ± 0.3	2.97 ± 0.22	107.0 ± 13.5	57	3
30.1 ± 0.3	5.61 ± 0.42	108.0 ± 13.2	54	3
34.2 ± 0.5	9.03 ± 0.50	48.5 ± 8.1	41	3
39.1 ± 0.2	0 ± 0	0 ± 0	32	2

Rearing chambers

All phenology experiments were conducted in environmental chambers (Percival Scientific Inc., Perry, IA) under 16L:8D photoperiod at ~70% relative humidity, and set at the respective experimental temperature. HOBO Pro temperature data recorders (Onset, Pocasset,

MA) were used to measure temperature every 30 minutes in each of the environmental chambers. Thirty-minute temperatures were averaged for each temperature treatment to determine actual chamber temperature for each replicate.

Developmental rate functions

F_0 *O. agrili*, F_1 *O. agrili*, and EAB adult emergence functions.

For EAB adult emergence and both generations of *O. agrili*, median developmental rates for each temperature treatment within each replicate were calculated as the inverse of the interpolated median development time (1/ median days). A nonlinear equation was fitted to the interpolated median developmental rates as a function of temperature for the following life stages: a) diapausing *O. agrili* larvae to F_0 adults, b) *O. agrili* parasitoid eggs to F_1 adults (F_2 development was assumed to be the same), and c) EAB diapausing larvae to adults. The following equation, which was first described by Logan et al. (1976) and algebraically modified by Régnière (1984), was used:

$$R(T) = P_1\{[1 + exp\,(P_2 - P_3 * \tau)]^{-1} - exp\,[(\tau - 1) / P_4]\} \quad (2)$$

where τ = (temperature − T_b) / (T_m − T_b); T_b = low temperature developmental threshold; and, T_m = high temperature developmental threshold. T_b and T_m were predetermined based on the minimum and maximum experimental temperatures. Model convergences were successful for fitting development rate equations for development to the adult stage for all life stages (Fig. 3.1 A, C, E; Table 3.3).

Variability in development rates for adult emergence of F_0 *O. agrili*, F_1 *O. agrili*, and EAB were modelled using the "same rate" method described in Gray (2012) (also see Wagner et

al. 1984b) for modeling variability in development rates. All development rates [$R(T)$] for each life stage were normalized by dividing the number of days to develop by the interpolated median days to develop for each temperature and replicate. The following Weibull function was fitted to the normalized rates:

$$F(t) = 1 - exp\,[-(x - \lambda/\beta)^{\alpha}] \quad (3)$$

where x represents the normalized development rates [$R(T)$]; λ, β and α are fitted parameters; and $F(t)$ is the cumulative probability of emergence. Weibull model convergences were successful for fitting the normalized adult development rates (Fig. 3.1B, D, F; Table 3.3).

Table 3.3. Parameter estimates and adjusted R^2 values for development rates and cumulative probability for *O. agrili* and EAB adult emergence.

	Development rate parameters							Cumulative probability parameters			
Insect	P_1	P_2	P_3	P_4	T_b	T_m	R^2	α	β	λ	R^2
F_0- *O. agrili*	0.060	3.871	9.290	0.0483	40	9	0.988	3.100	0.166	0.848	0.992
F_1- *O. agrili*	0.093	2.808	6.394	0.061	40	12	0.975	3.394	0.144	0.873	0.988
EAB	0.067	3.253	7.242	0.0362	40	10	0.984	6.086	0.343	0.675	0.991

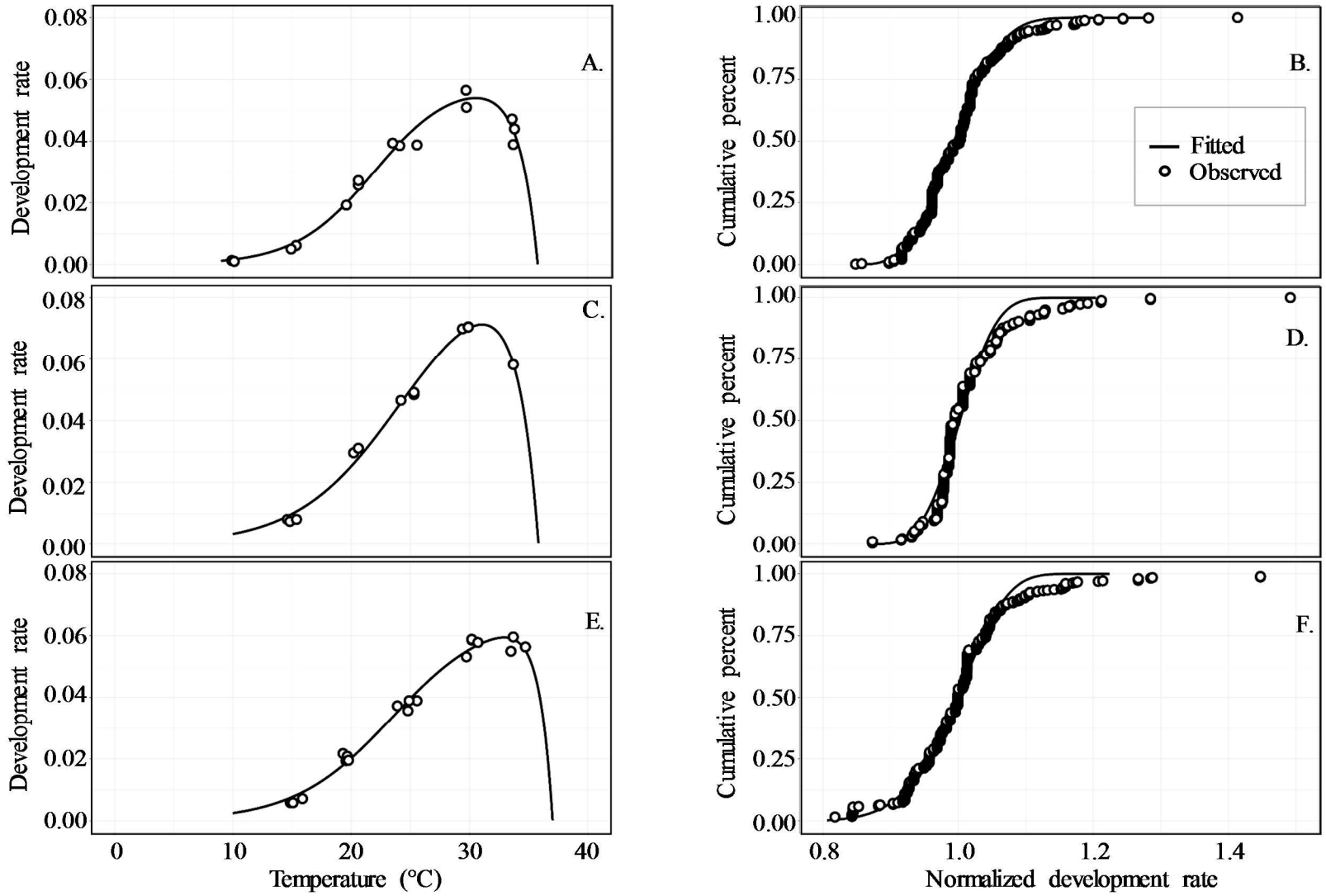

Figure 3.1. Functions fitted to observed development rates (1/days) and normalized cumulative development for F_0 *O. agrili* (A. and B.), F_1 *O. agrili* (C. and D.), and EAB development (E. and F.) to adult.

EAB oviposition and fecundity functions

A modified version of methods from Kim and Lee (2003) and Choi and Kim (2016) was used to model EAB oviposition. With their model, fecundity, oviposition longevity, cumulative oviposition, and cumulative survival were combined to predict oviposition over time as a function of temperature. The current model differs in that survival ends on the day that EAB adults stop laying fertile eggs (i.e., oviposition survival), rather than the day on which adults die. This was done because EAB adults often live several weeks in the laboratory after laying their last fertile egg and the objective was to model the EAB oviposition period rather than adult longevity.

EAB adult oviposition longevity is the time in days from when EAB females emerge as adults to the last day that fertile eggs are laid and includes the preoviposition period. A sigmoid function was fitted to the reciprocal of interpolated median days of EAB ovipositional longevity as a function of temperature:

$$r(T) = a + exp\,[-(b - T) / c] \quad (4)$$

where $r(T)$ is the oviposition longevity rate at temperature (T); and a, b and c are fitted parameters. Model convergence of oviposition longevity was successful (Fig. 2.2A; Table 3.4). Oviposition age (Px_i) was determined by summing oviposition longevity rates over time (i.e., days).

EAB fecundity was modeled by averaging total fertile eggs laid per female within each temperature treatment and replicate. The following function was fitted to the average number of eggs laid per female as a function of temperature:

$$f(T) = \omega * \exp\,[1 + (\varepsilon - T)/\kappa - \exp\,((\varepsilon - T)/\kappa] \quad (5)$$

where, $f(T)$ is lifetime fecundity at temperature T; ω is maximum fecundity; ε is temperature when maximum fecundity occurs; and κ is a fitted constant. Model convergence was successful for lifetime fecundity (Fig. 3.2C; Table 3.4).

EAB cumulative oviposition was modeled by first calculating the daily cumulative proportion of fertile eggs laid by all females within each temperature and each replicate. The cumulative proportion of eggs was then normalized by calculating daily oviposition age (Px_i) for each replicate and temperature. Eq. 3 was fitted to cumulative oviposition $p(Px_i)$ as a function of oviposition age (Px_i) (Fig. 3.2B).

Cumulative oviposition survival was determined by calculating the daily proportion of EAB females that had not terminated oviposition (i.e., EAB females laying fertile eggs) for each replicate and temperature. The oviposition survival was normalized by calculating oviposition age (Px_i) when each female stopped laying fertile eggs. Finally, the following modified Weibull function was fitted to the proportions of females that were ovipositing [$s(Px_i)$] as a function of oviposition age (Px_i):

$$s(Px_i) = exp\ \{-[(Px_i - \lambda) / \delta]\}^{\alpha} \quad (6)$$

where λ, δ, and α are fitted constants. Model convergence was successful for describing cumulative oviposition survival (Fig. 3.2D).

Female fecundity, cumulative oviposition, and cumulative oviposition survival were combined to model daily EAB oviposition [$ro(T)$] as a function of temperature as follows:

$$ro(T) = f(T) \times [p(Px_{i+1}) - p(Px_i)] \times s(Px_i) \quad (7)$$

Parameter estimates for all nonlinear models were determined using the nonlinear least squared (nls) method in the R statistical package (R Core Team, 2018). Adjusted R^2 values were calculated for all fitted models.

Table 3.4. Parameter estimates and adjusted R^2 for EAB oviposition.

Oviposition stage	Parameters and estimates			Adjusted R^2
Oviposition longevity	$a = 0.016$	$b = 46.327$	$c = 4.588$	0.800
Female fecundity	$\varepsilon = 26.360$	$\kappa = 6.000$	$\omega = 73.154$	0.601
Cumulative oviposition	$\alpha = 1.962$	$\beta = 0.736$	$\lambda = 0.188$	0.998
Oviposition termination	$\alpha = 1.836$	$\delta = 0.938$	$\lambda = 0.281$	0.998

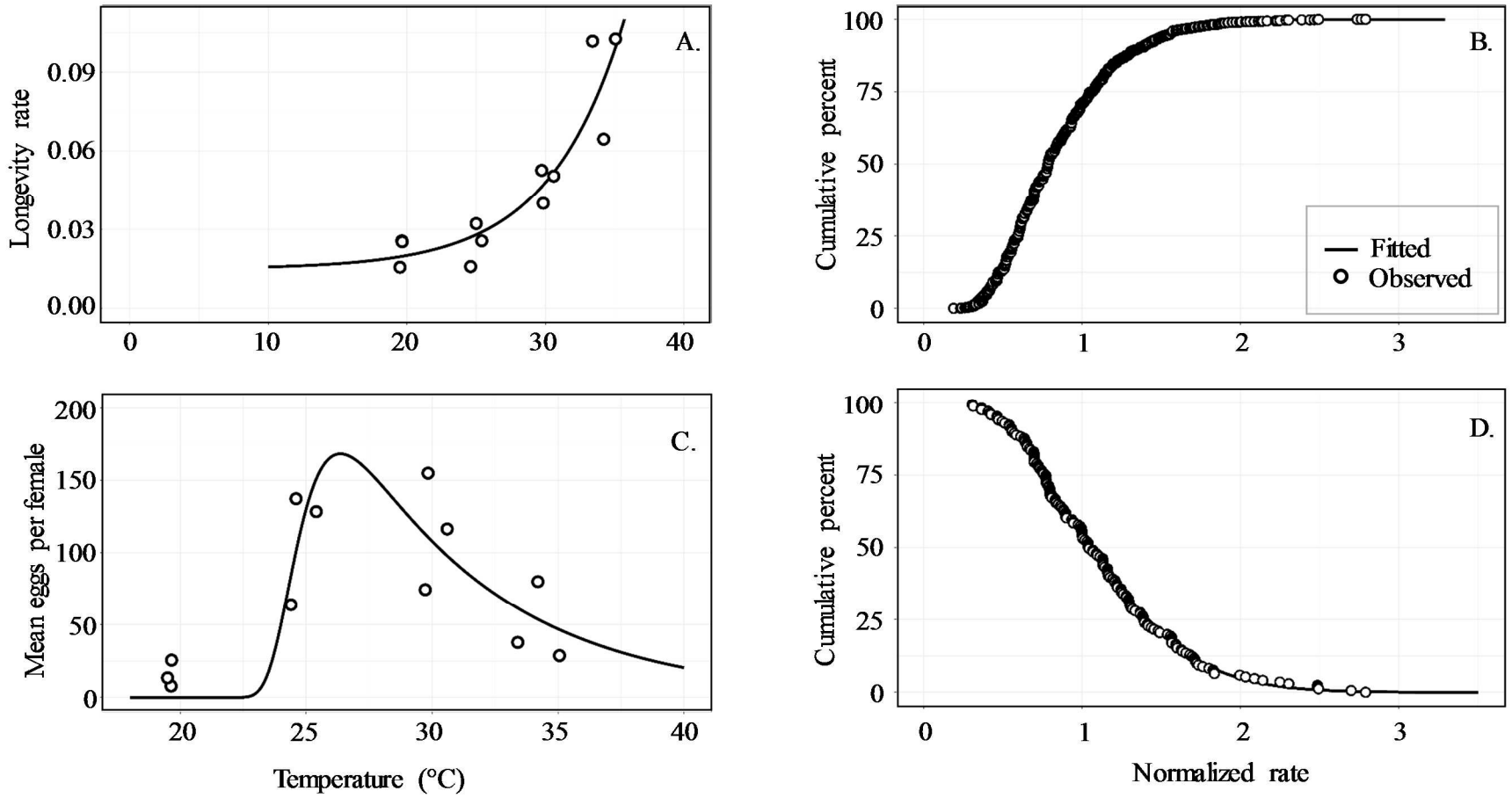

Figure 3.2. Functions fitted to predicted EAB oviposition longevity rate (1/days); A.), cumulative oviposition (B.), EAB fecundity (C.), and cumulative oviposition survival (D.).

Phenology model description and simulations

The rate equations for the five life stages described above were combined into a multiple cohort rate summation model that simulates the following: EAB adult emergence from diapause, EAB oviposition, *O. agrili* adult emergence from diapause (i.e., F_0-adults), and adult emergence of two *O. agrili* nondiapaused generations (i.e., F_1 and F_2). Development rates were calculated using hourly temperature data obtained from Cli-MATE (2019) and Michigan State University Enviroweather (2019) for weather stations nearest the sites selected for simulation runs (Fig. 2.3). Daily developmental rates and cumulative probabilities were divided by 24 to give hourly rates for simulations (model time step = 1 hr). Simulation runs began with all overwintering EAB and *O. agrili* larvae at the same stage of development (i.e., one cohort each). With each succeeding time step, developmental rates as a function of temperature were summed until each life stage was complete. Accumulated rates were input into the appropriate cumulative probability function to calculate the proportion of individuals that completed each life stage. The proportion of the population that completed development during each hourly time-step formed a cohort of recruits for the subsequent life stage and time-step. Hence, subsequent life stages were composed of the flow of cohorts arriving each hour. Cohorts maintained their own rate summation and developmental stage when they transitioned to the next life stage. Model outputs included emergence curves for each of the five life stages. The Julian days when day length were below critical day length for *O. agrili* diapause induction (i.e., 14.375 hours; Petrice et al. 2019) were also determined for each simulation run. Simulations were performed for four sites over a three-year period in south central and northwestern Michigan for comparison with *O. agrili* and EAB field-trapping data (see section *EAB and O. agrili field data*; Fig. 2.3). Simulations were also run for four locations where *O. agrili* was released along a north-south gradient in the

U.S. [Duluth, Minnesota (lat. 46.8369, long. -92.1833); Indianapolis, Indiana (lat. 39.8250, long. -86.2958); Knoxville, Tennessee (lat. 35.8181, long. -83.9858); and Shreveport, Louisiana (lat. 32.4469, -93.8242)] for broader comparisons of spatiotemporal host-parasitoid synchrony (Fig. 2.3). The simulation model was written with GNU Fortran 7.2.0 (Simply Fortran Ver. 2.41, Build 2564, GTK+).

EAB and *O. agrili* field data

EAB and *O. agrili* adult activity were monitored at three sites in south central Michigan [Gratiot-Saginaw (lat. 43.2337, long. -84.4477); Legg Park (lat. 42.69403, long. -84.3822), and Harris Nature Center (lat. 42.6965, long. -84.3752)] during 2016, 2017 and 2018, and one site in northwestern Lower Michigan [Eastport (lat. 45.1139, long. -85.3324)] during 2016, where both EAB and *O. agrili* are established (Fig. 2.3). EAB adult flight period was monitored using fluon-coated green funnel traps baited with cis-3-hexanol (Crook et al., 2014). Traps were suspended from the lower- or mid-canopy of ash trees and cis-3-hexanol lures were replaced after traps were in the field for ten weeks. Propylene glycol was placed in trap collection cups to kill and preserve insects. Trap contents were strained through a paint strainer and then placed in reclosable plastic bags and frozen until processed.

Oobius agrili were captured in unbaited yellow pan traps attached approximately 1.75m-high to the south-side of ash trees (USDA–APHIS/ARS/FS, 2019). A solution of 50:50 food-grade propylene glycol and H_2O, with a small amount of dish liquid (c.a., 2.5 mL/L) to reduce surface tension, was placed in yellow pan traps to capture and preserve insects. Trap contents were strained through a paint strainer and then placed in reclosable plastic bags and frozen until processed.

In 2016, 3 funnel traps and 40 yellow pan traps were placed at each of the 4 study sites (Fig. 3.3). At the south central sites, traps were placed in the field 24–27 May 2016 and removed 26–30 September 2016. Traps were deployed at the northwestern site on 10 June 2016 and removed 4 October 2016. Trap samples were collected bi-monthly in 2016. In 2017 and 2018, 3 funnel traps and 10 yellow pan traps were placed at each of the south central sites on 17–18 May and removed 28–29 September. Trap samples were collected weekly during 2017 and 2018. The northwestern site (Eastport) was excluded in 2017 and 2018 because of logistical constraints.

Figure 3.3. Map of eastern U.S. showing sites where *O. agrili* and EAB phenology were simulated and where adults of both species were trapped (red circles—Michigan only) for model validation.

Comparison of simulations with field data.

Percentages of EAB and *O. agrili* captured were calculated by dividing the number of individuals captured for each sample date by total number captured for each respective site and year. The percentage of EAB and *O. agrili* captured for each sample date were plotted over time to compare simulation outputs with field data. Mean absolute days between Julian dates for model predictions and trap captures were calculated for: A) the first day of predicted F_0 *O. agrili* and EAB adult emergence with the first field trap capture of each; B) 95% emergence of EAB and the peak EAB trap capture (i.e., sample date when the highest percentage was captured); C) 95% completion of EAB oviposition and the last trap capture of EAB females; and D) 95% emergence of each *O. agrili* generation and peak *O. agrili* trap capture. Also, the predicted percentage of EAB oviposition completed when 50% adult emergence was predicted for each *O. agrili* generation was calculated for each site.

Results

Model validation.

When simulation results were compared with trap captures at Michigan sites averaged over all sites and years, the predicted first emergence (absolute mean ± absolute se) of *O. agrili* was within 5.6 ± 2.1 days. The first trap capture of *O. agrili* occurred before model predictions for 2 out of 10 comparisons (Table 3.5). Predicted-initiation of EAB emergence had an absolute mean difference of 7.7 ± 2.1 days compared with first capture of EAB, and first capture of EAB occurred before model predictions for 4 of 10 comparisons (Table 3.5). Absolute mean difference between peak EAB trap captures and model predictions of 95% EAB emergence was 7.3 ± 1.8 days, with peak EABs captured occurring before model predictions for 4 out of 10 comparisons (Table 3.5). The last trap capture of EAB females compared to model predictions

of 95% of oviposition had an absolute mean difference of 11.0 ± 2.2 days, with the last female EAB captured before 95% of EAB oviposition was predicted for 7 out of 10 comparisons (Table 3.5). The difference between mean peak *O. agrili* trap captures and the predicted emergence of 95% *O. agrili* adults was lowest for emergence of the F_1 generation (8.8 ± 1.8; Table 3.5) compared to F_0 (30.4 ± 3.6; data not shown in table) and F_2 (28.9 ± 4.1; data not shown in table).

Table 3.5. Julian day comparisons of model predictions and trap captures for the beginning of *O. agrili* and EAB adult emergence with the first individuals of each captured in traps, 95% *O. agrili* F_1 emergence with the peak trap capture, 95% EAB adult emergence with the peak trap capture, and model prediction of 95% of EAB oviposition being completed and the last female captured in traps for three years at three sites in south central Michigan (Gratiot-Saginaw, Ithaca, MI; Harris Nature Center and Legg Park, Okemos, MI) and one site northwestern Michigan (Eastport, MI). Trapping was not done in Eastport in 2017 or 2018.

		O. agrili emergence begins and first trap capture			*O. agrili* F_1 95% emergence and peak trap capture			EAB emergence begins and first trap capture			EAB 95% emergence and peak trap capture			EAB 95% oviposition and last female EAB captured		
Year	Site	Trap	Model	Difference	Trap	Model	Difference	Trap	Model	Difference	Trap	Model	Difference	Trap	Model	Difference
2016	Eastport	175	179	-4	217	220	3	175	173	2	189	192	-3	231	250	-20
2016	Gratiot-Saginaw	173	167	6	200	203	3	160	161	-1	187	175	12	228	228	0
2016	Harris Nature Center	186	163	23	214	200	-14	172	156	16	186	172	14	214	222	-8
2016	Legg Park	160	163	-3	214	200	-14	172	156	16	172	172	0	228	222	6
2017	Eastport	NA	177	NA	NA	227		NA	167	NA	NA	189	NA	NA	257	NA
2017	Gratiot-Saginaw	166	164	2	215	205	-10	159	160	-1	166	174	-8	222	234	-12
2017	Harris Nature Center	166	163	3	215	203	-12	166	160	6	166	173	-7	215	233	-18
2017	Legg Park	166	163	3	186	203	17	153	160	-7	186	173	13	221	233	-12
2018	Eastport	NA	176	NA	NA	215		NA	168	NA	NA	183	NA	NA	242	NA
2018	Gratiot-Saginaw	165	164	1	200	199	-1	156	157	-1	172	174	-2	215	226	-11
2018	Harris Nature Center	172	163	9	200	197	-3	172	155	17	186	172	14	193	224	-31
2018	Legg Park	165	163	2	186	197	11	165	155	10	172	172	0	227	224	3
Mean ± se absolute difference				5.6 ± 2.1			8.8 ± 1.8			7.7 ± 2.1			7.3 ± 1.8			11.0 ± 2.2

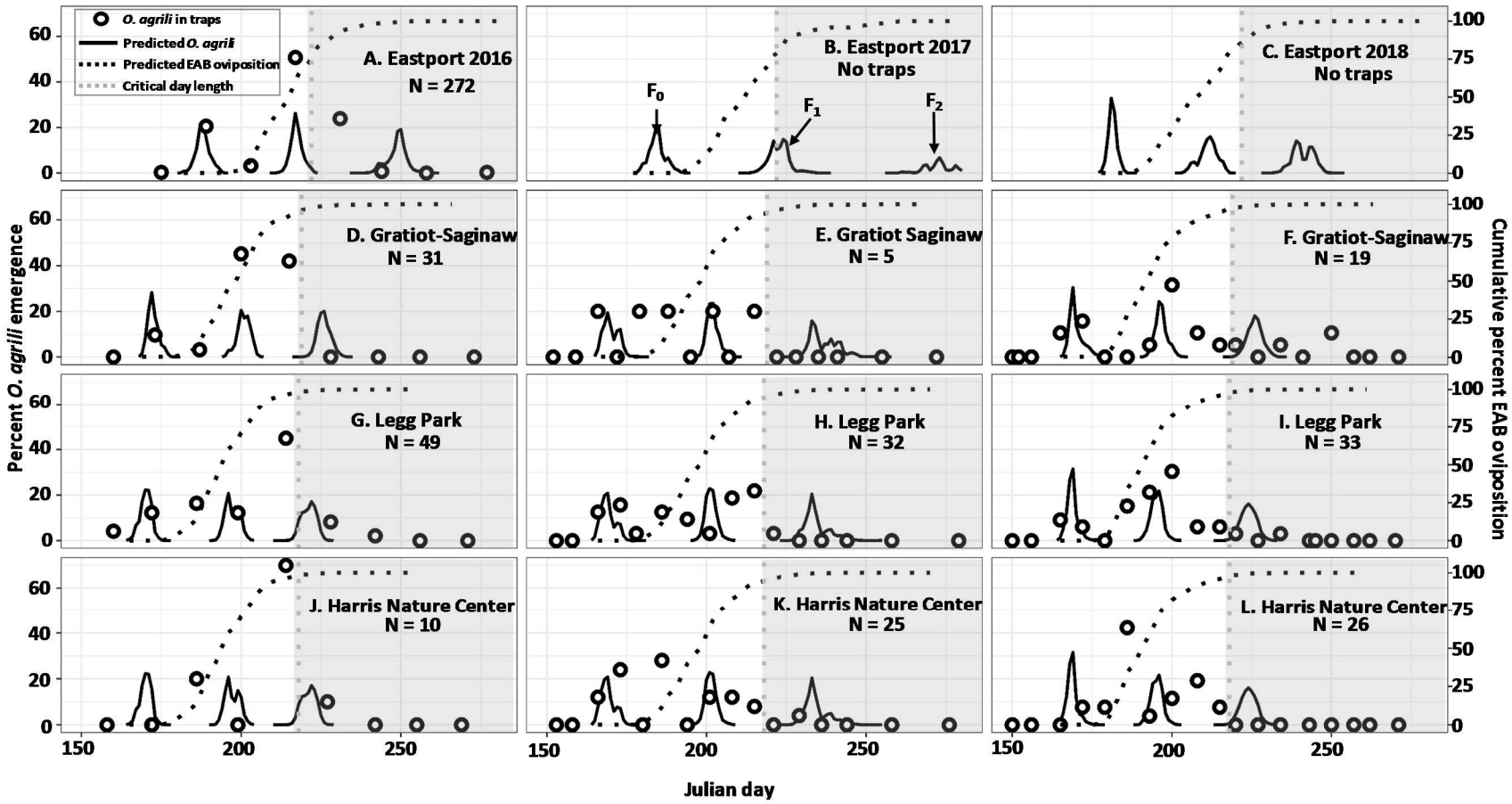

Figure 3.4. Percent incidence of predicted adult emergence of F_0, F_1, and F_2 *O. agrili,* cumulative percentage of EAB oviposition, and *O. agrili* adults captured in yellow pan traps by Julian day over three years for one site in northwestern Michigan [Eastport (A = 2016, B = 2017, C = 2018)], and three sites in south central Michigan [Gratiot-Saginaw, Ithaca, MI (D=2016, E = 2017, and F = 2018); Legg

Figure 3.4. (cont'd)

Park, Okemos, MI (G. = 2016, H = 2017, I = 2018); and Harris Nature Center, Okemos, MI (J = 2016, K = 2017, and L = 2018). N = the total number of *O. agrili* captured in traps for each site and year. See Fig. 2.3 for location of sites.

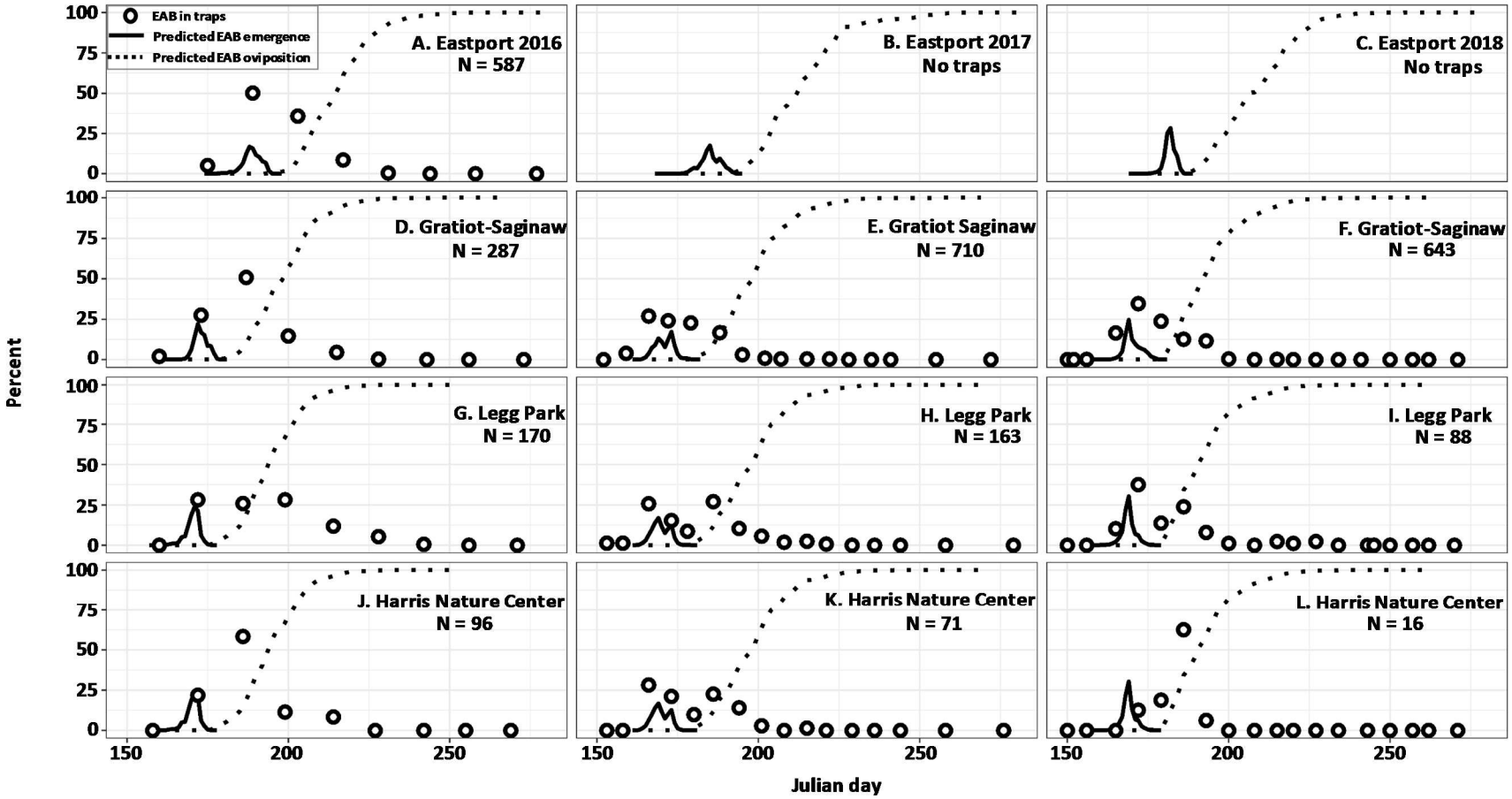

Figure 3.5. Percent incidence of predicted EAB adult emergence, cumulative percentage of EAB oviposition, and EAB adults captured in funnel traps by Julian day over three years for one site in northwestern Michigan [Eastport (A = 2016, B = 2017, C = 2018)], and three sites in south central Michigan [Gratiot-Saginaw, Ithaca, MI (D=2016, E = 2017, and F = 2018); Legg Park, Okemos, MI (G. =

Figure 3.5. (cont'd)

2016, H = 2017, I = 2018); and Harris Nature Center, Okemos, MI (J = 2016, K = 2017, and L = 2018). N = the total number of EAB captured in traps for each site and year. See Fig. 2.3 for location of sites.

Spatiotemporal comparison of simulations

At all eight sites (i.e., Michigan and north-south gradient) and years, simulated F_0 adult emergence began 0–3 days (mean = 1.8 ± 0.2) before EAB oviposition was predicted to begin (Figs. 3.4 and 3.6). When 50% of F_0 adult emergence was predicted, 0.0000006–0.0100000% of EAB oviposition was predicted to be complete (mean = 0.0013915 ± 0.0005732%), depending on site and year (Fig. 3.4 and 3.6). When 50% of F_1 adult emergence was predicted, 50.1–98.0% of EAB oviposition was complete (mean = 63.8 ± 2.0%), depending on site and year (Figs. 3.4 and 3.6). Almost all EAB oviposition (mean = 99.1 ± 0.1%) was complete when 50% F_2 adults had emerged for all sites and years. As expected, the interaction of critical day length with the emergence of *O. agrili* generations varied significantly among geographic locations (Fig. 3.4 and 3.6). At the most northern sites (i.e., Eastport, MI and Duluth, MN), day lengths fell below critical day length just before or after F_1 *O. agrili* adult emergence with the exception of Duluth, MN, in 2017 (Fig. 3.6B). Day lengths at the south central Michigan sites were above critical day length for F_0 and F_1 emergence, and below critical day length for the F_2 generation. At the central U.S. locations (Indianapolis, IN and Knoxville, TN), day lengths were above critical day length during F_1 and F_2 adult emergence except for the beginning of F_1 emergence at Knoxville, TN, in 2017 (Fig. 3.6H). Day lengths remained below critical day length during emergence of all *O. agrili* generations at Shreveport, LA (Fig. 3.6J, K, and L)

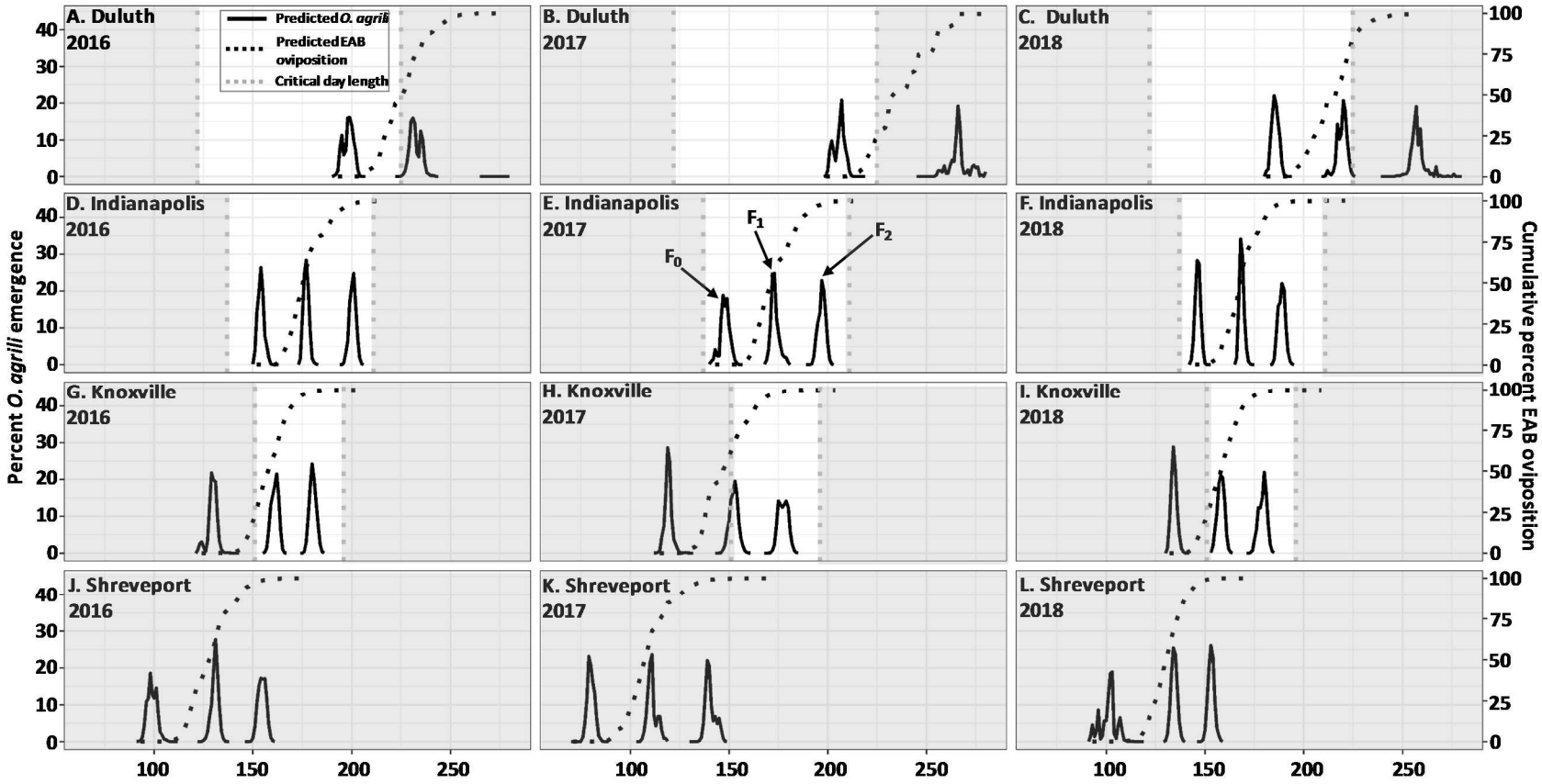

Figure 3.6. Percent incidence of predicted adult emergence of F_0, F_1, and F_2 *O. agrili,* and cumulative percentage of predicted EAB oviposition for three years at four locations along a north south gradient where *O. agrili* has been released: Duluth, Minnesota (A = 2016, B = 2017, C = 2018); Indianapolis, Indiana (D=2016, E = 2017, and F = 2018); Knoxville, Tennessee(G. = 2016, H = 2017, I = 2018); and Shreveport, Louisiana (J = 2016, K = 2017, and L = 2018). See Fig. 2.3 for location of sites.

Discussion

The ability to model parasitoid and host phenology is critical to understand how climate and geographic location can affect host-parasitoid synchrony. As the distribution of *O. agrili* in North America expands, interactions of climate and photoperiod could affect host-parasitoid synchrony by altering *O. agrili* emergence, voltinism, and diapause patterns. To examine these effects, a temperature-driven rate-summation model was developed to simulate *O. agrili* generations and EAB oviposition. Seasonal occurrences of critical day length for diapause induction were integrated into the model. Model realism was validated by comparing simulation results with seasonal trap collections of *O. ag*rili and EAB adults from four field sites in Michigan. Simulations for sites distributed across the current latitudinal distribution of EAB and *O. agrili* in the U.S. provide insight on spatiotemporal effects of temperature regimes and day lengths on parasitoid-host synchrony.

Model evaluation

Model predictions of *O. agrili* and EAB phenology aligned reasonably well with field data including first emergence of EAB and F_0 *O. agrili*, EAB peak emergence, and EAB ovipositional termination. However, there were some discrepancies between model predictions and field data. Some of these differences may be attributed to intrinsic factors such as the fit of the nonlinear functions that are the foundation of the model. For example, the EAB-adult-emergence cumulative probability function prematurely predicted the end of EAB emergence when compared to laboratory data (Fig. 3.1F) and, not surprisingly, EAB peak trap captures often occurred after the model predicted EAB emergence to end (Table 3.5). Similarly, the cumulative probability function predicted *O. agrili* adult F_1 and F_2 emergence to end earlier than

laboratory data (Fig. 3.1D). This may, in part, explain the high percentage of *O. agrili* captures after F_1 adult emergence was predicted but before the F_2 adult emergence was predicted (Fig. 3.4D,E,G,H,I, and J; Table 3.).

Considering trap captures at the Michigan sites, the model exaggerated the length of EAB oviposition, given that the last EAB females often were captured well before 95% EAB oviposition was predicted (Fig. 3.5). This overestimate seems plausible, given that EAB oviposition was measured under optimal conditions in the laboratory. For example, females were regularly provisioned with fresh foliage as opposed to them having to seek out suitable foliage. Also, males were always in close proximity for mating in the laboratory which is probably not the case in the field. Other factors that may have reduced the EAB oviposition period in the field include depletion of energy reserves used for locating suitable oviposition sites within and among different trees, as well as exposure to predators and adverse weather conditions such as precipitation and wind.

It is possible that the model failed to describe all of the *O. agrili* and EAB population variability. *Oobius agrili* is thelytokous parthenogenetic and, to our knowledge, all individuals released in North America are genetically identical. Similarly, genetic analyses found little variability in North American EAB populations (Bray et al., 2011; Keever et al., 2013). Therefore, the majority of variation in development rates of both species is most likely attributed to environmental factors, rather than genetic variability. One environmental factor is the effect of cold duration on diapause development which may affect diapause termination of both EAB and *O. agrili* diapausing larvae (Duan and Larson, 2019a; Duarte, 2013; Petrice et al., 2019). Development rates for the model were determined after diapausing individuals of both species were held at constant 4°C for several weeks or months, which may have synchronized diapause

development among individuals. In nature, a consistent chill period of 4°C does not occur, with daily temperatures fluctuating during the overwintering period. Therefore, variation in winter temperatures and duration of cold likely affect diapause development times and variability of adult emergence in the field.

Model predictions may also differ from trap captures because ambient air temperatures were assumed to be the same as those on the bark surface, where *O. agrili* develop, and under the bark, where EAB adults develop. Solar radiation may alter the temperatures on the surface and under the bark of trees from ambient air temperatures(Andresen et al., 2001; Potter and Andresen, 2002; Vermunt et al., 2012b, 2012a). Also, Wang et al. (2010) found EAB adults emerged earlier on the south side of trees compared to the north side. Solar radiation could have increased bark temperatures above ambient temperatures for insects developing on the south or west side of trees at trapping sites, resulting in faster development times for some individuals compared to model predictions.

Large differences between model predictions and trap captures were often associated with sites and years that captured lower numbers of individuals (Table 3.5, Figs. 3.4 and 3.5). Low *O. agrili* and EAB densities coupled with low trap efficiency may have inflated differences between model predictions and trap captures. Yellow pan traps are only visually attractive to *O. agrili* which would require adults to be close enough to "see" the traps. Also, *O. agrili*'s small size likely limits their flight distance. This makes it critical that traps are placed on trees where *O. agrili* are concentrated (Parisio et al., 2017). Green funnel traps were baited with plant host volatiles, thus providing both visual and olfactory stimuli for EAB adults. However, their effectiveness at trapping EAB at low populations densities is unclear (Poland et al., 2019). It is likely that capture rates did not accurately describe adult emergence patterns at some sites with

low *O. agrili* or EAB densities. Furthermore, some adults captured in traps could have emerged several days or weeks before they were captured because both EAB and *O. agrili* adults can survive for several weeks or months in the laboratory (Duan et al., 2014; Hoban et al., 2016; Larson and Duan, 2016; Petrice et al., 2019; Rutledge and Keena, 2012). Also, EAB adults must feed on foliage throughout their life which would continue to expose them to traps. Thus, temporal shifts of EAB or *O. agrili* attraction to specific trees coupled with low adult densities could have further confounded seasonal trap captures.

Another source of error between model predictions and trap captures could be from sampling frequency. Trap samples were collected every 2-weeks in 2016 and every week in 2017 and 2018. Therefore, the exact day within each trapping period that insects were actually captured cannot be determined. For sites and years where the model predicted the phenology event before it occurred in trap samples but the predicted date fell within that particular trapping period, differences may be lower than those calculated because the event may have occurred before traps were emptied. In contrast, when traps confirmed a phenological event occurred before the model predicted it should take place, then model predictions may differ even more than those calculated.

Spatiotemporal comparisons

The model predicted that emergence of F_0 adults and the beginning of EAB oviposition were temporally synchronized regardless of geographical location (Figs. 3.4 and 3.6). However, the model predicted that very little EAB oviposition was occurring when F_0-adults emerged. Early emergence of *O. agrili* would temporally position adults for the earliest possible egg parasitization and ensure the subsequent F_1 generation would emerge in close synchrony with

peak EAB oviposition. This would also increase the likelihood that there is still some EAB oviposition occurring when F_2 adults emerge. Premature emergence of F_0 adults should not reduce their physiological ability to parasitize EAB eggs if they are not immediately found because *O. agrili* adults can parasitize EAB eggs for several weeks in the laboratory (Hoban et al., 2016; Larson and Duan, 2016; Petrice et al., 2019). However, it could subject them to biotic and abiotic mortality factors before they locate and parasitize EAB eggs. Nevertheless, this suggests the F_0 generation should be released when EAB oviposition first begins.

Emergence of F_1 adults was most synchronized with peak EAB oviposition compared to other *O. agrili* generations. Therefore, the F_1 generation is likely responsible for the majority of EAB egg parasitization. The exception was for the most northern site (Duluth, MN) in 2017, where cool summer temperatures delayed development of the F_1 generation and resulted in EAB oviposition nearing completion when 50 % of F_1 adult emergence was predicted (Fig. 3.6B). F_1 adult emergence at the northwestern MI site was similarly, but slightly less, delayed in 2017. Overall parasitism of EAB eggs will likely be lower when F_1 adult emergence occurs when EAB oviposition is almost complete.

Model simulations predict the earliest possible emergence of F_1 and F_2 adults because *Oobius agrili* adult longevity and fecundity were not included in the model. One hour after a respective *O. agrili* cohort emerges, the models begins simulating development of subsequent F_1 or F_2 generations as long as EAB oviposition is occurring. Therefore, emergence of F_1 and F_2 adults should continue for several days after the model predicts adult emergence of each of these generations given that all generations of *O. agrili* can parasitize EAB eggs for several weeks after they emerge in the laboratory (Duan et al., 2014; Hoban et al., 2016; Larson and Duan, 2016; Petrice et al., 2019). At Michigan sites, the majority of *O. agrili* were captured in traps

before F_2 adult emergence was predicted. Also, *O. agrili* peak trap capture was closer to predicted F_1 emergence compared to F_2 emergence (Table 3.5). This demonstrates that primarily two generations of *O. agrili* adults (i.e., F_0 adults from diapausing larvae and F_1 adults from nondiapaused larvae) emerge per year in Michigan.

Critical day length occurred much closer to F_1 emergence for all years at the northwestern Michigan site compared to the three south central Michigan sites (Fig. 3.4). In addition, peak capture of *O. agrili* adults occurred just before day lengths decreased below critical day length at the northwestern Michigan site (Fig. 3.4A). Day lengths shorter than critical day length should induce diapause in F_2 progeny, presumably why only 1% (3 individuals) of *O. agrili* were captured at the northwestern site when F_2 adult emergence was predicted.

At all south central Michigan sites, peak F_1 emergence was predicted well before critical day length. Also, *O. agrili* were relatively abundant in traps at these sites during this period. It is conceivable that F_1 adults should have parasitized a substantial number of eggs during this period when EAB eggs were abundant. Given that developing progeny do not exhibit photoperiod-induced diapause after they are $\geq$ 6 days old (Petrice et al., 2019), a large number of F_2 adults should have emerged several weeks after F_1 emergence peaked at the south central Michigan sites. However, *O. agrili* trap captures were consistently low when F_2 adults were predicted to emerge, suggesting that developing F_2 progeny entered diapause earlier than predicted at south central sites. Diapause was likely induced by *O. agrili* larvae perceiving day lengths shorter than critical day length albeit ambient day lengths were above critical day length during this period.

Multiple factors may have caused developing F_2 progeny to enter diapause before critical day length occurred. Photoperiod exposure of larvae, rather than ovipositing adults, determines

diapause induction in *O. agrili* (Petrice et al. 2019). Given that EAB prefers to oviposit in cracks or between layers of bark (Wang et al., 2010), it is conceivable that photophases perceived by *O. agrili* larvae in hidden EAB eggs could be shorter than ambient photophases. Forest canopy cover at study sites could further reduce the amount of daylight that *O. agrili* larvae perceive. Also, critical day length for *O. agrili* diapause induction was determined at constant 25°C in the laboratory but critical day length for diapause induction in insects may increase with decreasing temperatures (Li et al., 2008). Therefore, lower night-time temperatures may lengthen the critical day length for *O. agrili* diapause induction. Alternatively, *O. agrili* larvae may also use thermoperiod in addition to photoperiod to measure day length as has been demonstrated in several insect species (Beck, 1982; Saunders, 2014; Wertman and Bleiker, 2019). If true, the day lengths measured by thermoperiod could be shorter than those based on ambient photoperiod. One or a combination of these factors are likely responsible for *O. agrili* entering diapause before critical day length is predicted by ambient photoperiod.

At all sites and for all years, EAB oviposition was near completion when 50% F_2 adult emergence was predicted. If large numbers of F_2 adults emerged when the model predicted, many would have been biologically stranded because of limited EAB eggs available for parasitism during this period. This would reduce the number of diapausing individuals that would perpetuate populations the following season. Therefore, it appears that in south central Michigan, diapause induction of F_2 larvae prior to ambient photophase falling below critical day length is important for *O. agrili*'s success. On some occasions, *O. agrili* adults were collected at Michigan sites during the period that coincided with model predictions of F_2 adult emergence, suggesting that some progeny perceived day lengths similar to ambient photophases and emerged as adults rather than entering diapause. Also, Abell et al. (2016) found that some *O. agrili* adults

parasitized sentinel EAB eggs in south central Michigan when F_2 *O. agrili* adults were expected to be present. These adults may have emerged from EAB eggs that received more day light such as open grown trees and/or eggs that were not completely hidden under bark.

The interaction of critical day length with predicted emergence of *O. agrili* generations varied considerably among geographic locations. The effects of these interactions are dependent on how *O. agrili* larvae measure day lengths in the field. If *O. agrili* perceive day lengths that are only slightly shorter than ambient day length, these interactions could significantly affect their population dynamics among different geographic locations. For example, at the Indianapolis, IN, and Knoxville, TN, sites, day lengths remained above critical day length during F_1 and F_2 emergence (Fig. 3.6). If *O. agrili* perceive day lengths that are longer than critical day length in these regions, most of the F_2 generation would emerge as adults even though very few EAB eggs may be present for them to parasitize. This would reduce the number of diapausing *O. agrili* that would overwinter and perpetuate populations the following year. At Shreveport, LA, day lengths are shorter than critical day length for emergence of all *O. agrili* generations. This would result in higher diapause rates and increase the number of diapausing individuals available to perpetuate populations the following season. However, it would also reduce overall parasitism rates because fewer adults would emerge from eggs parasitized by F_0 adults and all progeny produced by F_1 adults would enter diapause.

As discussed above, it is unclear how developing *O. agrili* progeny measure day length when EAB eggs are well hidden in crevices or between layers of bark. If most are unable to measure day length or perceive day lengths that are much shorter than ambient day lengths, it is possible that the critical day length for *O. agrili*'s diapause induction modulates a bet-hedging strategy for diapause. Parasitoids developing in well-hidden parasitized eggs may be more

protected from weather, predators and disease; increasing their probability of surviving as diapausing larvae until the following spring. These well-hidden individuals would perceive day lengths shorter than critical day length and enter diapause, regardless of ambient photoperiod. Alternatively, parasitoid progeny developing in exposed EAB eggs could suffer higher levels of biotic or abiotic sources of mortality. These individuals would perceive day lengths similar to ambient photophases and emerge as adults; a strategy to avoid mortality during the extended diapause period. However, these adults would risk becoming biologically stranded if no EAB eggs are present. Critical day length would be important for inducing diapause in these exposed parasitoid progeny by preventing them from developing to adults later in the season when little or no EAB eggs are available. If this hypothesis is true, geographic variation of day lengths may have less of an impact on *O. agrili* populations.

Model simulations can also estimate seasonal timing for releasing *O. agrili* in the field. For example, F_0 adults should be released during a short window of time (2–3 wk) beginning when EAB oviposition is predicted to initiate. This will allow sufficient time for their progeny to develop and emerge when EAB oviposition is peaking. If F_0 adults are released later during EAB oviposition (i.e., when F_1 adults are predicted to emerge), their progeny that emerge as adults will likely become biologically stranded because most EAB oviposition will be complete. Also, releases of F_1 adults (or other nondiapaused generations) should begin when EAB oviposition is near 50% complete when large numbers of EAB eggs are present, which will maximize parasitism and the number of progeny that enter diapause. Following these guidelines should help increase *O. agrili* establishment success at release locations.

Conclusion

A temperature driven multiple cohort rate summation model was developed to evaluate the synchrony of *O. agrili* generations with EAB oviposition and model predictions were validated with field collected data. Although there were some discrepancies between model predictions and trap captures, field data for most sites and years supported model predictions. Both model predictions and trap captures suggested that in south central and northwestern Michigan, *O. agrili* has primarily two generations, with the F_1 generation most synchronized with peak EAB oviposition. Geographic location does not appear to significantly affect temporal synchrony of the F_0 adult emergence with the initiation of EAB oviposition. However, the synchrony of F_1 emergence with peak EAB emergence was affected some years at the two most northern locations. More validation is necessary at a larger spatial scale to confirm that both species will respond similarly in different climates. For example, it is not clear how warmer winter temperatures in southern regions will affect diapause completion and this could alter emergence patterns of both species. It is unclear how ambient photoperiod will affect population dynamics of *O. agrili* at different latitudes, but it is possible that the critical day length for *O. agrili* diapause induction functions as a bet-hedging strategy for diapause which could reduce spatiotemporal effects on *O. agrili* population dynamics. A better understanding of how *O. agrili* measures day length in the field is needed to determine how *O. agrili* voltinism and diapause patterns will be affected in novel geographic locations.

The model developed in this study provides insight on the interactions of temperature and day length on the phenological synchrony of *O. agrili* and EAB at different geographic locations. As the distributions of EAB and *O. agrili* expand, this model will help predict their population

dynamics and synchrony. It can also help determine appropriate release times for diapaused and nondiapaused *O. agrili* phenotypes.

CHAPTER 4: EVALUATION OF SAMPLING METHODS, INTENSITY, AND TIMING FOR RECOVERING AND MONITORING *OOBIUS AGRILI* IN THE FIELD.

Abstract

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid being released as a biological control agent of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Detection and monitoring of *O. agrili* are challenging due to its small size (ca., 1mm) and the cryptic placement of host eggs. Four *O. agrili* recovery methods were compared in this study: 1) rearing adults from bark (bark rearing); 2) sifting parasitized eggs from bark (bark sifting); 3) sentinel EAB eggs in screened envelopes (sentinel eggs); and, 4) yellow pan traps. In 2016, all methods were applied to 40 trees within 0.25-hectare-plots at each of 4 sites in Michigan. In 2017 and 2018, methods were applied to 10 trees within the 0.25-hectare-plots at each of 3 sites. Sentinel eggs were not included in 2018. Mean number of *O. agrili* recoveries and trees positive for *O. agrili* were compared among sampling methods for each year. Bootstrap samples of 1–40 trees within each plot sampled in 2016 were iterated ×2000 and the number of iterations with at least one *O. agrili* recovery was used to calculate the percent probability of recovering *O. agrili* as a function of number of trees sampled. The coefficient of variation (CV) was calculated for each bootstrap sample. Fresh woodpecker feeding holes were highly correlated with *O. agrili* recovered for all methods. Bootstrap sampling was repeated using only trees with fresh woodpecker feeding holes to determine probability of *O. agrili* recovery and CVs. Results demonstrated that yellow pan traps and bark sifting recovered *O. agrili* at all sites and years, consistently had higher percentage of *O. agrili* positive trees, and required fewer trees sampled for >95% probability of *O. agrili* recovery compared to bark rearing and sentinel eggs. Bark rearing did not recover *O. agrili* at 1

site in 2016 when 40 trees were sampled, and 1 site in 2017 when 10 trees were sampled. Sentinel eggs recovered *O. agrili* at all sites in 2016 and 2017; however, low overall recovery numbers did not justify the amount of labor required. The probability of *O. agrili* recovery when sampling only trees with fresh woodpecker feeding holes increased the most for bark sifting and bark rearing, increased slightly for yellow pan traps, and decreased for sentinel eggs. Yellow pan traps and sentinel egg sampling should be conducted between 400–1200 DD_{10}, when most *O. agrili* adults are active in the field. The largest decrease in CVs occurred when approximately 10 trees were sampled for all sample methods and years. Advantages and disadvantages of each method are discussed.

Introduction

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a classical biological control agent of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), first released into the U.S. in 2007 (Bauer et al., 2015a). In its native range in northeastern China, *O. agrili* is an important natural enemy of EAB, with parasitism rates from 32–42% in some areas (Liu et al., 2007; Wang et al., 2015). *Oobius agrili* is a solitary egg parasitoid that reproduces by thelytokous parthenogenesis. Although males have been reported from China (Zhang et al., 2005), they are absent in populations introduced into North America. *Oobius agrili* is multivoltine and overwinters as diapausing larvae within EAB eggs.

As of February 2020, almost 2 million *O. agrili* have been released in 27 states and the District of Columbia in the U.S., and 3 Canadian provinces and its establishment has been confirmed in 13 states and 2 Canadian provinces (MapBioControl, 2020). Parasitism rates as high as 40% have been reported from some of the earliest release sites in Michigan (Abell et al., 2014). As the releases of *O. agrili* expand, reliable methods for monitoring its establishment and

abundance are essential for evaluating its efficacy. However, *O. agrili*'s small size (c.a., 1-mm long) makes adults particularly challenging to recover in the field. Immature *O. agrili* inside EAB eggs are also difficult to recover because EAB females lay their eggs in crevices or between layers of bark (Abell et al., 2014; Wang et al., 2010).

The Emerald Ash Borer Biological Control Release and Recovery Guidelines (referred to as *EAB Biocontrol Guidelines* hereinafter) (USDA–APHIS/ARS/FS, 2019) were developed to provide researchers and managers methods for releasing and recovering EAB biocontrol agents, including *O. agrili*. EAB Biocontrol Guidelines is a living document that incorporates the most current information available to maximize release and recovery success, albeit often based on limited studies and data, and is updated periodically as new research findings come to fruition. The three sampling methods recommended in EAB Biocontrol Guidelines for *O. agrili* recovery include a minimum of: 1) 15 yellow pan traps for capturing adults; 2) 10 bark samples sheered from the outer bark of ash trees and sifted for parasitized eggs (bark sifting), or 3) 30 bark samples sheered from the outer bark of ash trees for rearing adults (bark rearing).

Yellow pan traps are frequently used to collect hymenopteran parasitoids and Noyes (1990) found them to be more effective for sampling adult Encyrtidae and other Chalcidoidea, compared to other methods such as canopy fogging, malaise trapping, window pane trapping, and sweep netting. Yellow pan traps used for capturing *O. agrili* adults consist of yellow bowls filled with a solution that kills and preserves insects and attached to the lower trunk of ash trees (USDA–APHIS/ARS/FS, 2019). Trap samples are sorted under a dissecting scope and suspect individuals are collected and confirmed by examining morphological characters. Parisio et al. (2017) recovered *O. agrili* with yellow pan traps at release sites in New York and recoveries of *O. agrili* at several additional locations in the U.S. are reported in MapBioControl (2019).

However, Jones et al. (2019) recovered no *O. agrili* with yellow pan traps at sites where they were released.

Field collection of host eggs is frequently used for surveying egg parasitoids that attack exposed eggs that are relatively easy to locate, such as *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Jones et al. 2014) and *Anas tristis* (DeGreer) (Hemiptera: Coreidae) (Cornelius et al., 2016). EAB eggs parasitized by *O. agrili* have been recovered by visual inspection of host-tree bark in the field (Abell et al., 2014; Duan et al., 2012; Jennings et al., 2018; Liu et al., 2007; Wang et al., 2015). However, this method is challenging because EAB eggs are usually hidden in crevices or between layers of bark, requiring careful removal of the outer layer of corky bark without accidentally dislodging EAB eggs. After bark is removed and EAB eggs are exposed, their detection is subject to natural lighting conditions and inspectors' visual abilities (Abell et al. 2014). Bark sifting is a modified visual-inspection method that entails sheering off the outer corky-bark-layer of ash trees with a draw knife and collecting the bark with a plastic drop cloth or container (Abell et al. 2014). In the laboratory, bark sifting samples are shaken and sifted in a soil sieve to dislodge and separate smaller debris, which include EAB eggs, from larger bark pieces. EAB eggs are then sorted from bark debris under a dissecting microscope and their status determined (i.e., hatched or parasitized) (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). Abell et al. (2014) found the bark sifting method more effective compared to visual inspection for recovering EAB eggs parasitized by *O. agrili*. By contrast, Jennings et al. (2018) found visual inspection more effective compared to bark sifting. However, their bark sifting samples were one-fifth of the sample-surface area recommended by EAB Biocontrol Guidelines.

Oobius agrili can also be recovered by rearing adults in the laboratory from logs or bark collected from EAB infested trees. Bark or logs are collected in late winter or early spring, after diapausing parasitoids have been exposed to a cold period. Material is then reared indoors in containers equipped with emergence cups for collecting emerging parasitoids. Parisio et al. (2017) recovered more *O. agrili* adults by rearing logs (i.e., log rearing for adults) from EAB infested trees compared to captures in yellow pan traps at the same release sites. Abell et al. (2014) reared *O. agrili* adults from bark sifting samples prior to sifting and sorting for EAB eggs [i.e., bark rearing for adults (bark rearing)].

Sentinel host eggs is another effective sampling method for numerous egg parasitoids (Cornelius et al., 2016; Herlihy et al., 2016; Herz et al., 2007; Moya-Raygoza et al., 2012). Volatiles from host eggs can attract egg parasitoids (Michereff et al., 2016; Vinson, 1998), making them more effective than some passive sampling methods. Although this method is not recommended in EAB Biocontrol Guidelines, sentinel eggs have been used to successfully detect *O. agrili* in the field. For example, Duan et al. (2011) placed sentinel EAB eggs under bark flaps and recovered *O. agrili* at some study sites. Small ash bolts on which EAB females had oviposited eggs in the laboratory [i.e., sentinel egg bolts] were also used successfully to detect *O. agrili* in field (Duan et al. 2012, Abell et al. 2016). However, Parisio et al. (2017) recovered no *O. agrili* using sentinel eggs on ash bolts or sentinel eggs in plastic cups, even though *O. agrili* was reared from ash logs and collected in yellow pan traps at the same study sites. Aside from rearing and maintaining EAB adults for oviposition, preparing sentinel egg bolts is a lengthy process and EAB oviposition on sentinel egg bolts is highly variable (Abell et al., 2016; Duan et al., 2012; Parisio et al., 2017). Also, exposed EAB sentinel eggs often suffer high predation rates, possibly by ants, while in the field (Duan et al. 2011, 2012). Jennings et al. (2014)

developed a simple solution by cutting the desired number of EAB eggs from filter papers on which EAB had oviposited. EAB eggs were placed in screened envelopes with openings that allowed *O. agrili* to enter but deterred predators [i.e., sentinel EAB eggs in screened envelopes (sentinel eggs)]. Sentinel eggs recovered *O. agrili* when they were placed on the same trees on which parasitoids were released (Jennings et al., 2014).

Although each of the four sampling methods described above each has successfully recovered *O. agrili* in the field, it is unclear which method is most suitable when weighing their effectiveness and logistical requirements. It is also not known how many samples of each method are needed to be confident that sites have been adequately sampled for presence and abundance of *O. agrili*, as well as the optimal time for deploying methods that target *O. agrili* adults. Studies were conducted in 2016–2018 to address these questions. The first study objective was to compare bark rearing, bark sifting, sentinel eggs, and yellow pan trap methods for recovering *O. agrili* in the field. The second objective was to estimate the number of samples required to confidently survey for *O. agrili*. This was done by sampling all ash trees within discrete areas within sites where *O. agrili* was established and then bootstrap sampling (Simonoff et al., 1994) these data to predict how sample size affected the probability and number of *O. agrili* recoveries. The third objective was to determine the optimal seasonal-deployment of sample methods that target *O. agrili* adults (i.e., yellow pan traps and sentinel eggs). Results from this study will allow managers and researchers to select the most appropriate sampling-method, -size, and -period for recovering *O. agrili* in the field.

Materials and methods

Sampling methods

Each yellow pan trap consisted of a 355 mL (18 cm in diam.) yellow plastic bowl that was attached to a metal shelf bracket that was then secured with wood screws to the south side of ash trees approximately 1.75m above the ground (Fig. 4.1A). A second yellow bowl was nested inside the first bowl to capture insects and allow easy removal of samples. A solution of 50:50 food-grade propylene glycol and H_2O was added to the second bowl to kill and preserve insects. A small amount (c.a., 10ml per L) of unscented dish soap was added to reduce surface tension. Detailed instructions on yellow pan trap construction and sampling are given in EAB Biocontrol Guidelines. Trap samples were poured through paint strainers to separate insects from the trapping solution, placed in reclosable plastic bags, and frozen until processed. In the laboratory, contents from each trap were inspected under a dissecting microscope for suspect *O. agrili*. Identifications of *O. agrili* adults were confirmed using the key and description in Triapitsyn et al. (2015).

Sentinel eggs consisted of a 4 cm x 5 cm envelope made of nylon screening with 0.75 mm openings. Edges of envelopes were held closed with staples. Ten EAB eggs were placed in each envelope. Filter papers with EAB eggs were cut into small strips to fit inside envelopes. EAB eggs were produced by rearing EAB adults at 25°C as described by Rutledge and Keena (2012). EAB eggs were removed from rearing cups every 2–3 days and stored at 10°C within 24 hours to retard egg development. EAB eggs that were not immediately deployed to the field were stored a maximum of 3 days in 10°C. Therefore, EAB eggs had developed for 1–4 days before deployment. This was done because EAB eggs older than 8 days are rarely attacked by *O. agrili* (Duan et al., 2014). Sentinel eggs were stapled approximately 1.75 m above the ground

on the north side of the same trees that received yellow pan traps (Fig. 4.1B). Sentinel eggs were placed on the north side of trees to minimize the effect of direct sunlight on EAB eggs (Parisio et al., 2017). After collection, sentinel eggs were held at 25°C for a maximum of 7 days. EAB eggs were removed from screened envelopes and inspected for *O. agrili* parasitism. Signs and symptoms of parasitism included: chorion darkened, parasitoid egg stalk present, distorted aeropyles, and developing parasitoid visible through chorion (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). Parasitized eggs were placed in Petri dishes with friction-fitted lids (Fisher Scientific 50 X 9-mm dishes – catalog number 08-757- 105) to allow adults to eclose.

Bark sifting samples were collected by sheering off the outer corky bark-layer with a draw knife from a 1000 cm^2 area on the lower trunk of ash trees (Fig. 1C). When possible, bark sifting samples were taken 1.25–1.75-m above the ground from the south or west side of ash trees. However, for some situations the aspect and height was shifted if bark in the preferred position was not suitable for sampling (i.e., portion of the tree was dead, heavy scarring, or corky bark was removed during previous sampling). Bark was collected in a clear plastic tarp tightly wrapped around the trunk of the tree with the unattached edges raised to capture the sheered bark. Bark sifting samples were transferred from the plastic tarp to paper bags with the top folded over and held closed with paper clips. Detailed methods for bark sifting sampling are given in EAB Biocontrol Guidelines. After rearing was complete (see bark rearing methods below), bark sifting samples were placed in a No. 14 soil sieve with a tight fitting lid (Fig. 1C). Samples were aggressively shaken in the sieve for a 2-min-period, alternating from side-to-side- to up-and-down-motions every 15 seconds. The soil sieve was filled no more than half full for each two-minute shake period to increase the likelihood of dislodging eggs during the shaking

process. The shaking process was repeated until the entire sample had been shaken and sifted. Bark debris captured in the bottom of the sieve was removed and placed in small plastic containers with a screw top. Static reducing spray (StaticGUARD®, B&G Foods, Parsippany, NJ) was applied as needed on the sieve and plastic containers to reduce static electricity. Bark debris was sorted under a dissecting microscope to identify and remove EAB eggs (Fig. 4D). The fate of each EAB egg was determined by carefully examining for signs and symptoms of parasitism. EAB eggs that hatched usually contained lightly-colored frass and a jagged exit hole on their bottom surface where the EAB larva had exited the egg and entered the tree. Parasitized eggs were typically darker, contained dark globs of parasitoid meconium, and a round exit hole on the side or top (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). Other signs and symptoms of *O. agrili* attack included a parasitoid egg stalk and distorted aeropyles on the EAB egg surface from the developing parasitoid enlarging the egg during development. Occasionally, eggs contained dead EAB larvae or parasitoid adults, pupae, or larvae.

Bark rearing samples consisted of the bark sifting samples before they were sifted. Within 3 days of collection, bark was placed in rearing containers to allow parasitoids to develop and emerge. A supply list and instructions for constructing rearing containers are given in EAB Biocontrol Guidelines. Rearing containers consisted of 10-cm-diam cardboard tubes that were 25-cm-long (Fig. 4E). Plastic plugs, painted black to reduce light transmission, were placed in the ends of tubes. A 3.8-cm-hole was drilled in the center of one of the plastic plugs. The same size hole was drilled in a 133-mL plastic specimen cup lid. The top surface of the lid was attached to the plastic plug with glue after both holes were aligned. The large end of a clear plastic funnel (Maryland Plastics, Federalsburg, MD, Part # L-1000PP) that was slightly smaller

than the diameter of the specimen-cup lid was glued to the inside of the specimen cup lid. The funnel stem diam. was 7 mm. The specimen cup bottom was placed over the funnel and screwed into the specimen-cup lid. A small amount of honey was streaked onto the inside surface of the specimen cup bottom for emerging parasitoids to feed on. Bark rearing samples were reared a minimum of 8 weeks to allow diapausing *O. agrili* larvae to develop to adults and emerge. Adult parasitoid emergence was monitored every other day.

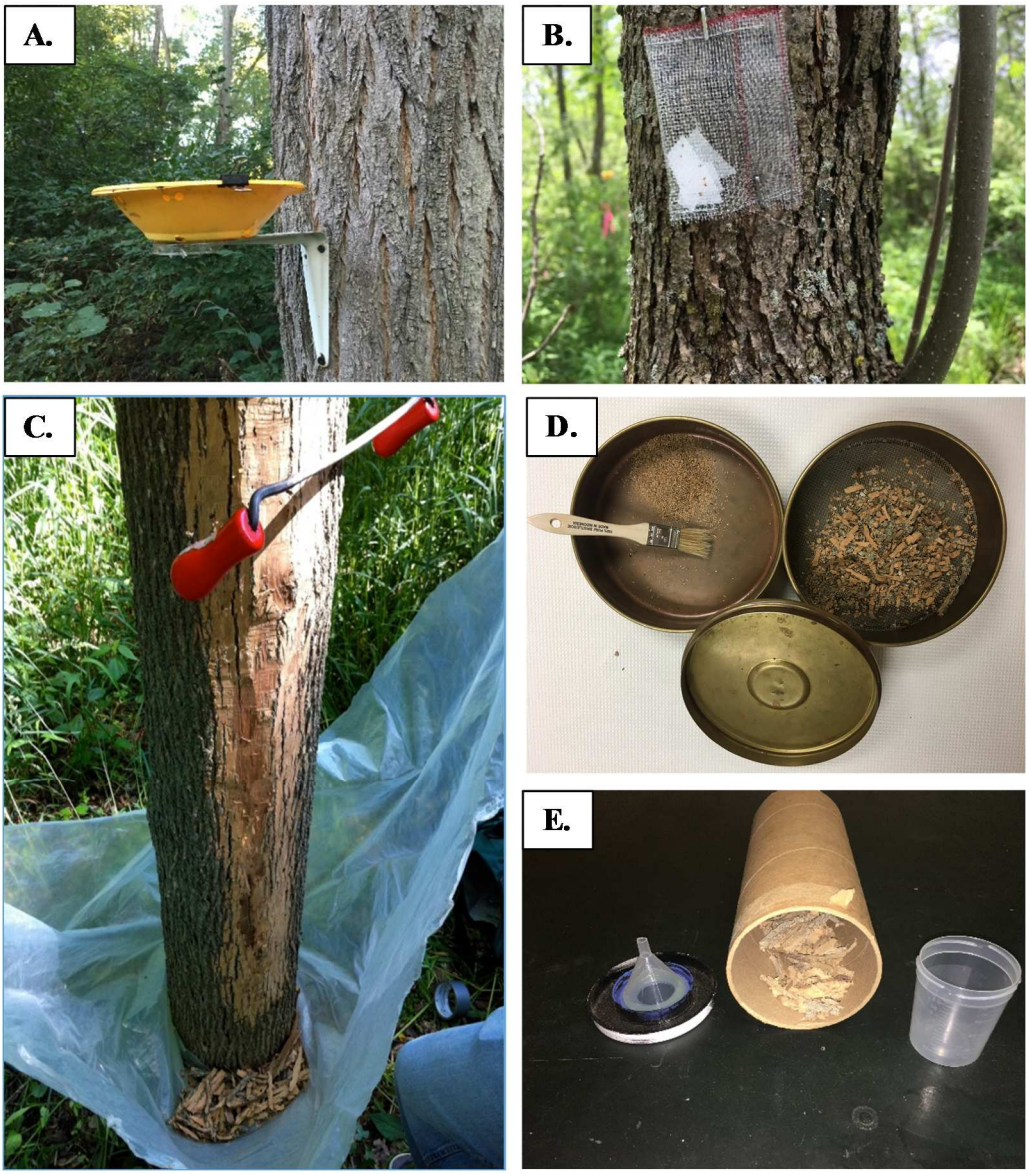

Figure 4. 1. Photos of different methods for sampling *O. agrili*: A) yellow pan trap; B) EAB eggs in screened envelopes (sentinel eggs); C) bark sheering (Photo by D.M. Miller); D.) sifting sheered bark for EAB eggs (bark sifting); sheered bark in rearing container (bark rearing).

Study sites

Studies were conducted in 2016–2018 at sites in Michigan where *O. agrili* was established. Three of these sites were in south central Michigan [Gratiot-Saginaw (near Ithaca;

lat. 43.2337, long. −84.4477); Legg Park (near Okemos; lat. 42.69403, long. −84.3822), and Harris Nature Center (near Okemos; lat. 42.6965, long. −84.3752)] and one site in northwestern Lower Michigan [Eastport (near Eastport; lat. 45.1139, long. −85.3324)] (Fig. 3.3; Table 4.1). All sites were dominated by ash trees and current EAB populations were moderate to high. *Oobius agrili* releases began in 2008 at Harris Nature Center and establishment was confirmed in 2012. No releases were conducted at Legg Park but establishment was confirmed in 2013, presumably spreading from Harris Nature Center (located only 1 km away). *Oobius agrili* releases at Gratiot-Saginaw began in 2009 and establishment was confirmed in 2010. Releases at Eastport were conducted in 2014 and establishment was confirmed in 2015.

2016 Sampling

In 2016, all four sampling methods were compared at Eastport, Gratiot-Saginaw, Harris Nature Center, and Legg Park. At each site, 40 trees were sampled which included all live ash trees that were 10-cm-DBH (diameter at breast height) or larger within a single 0.25-ha-plot (ca., 50m × 50m). Trees smaller than 10-cm-DBH were not sampled because: A) EAB attack densities are usually higher on larger trees; B) to ensure there was sufficient bark surface for bark rearing and bark sifting sampling; and C) bark of larger trees is corkier, which provides more oviposition sites for EAB and is more conducive to sheering bark for bark rearing and bark sifting sampling. For each tree, signs and symptoms of EAB attack were recorded including: a) fresh woodpecker feeding holes counted on the lower 4 m of the tree bole, b) epicormic shoots counted on the lower 4 m of the tree bole, c) EAB adult exit holes counted on the lower 2 m of the tree bole, and d) canopy dieback estimated to the nearest 10%. These data were recorded pre-season (i.e., in late spring 2016 when yellow pan traps and sentinel eggs were deployed), and post-season (i.e., winter or early spring 2017 when bark sifting and bark rearing samples were

collected), with the exception of canopy dieback that could not be recorded in winter or early spring because leaves had not flushed. Tree condition ranged from severe dieback with multiple fresh woodpecker feeding holes, and epicormic shoots, to apparently healthy with no evidence of EAB attack. All sampling methods were applied to each tree. Yellow pan traps and sentinel eggs were deployed 24–27 May 2016 and removed 26–30 September 2016 at Gratiot-Saginaw, Harris Nature Center, and Legg Park; and deployed 10 June 2016 and removed 4 October 2016 at Eastport. Every 2 weeks, yellow pan trap samples were collected and refilled with trapping solution, and sentinel eggs were removed and replaced. Bark sifting samples were collected 10–13 February 2017 at Gratiot-Saginaw, Harris Nature Center, and Legg Park; and 11 May 2017 at Eastport. Bark sifting samples were placed in rearing tubes for bark rearing sampling within 3 days of collection and removed after 60 days. Three fluon-coated green funnel traps baited with cis-3-hexanol (Crook et al., 2014) were used to monitor EAB adult density at each site. Traps were suspended from the lower- or mid-canopy of ash trees and cis-3-hexanol lures were replaced after traps were in the field for ten weeks. Propylene glycol was placed in trap collection cups to kill and preserve insects.

2017 and 2018 Sampling

In 2017, all four sampling methods were compared at Gratiot-Saginaw, Harris Nature Center, and Legg Park. Ten trees were selected within the same plots established in 2016. All sample trees selected were alive and showed signs and/or symptoms of EAB attack. All sampling methods were applied to each tree. Yellow pan traps and sentinel egg sampling was conducted 17–18 May through 28–29 September 2017. Bark sifting samples (also used for bark rearing sampling) were collected 7–9 May 2017. Three fluon-coated green funnel traps baited with cis-3-hexanol were used to monitor EAB adult densities at each site.

In 2018, only bark sifting, bark rearing, and yellow pan trap methods were compared at Gratiot-Saginaw, Harris Nature Center, and Legg Park. Sentinel eggs were excluded because adequate numbers of EAB eggs were not available. Similar to 2017, 10 live trees were selected within the plots that showed signs and/or symptoms of EAB attack. Bark sifting, bark rearing, and yellow pan trap sampling methods were applied to each tree. Yellow pan traps sampling was conducted 17–18 May 2018 through 28 September–1 October 2018. Bark sifting and bark rearing samples were collected 15–16 May 2019. Three fluon-coated green funnel traps baited with cis-3-hexanol were used to monitor EAB adult densities at each site.

Data analyses

Comparison of sample methods

The numbers of *O. agrili* adults (yellow pan traps and bark rearing) or parasitized eggs (bark sifting and sentinel eggs) recovered, referred to as "*O. agrili* recoveries", with each of the sample methods were summed for each sample tree within each year. Also, the percent of trees at each site that recovered at least one *O. agrili* adult (yellow pan traps and bark rearing) or parasitized egg (bark sifting and sentinel eggs), referred to as "*O. agrili* positive trees", was determined. The treatment effects on the total number of *O. agrili* recoveries and positive trees were analyzed with a generalized linear mixed model (PROC GLIMMIX) using SAS 9.4 for Windows (SAS Institute, 2012). *Oobius agrili* recoveries were analyzed with a negative binomial distribution and a log link function; data for *O. agrili* positive trees were analyzed with a binary distribution and a logit link function. Each sample year was analyzed separately. For 2016 data, a multi-location complete block model was used for comparisons with treatment as a fixed effect and site, sample tree within site, and the interaction of site × treatment as random effects. Because fewer trees and fewer sites were sampled in 2017 and 2018, data did not

support a multi-location complete block design and were analyzed instead with a complete block model with site as a fixed effect and sample tree within site as a random effect. Treatment least squared means that were significantly different ($P \leq 0.05$) were separated with Tukey-Kramer means comparison procedure.

Estimation of sample size

The probability of recovering *O. agrili* and the precision of *O. agrili* recoveries as a function of number of trees sampled was estimated for each sampling method by bootstrapping (Simonoff et al., 1994) the *O. agrili* recovery data for the 40 trees sampled at each site in 2016. First, bootstrap sample sizes of 1–40 trees were each iterated 2000 times for each sample method within each site using PROC SURVEYSELECT (SAS Institute, 2012). The percentage probability of at least one tree positive for *O. agrili* was calculated for each bootstrap sample size for each sample method within each site (i.e., number of iterations that recovered *O. agrili*/2000 iterations). A four-parameter Weibull probability function was fitted to the percent probability as a function of number of trees sampled from all four sites using the "drc" package in R (R Core Team, 2018; Ritz et al., 2015) for each of the sampling methods. The coefficient of variation (CV) was calculated for the mean number of *O. agrili* recoveries from each bootstrap iteration within each bootstrap sample size from each site and sample method as an estimate of change in precision as a function of trees sampled (Stanovick et al., 2002). Next, a Pearson correlation analysis (PROC CORR; SAS Institute 2012) was used to determine which signs or symptoms of EAB attack recorded during pre-season (late spring 2016) and post-season (late winter/early spring 2017) were correlated with *O. agrili* recoveries for each sampling method in 2016. The bootstrapping procedure for sampling all trees as described above was repeated but included only trees with the sign or symptom of EAB attack that was most correlated with *O. agrili* recoveries.

Probability curves were fitted to percent probability of recovery and CVs were calculated for number of recoveries for these bootstrap data.

Sampling period

Growing degree days (base 10°C; GDD_{10}), calculated using the Baskerville-Emin method, were obtained from Michigan State University Enviroweather (2019) for weather stations nearest to each site for sentinel egg collection dates in 2016 and 2017, and yellow pan traps in 2016, 2017, and 2018. Cumulative sentinel egg and yellow pan trap *O. agrili* recoveries were calculated as a function of GDD_{10} to determine the range of GDD_{10} when *O. agrili* adults were active. Mean GDD_{10} when: 1) *O. agrili* was first recovered, 2) maximum number (i.e., peak) were recovered, and 3) ≥95% were recovered, was calculated for sentinel eggs and yellow pan traps to estimate optimal sampling time.

Results

Comparison of sample methods

In 2016, *O. agrili* was recovered with each of the four sampling methods (Table 4.2). Also, all methods recovered *O. agrili* at each site with the exception of bark rearing at Harris Nature Center. Parasitized eggs with exit holes were recovered at Harris Nature Center using the bark sifting method, but no overwintering *O. agrili* were present in these eggs (Table 4.2). Mean *Oobius agrili* recoveries per tree were overall very low and varied significantly among sampling methods ($F = 10.33$; $df = 3, 9.5$; $P = 0.0024$; Fig. 4.2). Mean *O. agrili* recoveries per sample tree were highest for yellow pan traps (mean ± se per tree = 0.52 ± .29) and bark sifting (0.32 ± .18), and lowest for bark rearing (0.32 ± 0.18) and sentinel eggs (0.08 ± 0.05; Fig. 4.2). Percent *O. agrili* positive trees also varied significantly among treatments in 2016 ($F = 7.95$; $df = 3, 9.6$; $P =$

0.0057, Fig. 4.2). Yellow pan traps had the highest percentage of *O. agrili* positive trees (33.8 ± 13.6%), and bark rearing (6.9 ± 4.1%) and sentinel eggs (4.8 ± 4.1%) had the lowest percentages (Fig. 4.2). Percentage of *Oobius agrili* positive trees were intermediate for bark sifting (23.0 ± 10.8%).

In 2017, all four sampling methods recovered *O. agrili* from the three study sites, with the exception of bark rearing at Gratiot-Saginaw (Table 4.2). Mean *Oobius agrili* recoveries per tree varied significantly among sampling methods (F = 4.83; df = 3, 89; *P* = 0.0037; Fig. 4.2) and overall recoveries were very low (Table 4.2). Mean *O. agrili* recoveries was significantly higher for yellow pan traps (0.93 ± 0.53 per tree) compared to bark rearing (0.08 ± 0.07). Mean recoveries for sentinel eggs (0.44 ± 0.27) and bark sifting (0.33 ± 0.20) were intermediate between yellow pan traps and bark rearing. Mean percent *O. agrili* positive trees also varied significantly among methods (F = 4.43; df = 3, 104; *P* = 0.0057; Fig. 4.2). Yellow pan traps had significantly higher percent *O. agrili* positive trees (53.1 ± 18.3%) compared to sentinel eggs (14.4 ± 10.1%) and bark rearing (7.8 ± 6.5%) samples. Mean percentage positive trees was intermediate for bark sifting (22.1 ± 13.3%).

In 2018, *O. agrili* was recovered with all three sampling methods (bark rearing, bark sifting, and yellow pan traps) at all sites and overall *O. agrili* recoveries and positives were higher compared to 2016 and 2017. Mean *O. agrili* recoveries varied significantly among sampling methods (F = 10.11; df = 2, 82.7; *P* = 0.0001). *Oobius agrili* recoveries were significantly higher for yellow pan traps (2.22 ± 0.59 per tree) and bark sifting (2.06 ± 0.55) methods compared to the bark rearing (0.33 ± 0.13) (Fig. 4.2). *Oobius agrili* positive trees were also significantly higher for yellow pan traps (64.0 ± 10.1%) and bark sifting 67.5 ± 9.8%) compared to bark rearing (22.3 ± 8.4%) (Fig. 4.2; F = 6.73; df = 2, 87; *P* = 0.0019).

Table 4. 1. Data collected in 2016 from Michigan study sites (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) including: dominant ash species, mean tree diameter, site was lowland or upland, year that EAB was detected in the county in which site was located, year *Oobius agrili* was released, mean percent of canopy dieback of all live ash trees, percent of trees with fresh woodpecker feeding holes, and total EAB captured in green funnel traps (three traps at each site).

Site	Dominant ash species	Mean tree diameter	Lowland or upland	Year EAB detected	Year *O. agrili* released	Mean percent dieback	Percent trees with fresh woodpecker holes	Total EAB captured
Eastport	White	19.8 ± 1.0	Upland	2004	2014	24 ± 4	73	584
Gratiot-Saginaw	Green	10.3 ± 0.2	Lowland	2004	2009	25 ± 6	10	287
Harris Nature Center	White	17.8 ± 0.9	Upland	2003	2008	30 ± 6	18	96
Legg Park	Green	13.9 ± 0.4	Lowland	2003	No release	13 ± 5	25	170

Table 4. 2. Total number of *O. agrili* recoveries and positive trees for four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs; yellow pan traps) at four sites (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) for three years. Eastport was only sampled in 2016. Number of trees sampled at each site was 40 in 2016 and 10 in 2017 and 2018.

		Sampling method							
		Bark rearing adults		Bark sifting eggs		Sentinel eggs		Yellow pan traps	
Year	Site	Total recovered	No. positive trees	Total recovered	No. positive trees	Total recovered	No. positive trees	Total recovered	No. positive trees
2016	Eastport	34	11	95	18	13	5	272	33
	Gratiot-Saginaw	11	3	33	14	5	3	31	8
	Harris Nature Center	0	0	8	3	5	2	10	7
	Legg Park	4	4	33	9	5	2	49	10
2017	Gratiot-Saginaw	0	0	4	2	9	2	5	3
	Harris Nature Center	1	1	4	1	1	1	25	4
	Legg Park	4	2	9	4	14	4	32	9
2018	Gratiot-Saginaw	5	3	37	9	NA	NA	19	7
	Harris Nature Center	4	1	9	4	NA	NA	27	8
	Legg Park	3	3	31	7	NA	NA	32	4

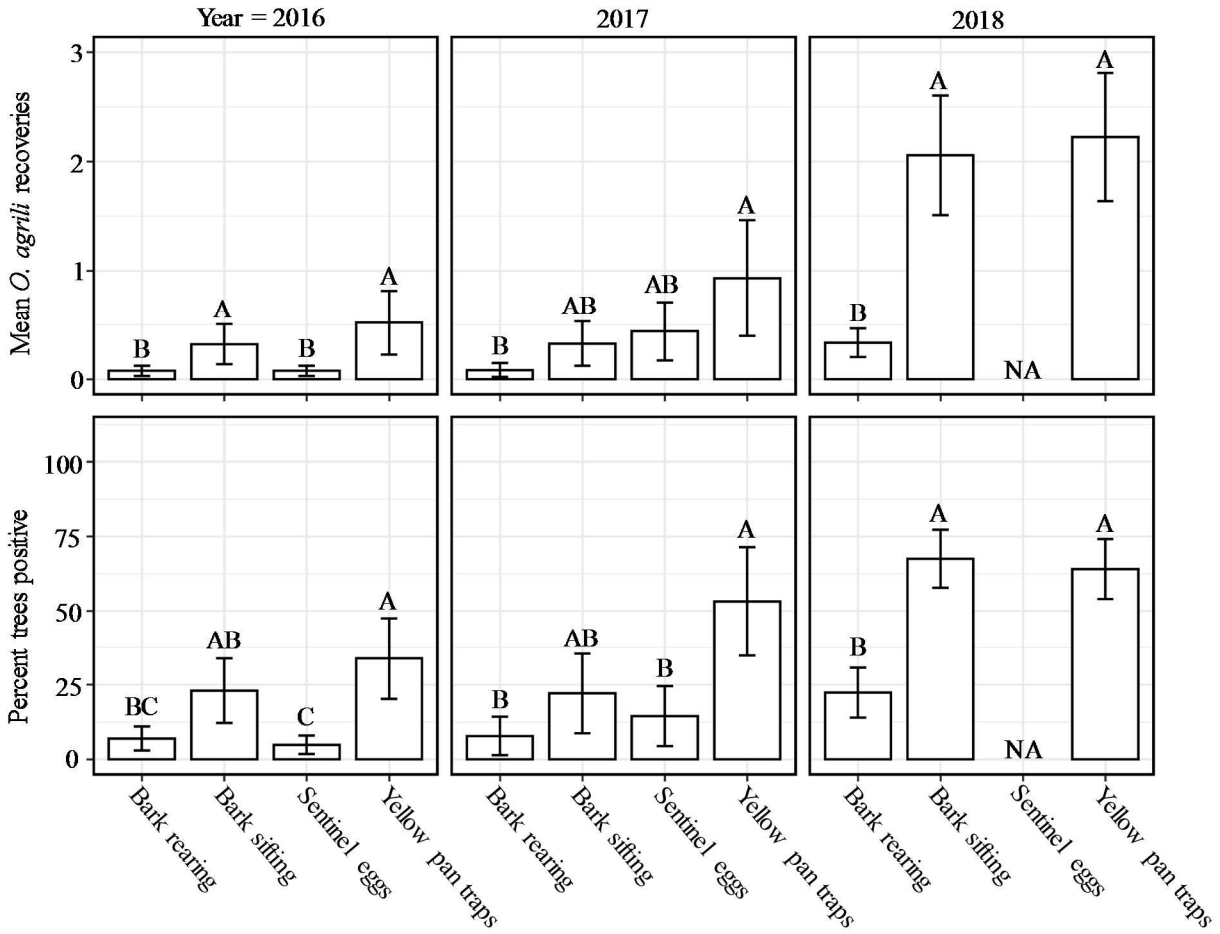

Figure 4. 2. Mean number of recoveries (top row) and mean percentage of positive trees (bottom row) for *O. agrili* by sampling method (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps) for three sample years. Means with different letters within each year and row are significantly different ($P \leq 0.05$; Tukey-Kramer means separation).

Estimation of sample size

Bootstrapping results demonstrated that the probability of recovering *O. agrili* as a function of number of trees sampled varied among sampling methods and sites (Fig. 4.3; Table 4.4). For yellow pan traps, when all trees within plots were bootstrap sampled, the probability

function estimated that a sample size of 13 trees [confidence interval (c.i.) = 10–16] had a 95% probability of recovering *O. agrili*. Bark sifting required 16 trees (c.i. = 6–22) and sentinel eggs required 43 trees (c.i. = 27–70) to reach 95% probability of recovery when all trees were sampled. The probability of recovery never reached 95% for bark rearing due to failure to recover *O. agrili* at Harris Nature Center in 2016. CVs for number of *O. agrili* recoveries when all trees within sites were randomly sampled were very high for all methods and sites (Fig. 4.4). CVs for the number of *O. agrili* recovered decreased as the number of trees sampled increased but the most dramatic decrease in CVs occurred when sample size increased from 1 to approximately 10 trees regardless of sampling method or site (Fig. 4.4).

Several signs and symptoms were significantly correlated with *O. agrili* recoveries for the different sampling methods (Table 4.4). Fresh woodpecker feeding holes was the most consistent sign or symptom correlated with *O. agrili* recoveries among sampling methods with correlations highly significant (P <0.0001) for bark rearing, bark sifting, and yellow pan traps, and marginally significant (P = 0.0551) for sentinel eggs (Table 4.4). Therefore, bootstrap sampling was performed using only trees with fresh woodpecker feeding holes. Trees with pre-season fresh woodpecker feeding holes in 2016 were used to subsample for yellow pan trap and sentinel egg methods because these were observed when yellow pan traps and sentinel eggs were first installed. Trees with post-season fresh woodpecker feeding holes were subsampled for bark rearing and bark sifting methods because these were observed in late-winter or early-spring 2017 when bark samples were collected. For all methods except for sentinel eggs, percentage probability of recovery increased with increasing number of sample trees that had fresh woodpecker feeding holes compared to including all trees (Fig. 4.3; Table 4.3). The number of trees required to reach 95% probability of *O. agrili* recovery was 7 (c.i. = 6–8) and 10 (c.i. = 7–

13) for bark sifting and yellow pan traps, respectively (Fig. 4.3). Of the 3 sites that bark rearing recovered *O. agrili*, sampling trees with fresh woodpecker feeding holes increased the probability of recovery but 95% was never reached because *O. agrili* was not recovered with bark rearing at Harris Nature Center in 2016 (Fig. 4.3; Table 4.3). For sentinel eggs, probability of recovery was lower when only trees with fresh woodpecker feeding holes were sampled because no *O. agrili* were recovered on trees with fresh woodpecker feeding holes at two of four sites (Fig. 4.3).

Table 4. 3. Percent probability of *O. agrili* recover and 95% confidence interval as a function of number of trees that were bootstrap sampled from data that included all trees or only trees with fresh woodpecker feeding holes within plots for four different sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps).

	Mean percent probability of recovery ± 1/2 95% confidence interval							
	Bark rearing		Bark sifting		Sentinel eggs		Yellow pan traps	
No. trees	All trees	Trees with fresh wood-pecker feeding holes	All trees	Trees with fresh wood-pecker feeding holes	All trees	Trees with fresh wood-pecker feeding holes	All trees	Trees with fresh wood-pecker feeding holes
5	38.8 ± 15.0	43.1 ± 15.6	68.8 ± 4.7	87.8 ± 1.8	31.1 ± 4.0	30.7 ± 12.0	76.6 ± 3.5	85.0 ± 3.4
10	54.3 ± 10.3	58.4 ± 10.7	85.6 ± 3.6	97.9 ± 1.2	51.5 ± 3.1	38.3 ± 8.3	92.4 ± 2.7	95.7 ± 2.8
15	62.4 ± 10.0	65.8 ± 10.4	92.2 ± 3.1	99.6 ± 0.7	65.4 ± 2.8	42.9 ± 7.3	97.4 ± 2.1	98.5 ± 1.9
20	67.1 ± 9.0	69.6 ± 8.9	95.2 ± 2.5	99.9 ± 0.8	75.0 ± 2.8	46.2 ± 7.4	99.1 ± 1.6	99.4 ± 1.6
25	69.9 ± 7.7	71.8 ± 7.6	96.8 ± 2.3	99.9 ± 0.8	81.8 ± 2.5	48.7 ± 8.1	99.7 ± 1.7	99.7 ± 1.7
30	71.7 ± 8.0	73.1 ± 8.3	97.7 ± 2.6	100 ± 0.8	86.5 ± 2.4	50.9 ± 8.9	99.9 ± 1.9	99.9 ± 1.9
35	72.8 ± 10.3	73.9 ± 10.4	98.2 ± 3.1	100 ± 0.8	89.9 ± 3.1	52.7 ± 9.9	100 ± 2.0	99.9 ± 2.0
40	73.6 ± 13.1	74.3 ± 12.7	98.5 ± 3.6	100 ± 0.8	92.2 ± 4.4	54.2 ± 10.8	100 ± 2.1	99.9 ± 2.1

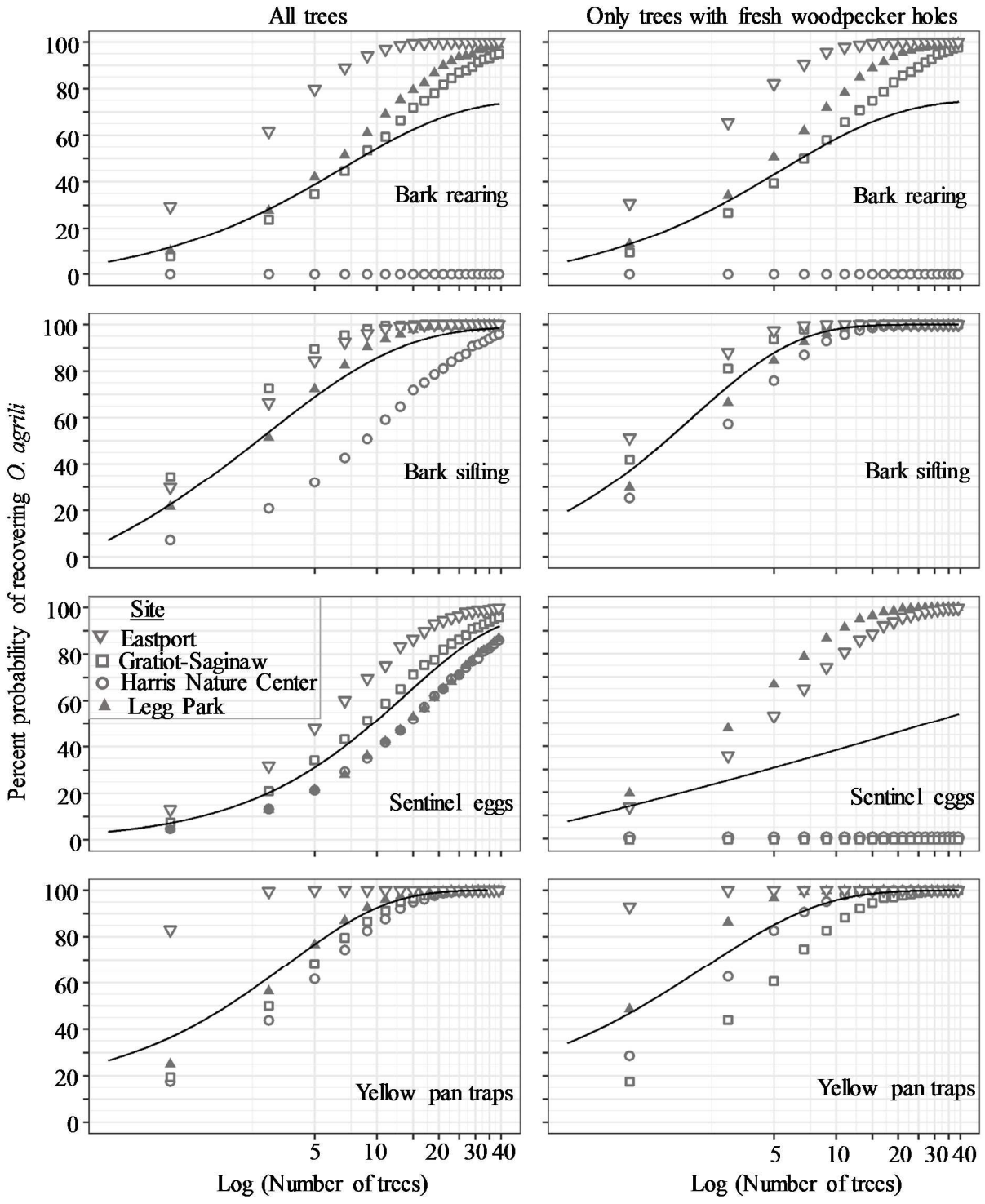

Figure 4. 3. Four-parameter Weibull probability function fitted to percentage of bootstrapping iterations with *O. agrili* recoveries for samples of 1–40 trees at four different sites [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI, 3) Harris Nature Center and 4) Legg Park:

Figure 4.3. (cont'd)

Okemos, MI] for each of four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all trees sampled at each site and right column includes only trees that had fresh woodpecker feeding holes.

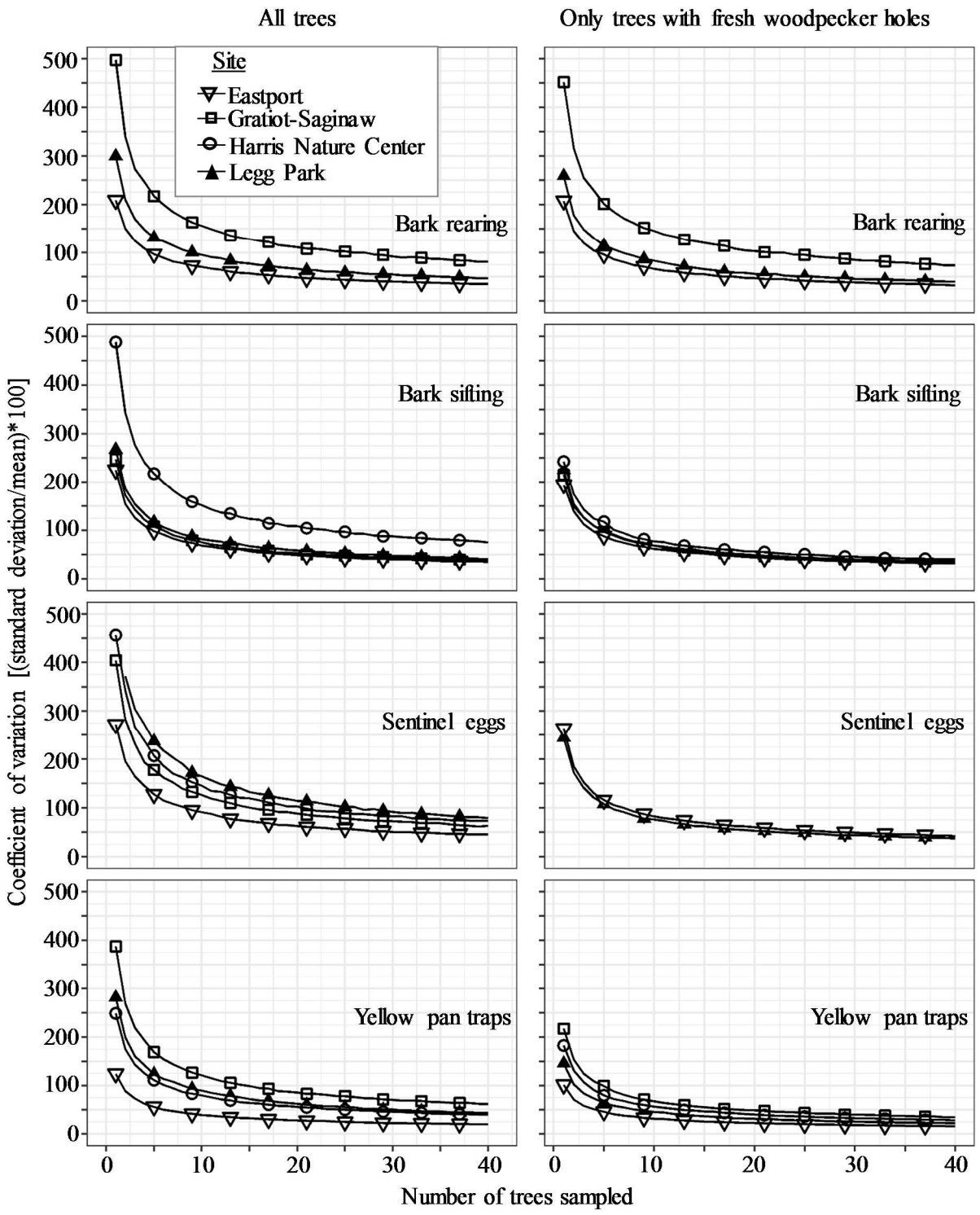

Figure 4. 4. Coefficient of variation of mean *O. agrili* recoveries for bootstrapped samples of 1-40 trees each iterated 2000 times for 4 sites in Michigan [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI, 3) Harris Nature Center and 4) Legg Park: Okemos, MI] and for 4

Figure 4.4. (cont'd)

different sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all 40 trees within sample plots at each site and right column includes only trees within each plot that had fresh woodpecker feeding holes. CVs are only shown for sites and methods that recovered *O. agrili* (see Fig. 4.3 and Table 4.1).

Table 4. 4. Correlation matrix showing p-value (*P*) and correlation coefficient (R) of the relationship between signs or symptoms of EAB attack (woodpecks = fresh woodpecker feeding holes; epicormics shoots; exits = EAB exit holes; EAB eggs= total number of EAB eggs collected from bark samples) and recoveries of *O. agrili* adults or EAB eggs (parasitized and total) using four different sampling methods (bark rearing= bark rearing for adult *O. agrili*; bark sifting = bark sifting for parasitized EAB eggs; sentinel eggs = sentinel EAB egg screened envelopes; and yellow pan traps).

	Canopy dieback		**Woodpecks pre-season**		**Epicormic shoots pre-season**		**Exits pre-season**		**Woodpecks post-season**		**Epicormic shoots post-season**		**Exits post-season**		**EAB eggs**	
	P	**R**	***P***	**R**	***P***	**R**	***P***	**R**	***P***	**R**	***P***	**R**	***P***	**R**	*P*	**R**
Bark rearing recoveries	0.441	0.06	0.0008	0.26	0.011	0.20	0.855	0.01	<.0001	0.40	0.298	0.08	<.0001	0.30	<.0001	0.40
Bark sifting recoveries	0.072	0.14	<.0001	0.50	0.009	0.21	0.613	-0.04	<.0001	0.46	0.635	0.04	<.0001	0.50	<.0001	0.74
Sentinel eggs recoveries	0.719	0.03	0.0551	0.15	0.878	-0.01	0.133	0.12	0.011	0.20	0.287	-0.08	0.248	0.09	0.097	0.13
Yellow pan trap recoveries	0.357	0.07	<.0001	0.67	0.438	0.06	0.527	-0.05	<.0001	0.63	0.317	0.08	<.0001	0.45	<.0001	0.33
EAB eggs	<.0001	0.33	0.0003	0.29	<.0001	0.42	0.813	-0.02	<.0001	0.35	0.931	0.01	<.0001	0.40		

Similar to subsampling all trees within plots, CVs for *O. agrili* recoveries when only trees with fresh woodpecker feeding holes were bootstrap sampled were very high for all methods and sites (Fig. 4.4). For sites and methods where *O. agrili* was recovered on trees with fresh woodpecker feeding holes, CVs decreased dramatically as sample size increased to 10 trees and then decreased at a slower rate as sample size increased through 40 trees.

Sample timing

Mean GDD_{10} when yellow pan traps first collected *O. agrili* was 433 ± 25 (range: 342–625; Fig. 4.5). Peak *O. agrili* were captured in yellow pan traps at 845 ± 43 GDD_{10} (range: 617–1003; Fig. 4.5). Mean GDD_{10} when 95% *O. agrili* were collected in yellow pan traps was 1068 ± 47 (range: 958–1407). Last capture of *O. agrili* in yellow pan traps was 1158 ± 56 GDD_{10} (range: 963–1407).

Mean GDD_{10} when parasitized sentinel eggs were first recovered was 774 ± 99 (range:418–1185; Fig. 4.6). Mean GDD_{10} for peak sentinel egg parasitism was 891 ± 122 (range: 418–1199). Cumulative sentinel egg parasitism reached 95% at 984 ± 107 (range: 418–1199; Fig. 4.6).

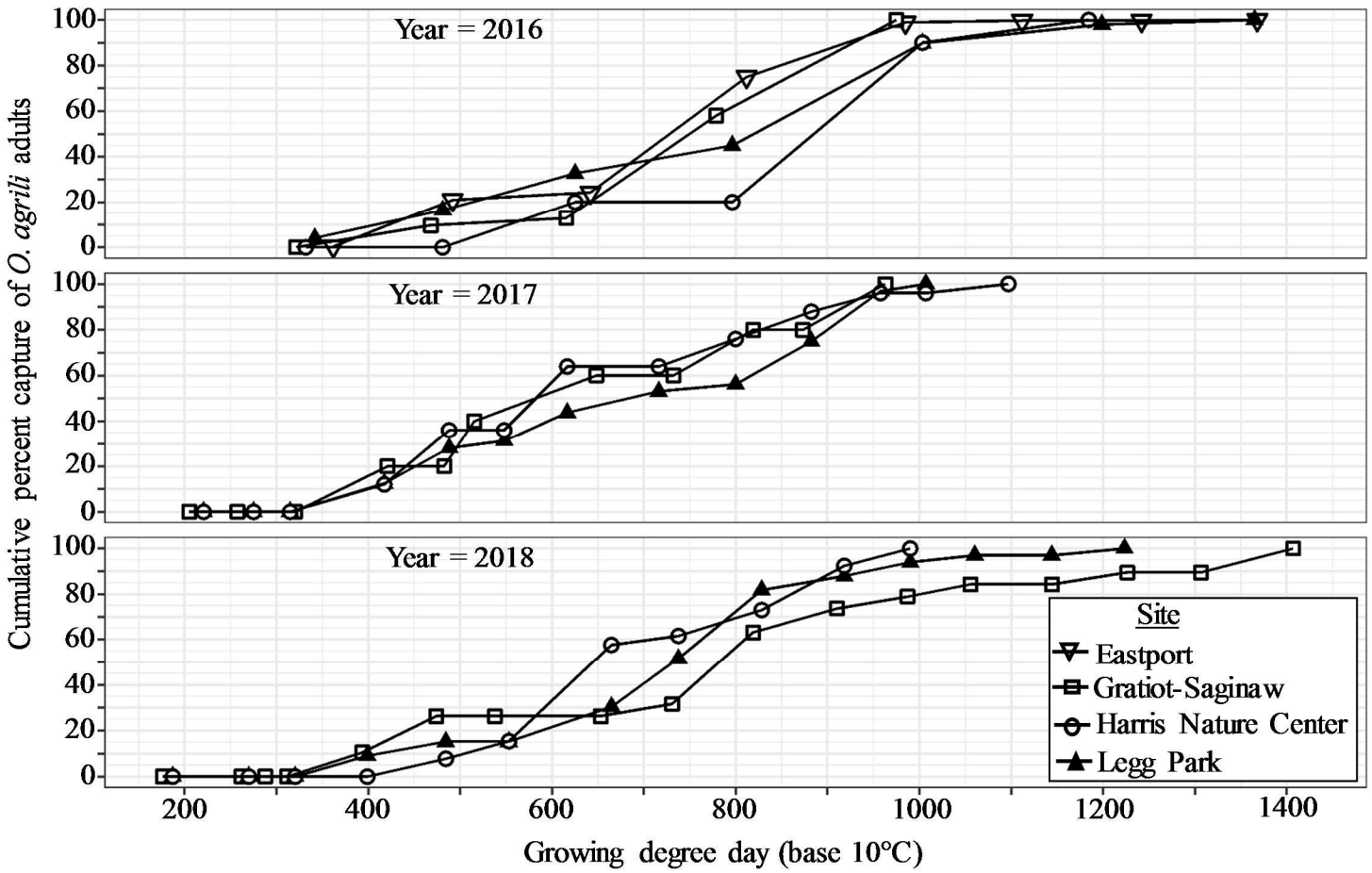

Figure 4. 5. Cumulative percent capture of *O. agrili* adults in yellow pan traps by growing degree days (base =10°C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a three-year period. Samples collected once every two weeks in 2016 and once every week in 2017 and 2018. Eastport was only sampled in 2016.

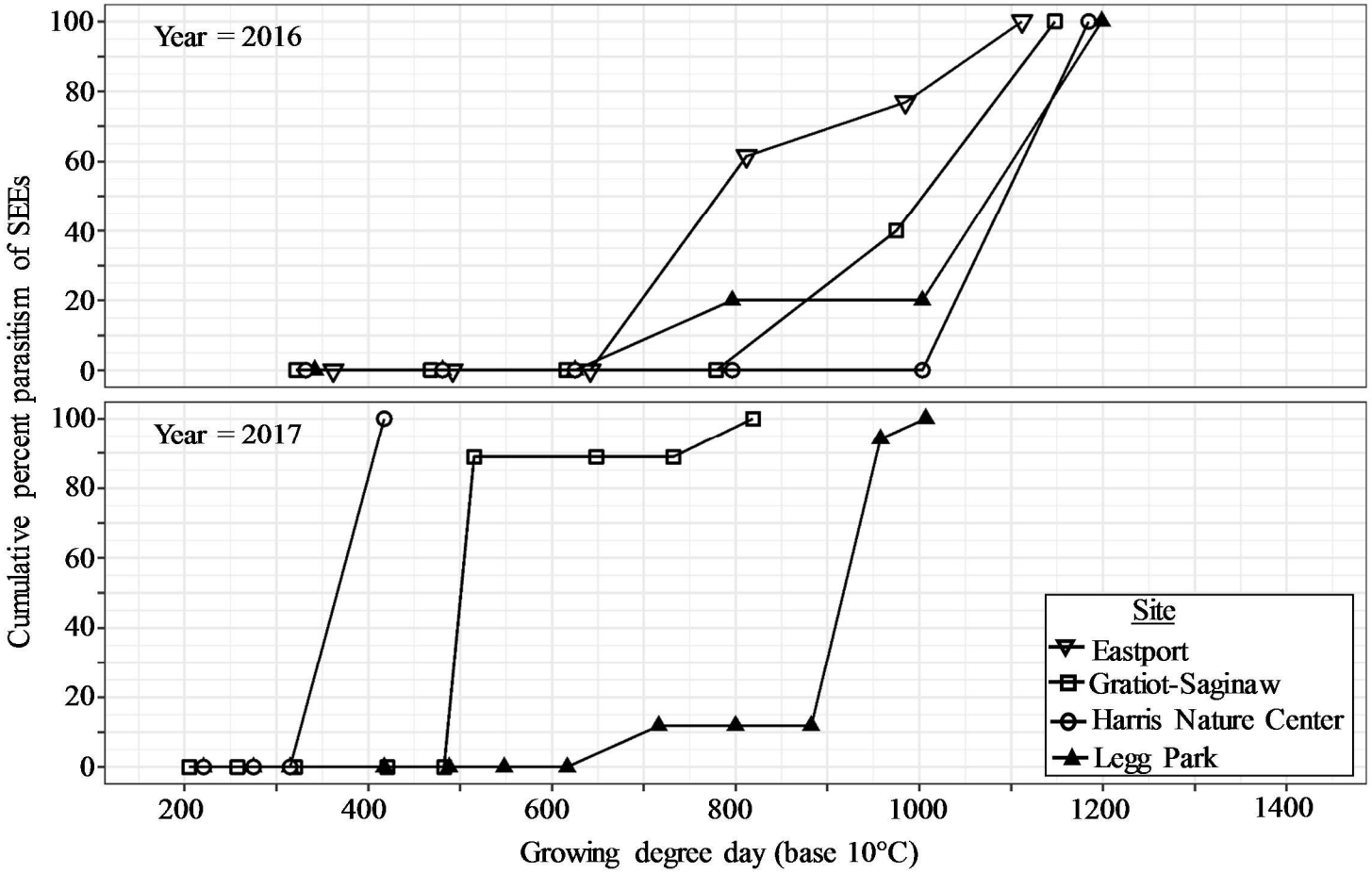

Figure 4. 6. Cumulative percent parasitism of sentinel eggs by growing degree days (base =10°C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a two-year period. Samples collected once every two weeks in 2016 and once every week in 2017. Eastport was only sampled in 201

Table 4. 5. Information provided, site visits required, and relative labor and technology required for each of four *O. agrili* sampling method.

	Sampling methods			
	Bark rearing	Bark sifting	Sentinel eggs	Yellow pan traps
Confirms *O. agrili* currently present at site	Yes	No	Yes	Yes
Estimates percent parasitism	No	Yes	Yes	No
Determines seasonal activity of adults	No	No	Yes	Yes
Collects other EAB parasitoids	No	No	No	Yes
Site visits required	One	One	Several	Several
Technology required	Low	Low	High	Medium
Labor required	Low	Low	High	Medium

Discussion

This study compared four sampling methods for detecting and recovering *O. agrili* in the field, including three methods recommended by EAB Biocontrol Guidelines. The probability of recovering a positive *O. agrili* sample and the precision of number of *O. agrili* recoveries as a function of total trees sampled was estimated for each of these methods. Also, the GDD_{10} when *O. agrili* adults were active was determined for methods that target *O. agrili* adults (i.e., yellow pan traps and bark rearing). Of the methods tested, yellow pan traps and bark sifting methods consistently had higher *O. agrili* recoveries and positive trees compared to bark rearing and sentinel eggs, and recovered *O. agrili* at all sites and all years. Both yellow pan traps and bark sifting had higher probability of recoveries with fewer sampled trees, compared to bark rearing and sentinel eggs. Focusing sample efforts on trees with fresh woodpecker feeding holes increased the probability of recovering *O. agrili* for all methods except sentinel eggs, and decreased the CVs for *O. agrili* recoveries. Each of these methods has advantages and

disadvantages, and each provides slightly different information regarding *O. agrili* populations (Table 4.5).

Capture of adult *O. agrili* in yellow pan traps obviously confirms they are present at sites during the sampling season. Yellow pan traps also provide information on *O. agrili* abundance and seasonal activity at release sites. Another advantage of yellow pan traps is they also capture other EAB parasitoid species (Jones et al., 2019; Parisio et al., 2017). A disadvantage is yellow pan traps must be assembled because they are not commercially available. Also, yellow pan traps require installation and periodic collection when *O. agrili* adults are active; during spring and/or summer depending on geographic location. Yellow pan trap samples should be collected a minimum of once every 2 weeks; the sampling interval for this study in 2016 using a solution of 50:50 propylene glycol and H_2O. EAB Biocontrol Guidelines recommends sampling every week using a solution of 25:75 propylene glycol and H_2O. Although the condition of samples was acceptable for two-week samples when a 50:50 solution was used, samples collected weekly during 2017 and 2018 were less decayed. Most *O. agrili* were captured in yellow pan traps between 433–1068 DD_{10}, a mean trapping period of 54 ± 4 days (range =41–85). This would require approximately 4 collections at 2-wk intervals; or 8 collections at 1-wk intervals under Michigan conditions. After collection, sorting samples for suspect parasitoids can be challenging because large numbers of nontarget insects in samples can make recognizing *O. agrili* difficult. Also, the small size of *O. agrili* allows them to become entangled with legs or other body parts of larger insects. Furthermore, several congeners and close relatives have similar morphology to *O. agrili*, and specimens may require confirmation by specialists.

Bark sifting was another effective method for recovering *O. agrili*. A major advantage of this method is that all samples can be collected during a single site visit and at any time of the

season. However, bark sifting samples are usually collected during fall–spring when *O. agrili* are diapausing. Also, percentage parasitism of EAB eggs can be calculated by dividing total EAB eggs recovered by the number parasitized, providing an estimate of the parasitoid's impact on EAB populations. A significant disadvantage of bark sifting is if parasitoids have already emerged from parasitized eggs, presence of parasitized eggs with exit holes confirms only that *O. agrili* were present at some previous time, given that EAB eggs may persist on trees for more than one year (Abell et al. 2014). Collecting and sifting bark sifting samples take only a few minutes for each. However, sorting bark debris for EAB eggs can be tedious and requires approximately 1hr per sample (Jennings 2018; Pers. obs.), especially if there is a large volume of bark debris. Also, if eggs are damaged during collection or sifting, determining their status can be challenging.

Bark rearing was one of the least effective methods tested and failed to recover *O. agrili* adults in 2016 at Harris Nature Center despite that recoveries were made with the other 3 methods (Table 4.2). As a result, the overall probability of recovery estimated using the probability function did not reach 95% (Fig. 4.3, Table 4.3). *Oobius agrili* abundance was very low at Harris Nature Center in 2016, with only 10 *O. agrili* adults recovered in yellow pan traps and 8 parasitized EAB eggs recovered with bark sifting (Table 4.2). Bark rearing also recovered no *O. agrili* at Gratiot-Saginaw in 2017, which also had low overall recoveries of *O. agrili* that year (Table 4.2). However, only 10 bark rearing samples were taken from each site in 2017. Nevertheless, the bark rearing method may not be effective for sampling areas with very low *O. agrili* densities. An advantage of bark rearing is that bark sifting samples can be reared prior to sifting to quickly assess if *O. agrili* successfully overwintered at release sites. However, bark samples used for bark rearing should be collected after *O. agrili* had experienced a period of cold

temperatures (i.e., winter–spring), allowing completion of diapause development (Duan and Larson, 2019b); but before adult *O. agrili* emerge in the spring. If adult *O. agrili* emerge from bark rearing samples in the laboratory, presence at a site is confirmed and bark can be processed with the bark sifting method to estimate percent parasitism. Recovery of *O. agrili* with bark rearing method can be improved by collecting larger bark samples or sampling from more trees. Alternatively, logs can be reared from felled ash trees (log rearing for adults), which Parisio et al. (2017) found to be effective for recovering *O. agrili*. The bark rearing method requires initial investment in labor and materials to construct rearing containers. However, rearing containers can be reused for future sampling efforts.

Unfortunately, sentinel eggs was not very effective at sampling *O. agrili* in this study. Parisio et al. (2017) failed to recover *O. agrili* with sentinel EAB eggs on ash bolts or in cups. Sentinel eggs was the only method tested in this study that included a possible olfactory attractant (i.e., volatiles from fresh EAB eggs). Volatiles from host eggs provide important host location cues for egg parasitoids (Vinson, 1998); however, volatiles produced by ovipositing adults may provide additional host location cues (Colazza et al., 1999; Peri et al., 2006). For this study, EAB eggs deposited on filter papers were used, so there were likely little or no volatiles from EAB adults present. Sentinel eggs did recover *O. agrili* at all sites, including Harris Nature Center in 2016 and Gratiot-Saginaw in 2017, both with very low overall *O. agrili* recoveries for all methods (Table 4.2). During 2016 and 2017, all positive sentinel eggs were recovered between 418–1185 DD_{10}, similar to when yellow pan traps captured most *O. agrili* adults. A major advantage of sentinel eggs is since *O. agrili* is the only egg parasitoid known to attack EAB in North America, *O. agrili*'s presence can be confirmed by the presence of parasitized eggs, which usually darken several days after attack (Abell et al., 2014). Sentinel eggs can also

provide data on *O. agrili* seasonal activity and parasitism rates (Abell et al., 2016; Duan et al., 2012). However, given the amount of labor and technology required for rearing EAB eggs, the multiple trips required for deployment and collection, and low overall recovery rates, sentinel eggs is the least time- and cost-effective of the methods compared if the primary objective is to determine *O. agrili* presence at release sites.

Sampling only trees with fresh woodpecker feeding holes improved detection rates for most sampling methods. Woodpeckers primarily forage on late instar EAB larvae, and fresh feeding holes confirms trees were recently attacked if EAB is established in the area (Cappaert et al., 2005; Duan et al., 2010). The probability of detecting *O. agrili* on trees with fresh woodpecker feeding holes increased most for bark sifting, compared to the other sampling methods. For example, the percent probability of recovering *O. agrili* for 10 bark sifting samples, as recommended by EAB Biocontrol Guidelines, increased from 85.6 ± 3.6% when all trees were subsampled, to 97.9 ± 1.2% when only trees with fresh woodpecker feeding holes were sampled (Table 4.3). Since the bark sifting method targets EAB eggs, which are immobile, it is intuitive that focusing on trees that were recently attacked by EAB improved detection because host eggs must be present for parasitized eggs to be recovered. Similarly, the probability of detection for BSA increased when sampling trees with fresh woodpecker feeding holes for the three sites that BSA recovered *O. agrili* (Fig. 4.3). Post-season EAB exit holes were also significantly correlated with *O. agrili* recoveries for bark sifting and bark rearing (Table 4.4). Including these trees may be considered when using these methods, but care must be taken to ensure that live phloem was available for EAB oviposition the previous season.

Sampling trees with fresh woodpecker feeding holes increased detection only slightly for yellow pan traps. The percent probability of detection for 15 yellow pan traps samples, the

minimum number recommended by EAB Biocontrol Guidelines, increased from 97.4 ± 2.1 for sampling all trees to 98.5 ± 1.9 for only trees with fresh woodpecker feeding holes (Table 4.3 and 4.5). Yellow pan traps target *O. agrili* adults which can move freely throughout the environment in search of EAB eggs. Therefore, some *O. agrili* may be recovered on trees that were not recently attacked by EAB. Nevertheless, sampling trees with multiple fresh woodpecker feeding holes should increase the number of *O. agrili* collected, given the relatively high correlation coefficient (R = 0.6675; Table 4.4) that described the relationship between fresh woodpecker feeding holes and capture of *O. agrili* in yellow pan traps.

The probability of *O. agrili* detection decreased for sentinel eggs when only trees with fresh woodpecker feeding holes were sampled; due to no *O. agrili* recovered with the sentinel eggs method on trees with fresh woodpecker feeding holes at two of the four sites (Gratiot-Saginaw and Harris Nature Center). Both of these sites had low numbers of trees with fresh woodpecker feeding holes (six and seven trees, respectively) when sentinel eggs were installed in 2016. Also, the correlation between fresh woodpecker feeding holes and sentinel egg recoveries was only marginally significant ($P = 0.0551$; Table 4.4). Placing sentinel eggs on trees with fresh woodpecker feeding holes may not be as important compared to other methods because volatiles associated with host eggs likely attract *O. agrili* adults. However, *O. agrili*'s limited flight capability would require sentinel eggs to be deployed relatively close to trees where *O. agrili* adults are present.

Regardless of the sampling method, CVs for *O. agrili* recoveries were very high. Some of this variation may be attributed to the inefficiency of the sampling methods used. Given the small size and limited flight capabilities of *O. agrili*, it is possible that *O. agrili* was present on additional trees that were sampled at study sites but remained higher in trees, preventing their

detection with sampling methods applied to the lower trunk of trees. Also, *O. agrili* tends to be distributed unevenly within sites (Abell et al. 2014), likely contributing to the high capture variation among trees. As expected, CVs decreased as the number of sample trees increased, but the decrease in CVs was less dramatic after >10 trees were sampled. Also, CVs were lower when trees with fresh woodpecker feeding holes were sampled compared to sampling all trees within sites. Given these results, a minimum of ten trees with fresh woodpecker feeding holes should be sampled at each site regardless of sampling method. As sample size increases above ten trees, CVs should continue to decrease and precision of recovery estimates increase; however, these improvements will diminish as sample size increases.

Conclusion

Each of the methods tested recovered *O. agrili* adults or parasitized eggs. The yellow pan trap and bark sifting methods consistently performed the best for recovering *O. agrili*. CVs of *O. agrili* recoveries increased with increasing sample size, but the most dramatic increases occurred as sample sizes increased from 1 to 10 trees. The recommended EAB Biocontrol Guidelines sample size for yellow pan traps (15 traps per site) and bark sifting (10 samples per site when trees with fresh woodpecker feeding holes are sampled) should be adequate for detecting *O. agrili* at release sites if *O. agrili* is established. However, if only bark rearing is used, the recommended minimum of 30 samples per site may fail to recover *O. agrili* if it is established. Sentinel eggs recovered *O. agrili* at all sites; however, the technology and labor required makes this method impractical for most situations. Success and precision of *Oobius agrili* recoveries can be improved by selecting sites with an abundance of ash trees that have fresh woodpecker feeding holes. Focusing sampling efforts on trees within sites with large

numbers of fresh woodpecker feeding holes, but trees that are still living or that died during the current season, can further improve recovery success and precision, especially for the bark sifting and bark rearing methods. Most *O. agrili* adults were active between 400–1200 DD_{10} and methods that target adults should be conducted during this period. Results and discussion in this paper can help select an appropriate sampling method, sample size, and optimal deployment time to meet project objectives and satisfy logistical constraints for monitoring *O. agrili*.

CHAPTER 5: CONCLUSION

The distribution of EAB and *O. agrili* in North America now expands well beyond their known endemic climatic ranges in northeastern China and this may lead to host-parasitoid asynchrony in some areas. Geographic variation in day length could further confound synchrony given that photoperiod modulates *O. agrili*'s diapause induction. Research presented here was aimed at addressing questions and concerns regarding *O. agrili* spatiotemporal synchrony with EAB oviposition across current and potential distributions in North America and to evaluate methods for monitoring its establishment.

Results from laboratory experiments demonstrated that diapause is directly induced in developing *O. agrili* larvae when exposed to short day lengths. Response of larvae to photoperiod induced diapause ends when larvae are 6–7 days old. Adult photoperiod-exposure has no significant effect on diapause response of their progeny but diapause history of parasitoid adults can affect the proportion of their progeny that responds to photoperiod induced diapause. Only a small percentage of progeny produced by F_0 adults, which undergo diapause as larvae, enter diapause under short day photoperiod and this percentage increases as adult age increased. This effect is attributed to an "interval-timer" for diapause response that prevents populations from entering diapause too early in the season (Dixon, 1972; Lees, 1960; Tauber et al., 1986). In comparison, all progeny produced by nondiapaused *O. agrili* adults enter photoperiod-induced diapause when exposed to short day photoperiods. The critical day length when 50% of the *O. agrili* population enters diapause is between 14.25 and 14.5 hours of daylight. Photoperiod-induced diapause can be reversed by exposing diapausing larvae to day lengths above the critical day length and does not require exposure to a chill period. Also, emergence-times for adults

developing from diapause is affected by photoperiod and length of chill period, and this affect appears nonlinear.

A multiple cohort rate summation model was developed to simulate *O. agrili* phenology and synchrony with EAB oviposition across different climate regimes. *Oobius agrili* and EAB adult trapping data from Michigan sites were used to validate the model. In general, model predictions compared favorably with field data collected from four Michigan sites. Most of the differences between predicted and field data were likely attributed to: 1) lack of fit to certain probability distributions and failure of the model to describe all population variability; 2) trapping efficiency and low host and parasitoid population densities; 3) solar radiation altering bark temperatures from ambient temperature; 4) adult longevity and behavior affecting seasonal capture rates; and 5) *O. agrili* larvae developing in EAB eggs hidden under bark perceiving day lengths shorter than ambient photophases.

Model simulations demonstrated that *O. agrili* emergence from diapause remains temporally synchronized with initiation of EAB oviposition across a north-south gradient and trapping data in Michigan supported this prediction. Simulations also demonstrated that the first generation of non-diapaused adults (F_1 generation) is most synchronized with peak EAB oviposition and EAB oviposition is near completion when the second nondiapaused generation (F_2) emerges; these predictions were also supported by trap captures in Michigan. At the most northern locations, cool summer temperatures could reduce synchrony of the F_1 generation with peak EAB oviposition and reduce the probability of F_2 adult development and emergence. More trapping data is needed at a broader scale to further validate these model predictions. For example, it is possible that variation in winter temperatures across different climates may alter diapause development rates and adult emergence times, reducing model accuracy.

Comparison of trapping data with model predictions demonstrates that primarily two generations of *O. agrili* adults emerge each season in Michigan (F_0- and F_1-adults). Almost all progeny produced by F_1 adults apparently enter diapause despite ambient photophases that are longer than critical day length in south central Michigan. Diapause may be induced earlier than predictions because developing *O. agrili* perceive day lengths that are shorter than ambient photophases. There are two major hypotheses that may explain this: 1) diapause is induced because the hidden location of EAB eggs reduces or eliminates photophase lengths perceived by developing *O. agrili*; or 2) developing larvae also use thermoperiod to measure day lengths and these day lengths differ from ambient photophases.

The temporal occurrence of critical day length during emergence of *O. agrili* generations varies across geographic locations. Day lengths that are perceived by developing *O. agrili* will determine the significance of these effects on *O. agrili* population dynamics. If perceived day lengths are substantially shorter than ambient photophases, then spatiotemporal photoperiod variation may have relatively minimal effects on *O. agrili* population dynamics. However, if *O. agrili* perceive day lengths that are similar but slightly shorter than ambient photophases, diapause patterns could be significantly disrupted at some locations. Therefore, determining the environmental cues that developing *O. agrili* larvae use to measure day length in the field is crucial for understanding how spatiotemporal photoperiod variation will affect *O. agrili* population dynamics.

All sampling methods compared in this study recovered *O. agrili* at release sites. Yellow pan trap and bark sifting methods were the most consistent and required the least trees sampled to be >95% successful at recovering *O. agrili*. Following EAB Biological Control Release and Recovery Guidelines protocols for yellow pan traps and bark sifting sampling should be

adequate for recovering *O. agrili* if it is established. The bark rearing method may fail to recover *O. agrili* when following EAB Biological Control Release and Recovery Guidelines protocols of 30 samples; bark rearing recovery success may be improved by increasing the surface area and/or number of trees sample per site. Sentinel eggs are effective at recovering *O. agrili* but the labor and technology required makes them impractical for most situations. Regardless of sampling method, the largest increase in precision of *O. agrili* recoveries occurred when sample size was increased to 10 trees. Sampling efforts should focus on sites with an abundance of ash trees that have fresh woodpecker feeding holes. Sampling ash trees that are still living and have the most woodpecker feeding holes is especially important for bark sifting and bark rearing sampling. Yellow pan traps and sentinel egg sampling should be conducted between 400–1200 DD_{10}, when most *O. agrili* adults are active in the field.

EAB is a destructive invasive forest insect pest and has the potential to functionally extirpate several ash species that are native to eastern North America. As its range expands, more ash species will certainly be at risk. Currently, biological control is the only long-term self-perpetuating option for managing EAB. Understanding how introduced EAB-parasitoids will perform throughout their current and potential distribution in North America is crucial for evaluating their role at reducing impacts of EAB. This research focused on the EAB egg parasitoid, *O. agrili*, and results of this work provide insight and tools for further evaluation of spatiotemporal variation in *O. agrili* phenology and population dynamics within its current and potential distribution. Simulations suggest that different temperature regimes will not significantly affect the temporal synchrony of *O. agrili* with EAB oviposition, with the exception of some northern locations. However, the effects of photoperiod on *O. agrili* diapause induction in nature is still unclear. More research is needed to determine day-length perception of

developing *O. agrili* progeny in the field to improve model realism for simulating *O. agrili* phenology. Furthermore, the phenology and population dynamics of *O. agrili* and EAB oviposition requires validation across a broader spatial scale. If the spatiotemporal interactions of climate and photoperiod negatively impact *O. agrili* population dynamics at some geographic locations, then more suitable strains or species may need to be explored in Asia. Similar methods as those developed during this project should be applied to the additional three EAB biological control agents that are endemic to northeastern China and Russian Far East and being released across a broad range of climates in North America.

REFERENCES

REFERENCES

Abell, K.J., Bauer, L.S., Duan, J.J., Van Driesche, R., 2014. Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique. Biol. Control 79, 36–42. https://doi.org/10.1016/j.biocontrol.2014.08.002

Abell, K.J., Bauer, L.S., Miller, D.L., Duan, J.J., Driesche, R.G. Van, 2016. Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae. Florida Entomol. 99, 667–672. https://doi.org/10.1653/024.099.0413

Abell, K.J., Duan, J.J., Bauer, L., Lelito, J.P., Van Driesche, R.G., 2012. The effect of bark thickness on host partitioning between <>Tetrastichus planipennisi</i> (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biol. Control 63, 320–325. https://doi.org/10.1016/j.biocontrol.2012.08.009

Anderson, J.F., Kaya, H.K., 1974. Diapause induction by photoperiod and temperature in the elm spanworm egg parasitoid, *Ooencyrtus* sp. Ann. Entomol. Soc. Am. 67, 845–849. https://doi.org/10.1093/aesa/67.6.845

Andresen, J.A., McCullough, D.G., Potter, B.E., Koller, C.N., Bauer, L.S., Lusch, D.P., Ramm, C.W., 2001. Effects of winter temperatures on gypsy moth egg masses in the Great Lakes region of the United States. Agric. For. Meteorol. 110, 85–100. https://doi.org/10.1016/S0168-1923(01)00282-9

Bauer, L.S., Duan, J.J., Gould, J.R., Van Driesche, R., 2015a. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Can. Entomol. 147, 300–317. https://doi.org/10.4039/tce.2015.18

Bauer, L.S., Duan, J.J., Lelito, J.P., Liu, H., Gould, J.R., Bauer, L.S., Duan, J.J., Lelito, J.P., Liu, H., Gould, J.R., 2015b. Biology of emerald ash borer parasitoids, in: van Driesche, R.G., Reardon, R. (Eds.), Biology and Control of Emerald Ash Borer. USDA FS FHTET-2014-09, Morgantown, Wv, pp. 97–112.

Bauer, L.S., Liu, H., Miller, D., Gould, J., 2008. Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America. Newsl. Michigan Entomol. Soc. 53, 38–39.

Beck, S.D., 1982. Thermoperiodic induction of larval diapause in the European corn borer, *Ostrinia nubilalis*. J. Insect Physiol. 28, 273–277. https://doi.org/10.1016/0022-1910(82)90087-7

Beck, S.D., 1980. Insect Photoperiodism, second. ed. Academic Press, New York.

Belokobylskij, S.A., Yurchenko, G.I., Strazanac, J.S., Zaldívar-Riverón, A., Mastro, V., 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Ann. Entomol. Soc. Am. 105, 165–178. https://doi.org/10.1603/an11140

Bick, E.N., Forbes, N.J., Haugen, C., Jones, G., Bernick, S., Miller, F., 2018. Seven-year evaluation of insecticide tools for emerald ash borer in *Fraxinus pennsylvanica* (Lamiales: Oleaceae) Trees. J. Econ. Entomol. 111, 732–740. https://doi.org/10.1093/jee/toy018

Boivin, G., 1994. Overwintering strategies of egg parasitoids, Biological Control with Egg Parasitoids. CAB, Wallingford.

Bray, A.M., Bauer, L.S., Poland, T.M., Haack, R.A., Cognato, A.I., Smith, J.J., 2011. Genetic analysis of emerald ash borer (*Agrilus planipennis* Fairmaire) populations in Asia and North America. Biol. Invasions 13, 2869–2887. https://doi.org/10.1007/s10530-011-9970-5

Brown-Rytlewski, D., Wilson, M., 2004. Tracking the emergence of emerald ash borer adults, in: Mastro, B., Reardon, R. (Eds.), Proceedings of the Emerald Ash Borer Research and Technology Meeting. USDA FS FHTET-2004-15, Romulus, MI, pp. 13–14.

Cappaert, D., McCullough, D.G., 2009. Occurrence and seasonal abundance of *Atanycolus cappaerti* (Hymenoptera: Braconidae) a native parasitoid of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Gt. Lakes Entomol. 42, 16–29.

Cappaert, D., Mccullough, D.G., Poland, T.M., Siegert, N.W., 2005. Emerald ash borer in North America: a research and regulatory challenge. Am. Entomol. 51, 152–165.

Chamorro, M.L., Jendek, E., Haack, R.A., Petrice, T.R., Woodley, N.E., Konstantnov, A.S., Volkovitsh, M.G., Yang, X.-K., Grebennikov, V. V., Lingafelter, S.W., 2015. Illustrated guide to the emerald ash borer, *Agrilus planipennis* Fairmaire, and related species (Coleoptera, Buprestidae). Pensoft Publishers, Sofia, Bulgaria.

Choi, K.S., Kim, D.S., 2016. Effect of temperature on the fecundity and longevity of *Ascotis selenaria* (Lepidoptera: Geometridae): developing an oviposition model. J. Econ. Entomol. 109, 1267–1272. https://doi.org/10.1093/jee/tow029

Cli-MATE, 2019. MRCC's application tools environment for accessing climate data and value-added tools [WWW Document]. URL https://mrcc.illinois.edu/CLIMATE/ (accessed 1.16.20).

Colazza, S., Salerno, G., Wajnberg, E., 1999. Volatile and contact chemicals released by *Nezara viridula* (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). Biol. Control 16, 310–317. https://doi.org/10.1006/bcon.1999.0763

Cornelius, M.L., Buffington, M.L., Talamas, E.J., Gates, M.W., 2016. Impact of the egg parasitoid, *Gryon pennsylvanicum* (Hymenoptera: Scelionidae), on sentinel and wild egg masses of the squash bug (Hemiptera: Coreidae) in Maryland. Environ. Entomol. 45, 367–

375. https://doi.org/10.1093/ee/nvv228

Crook, D.J., Francese, J.A., Rietz, M.L., Lance, D.R., Hull-Sanders, H.M., Mastro, V.C., Silk, P.J., Ryall, K.L., 2014. Improving detection tools for emerald ash borer (Coleoptera: Buprestidae): comparison of multifunnel traps, prism traps, and lure types at varying population densities. J. Econ. Entomol. 107, 1496–1501. https://doi.org/10.1603/ec14041

Damos, P., Savopoulou-Soultani, M., 2012. Temperature-driven models for insect development and vital thermal requirements. Psyche (Stuttg). 2012, 1–13. https://doi.org/10.1155/2012/123405

Dixon, A.F.G., 1972. The "interval timer", photoperiod and temperature in the seasonal development of parthenogenetic and sexual morphs in the lime aphid, *Eucallipterus tiliae* L. Oecologia 9, 301–310. https://doi.org/10.1007/BF00345334

Duan, J.J., Bauer, L.S., Abell, K.J., Ulyshen, M.D., Van Driesche, R.G., 2015. Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: Implications for biological control. J. Appl. Ecol. 52, 1246–1254. https://doi.org/10.1111/1365-2664.12485

Duan, J.J., Bauer, L.S., Hansen, J.A., Abell, K.J., Van Driesche, R., 2012. An improved method for monitoring parasitism and establishment of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid introduced for biological control of the emerald ash borer (Coleoptera: Buprestidae) in North America. Biol. Control 60, 255–261. https://doi.org/10.1016/j.biocontrol.2011.11.007

Duan, J.J., Bauer, L.S., Van Driesche, R.G., 2017. Emerald ash borer biocontrol in ash saplings: The potential for early stage recovery of North American ash trees. For. Ecol. Manage. 394, 64–72. https://doi.org/10.1016/j.foreco.2017.03.024

Duan, J.J., Bauer, L.S., van Driesche, R.G., Gould, J.R., 2018. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests 9, 1–17. https://doi.org/10.3390/f9030142

Duan, J.J., Jennings, D.E., Williams, D.C., Larson, K.M., 2014. Patterns of parasitoid host utilization and development across a range of temperatures: implications for biological control of an invasive forest pest. BioControl 59, 659–669. https://doi.org/10.1007/s10526-014-9604-9

Duan, J.J., Larson, K.M., 2019a. Effects of chilling on diapause development and reproductive fitness of two congeneric species of encyrtid parasitoids (Hymenoptera: Encyrtidae)of the emerald ash borer. Biol. Control 134, 163–169. https://doi.org/10.1016/j.biocontrol.2019.04.010

Duan, J.J., Larson, K.M., 2019b. Effects of chilling on diapause development and reproductive fitness of two congeneric species of encyrtid parasitoids (Hymenoptera: Encyrtidae) of the emerald ash borer. Biol. Control 134, 163–169. https://doi.org/10.1016/j.biocontrol.2019.04.010

Duan, J.J., Ulyshen, M.D., Bauer, L.S., Gould, J., Van Driesche, R., 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). Environ. Entomol. 39, 1513–1522. https://doi.org/10.1603/en10023

Duan, J.J., Van Driesche, R.G., Crandall, R.S., Schmude, J.M., Rutledge, C.E., Slager, B.H., Gould, J.R., Elkinton, J.S., Bernal, J., 2019. Establishment and early impact of *Spathius galinae* (Hymenoptera: Braconidae) on emerald ash borer (Coleoptera: Buprestidae) in the Northeastern United States. J. Econ. Entomol. 112, 2121–2130. https://doi.org/10.1093/jee/toz159

Duan, J.J., Watt, T., Taylor, P., Larson, K., Lelito, J.P., 2013. Effects of ambient temperature on egg and larval development of the invasive emerald ash borer (Coleoptera: Buprestidae): implications for laboratory rearing. J. Econ. Entomol. 106, 2101–2108. https://doi.org/10.1603/ec13131

Duarte, S.A.D., 2013. Characterizing prepupal diapause and adult emergence phenology of emerald ash borer. Ohio State University.

Emerald Ash Borer Information Network, 2020. Emerald ash borer information from Michigan State University, Purdue University, the Ohio State University, the Michigan and Ohio Departments of Agriculture; the Michigan, Indiana and Ohio Departments of Natural Resources; the USDA Forest Service; the USD [WWW Document]. URL http://www.emeraldashborer.info/ (accessed 1.1.20).

Fantinou, A.A., Alexandri, M.P., Tsitsipis, J.A., 1998. Adult emergence rhythm of the egg-parasitoid *Telenomus busseolae*. BioControl 43, 141–151. https://doi.org/10.1023/A:1009984318289

Federal Register, 2015. Field release of the parasitoid *Spathius galinae* for the biological control of the emerald ash borer (*Agrilus planipennis*)in the continental United States. Federal Register, 80: 7827 [docket number APHIS-2014-0094]. [WWW Document]. URL http://www.regulations.gov/#!docket Detail;D=APHIS-2014-0094

Federal Register, 2007. Availability of an environmental assessment for the proposed release of three parasitoids for the biological control of the emerald ash borer (*Agrilus planipennis*) in the continental U.S. Federal Register, 72: 28947–28948. [docket number APHIS-2007 [WWW Document]. URL http://www.regulations.gov/#!documentDetail; D=APHIS-2007-0060-0043

Gray, D.R., 2012. Using geographically robust models of insect phenology in forestry, Phenology and Climate Change. Intech, Rijeka. https://doi.org/10.5772/35710

Gray, D.R., 2009. Age-dependent postdiapause development in the gypsy moth (Lepidoptera: Lymantriidae) life stage model. Environ. Entomol. 38, 18–25. https://doi.org/10.1603/022.038.0104

Haack, R., Jendek, E., Liu, H., Marchant, K.R., Petrice, T.R., Poland, T.M., Ye, H., 2002. The emerald ash borer: a new exotic pest in North America. Newsl. Michigan Entomol. Soc. 47,

1–5.

Herlihy, M. V., Talamas, E.J., Weber, D.C., 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11, 1–9. https://doi.org/10.1371/journal.pone.0150275

Herms, D.A., McCullough, D.G., 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. 59, 13–30. https://doi.org/10.1146/annurev-ento-011613-162051

Herz, A., Hassan, S.A., Hegazi, E., Khafagi, W.E., Nasr, F.N., Youssef, A.I., Agamy, E., Blibech, I., Ksentini, I., Ksantini, M., Jardak, T., Bento, A., Pereira, J.A., Torres, L., Souliotis, C., Moschos, T., Milonas, P., 2007. Egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) in olive groves of the Mediterranean region. Biol. Control 40, 48–56. https://doi.org/10.1016/j.biocontrol.2006.08.002

Hoban, J., Duan, J.J., Hough-Goldstein, J., 2016. Effects of temperature and photoperiod on the reproductive biology and diapause of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid of emerald ash borer (Coleoptera: Buprestidae). Environ. Entomol. 45, 726–731. https://doi.org/10.1093/ee/nvw034

Ikeno, T., Tanaka, S.I., Numata, H., Goto, S.G., 2010. Photoperiodic diapause under the control of circadian clock genes in an insect. BMC Biol. 8. https://doi.org/10.1186/1741-7007-8-116

Jackson, D.J., 1963. Diapause in *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae), a parasitoid of the eggs of Dytiscidae (Coleoptera). Parasitology 53, 225–251. https://doi.org/10.1017/S0031182000072711

Jennings, D.E., Duan, J.J., Larson, K.M., Ito, J.P.L., Shrewsbury, P.M., 2014. Evaluating a new method for monitoring the field establishment and parasitism of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid of emerald ash borer (Coleoptera: Buprestidae). Florida Entomol. 97, 1263–1265. https://doi.org/10.1653/024.097.0339

Jennings, D.E., Duan, J.J., Shrewsbury, P.M., 2018. Comparing methods for monitoring establishment of the emerald ash borer (*Agrilus planipennis*, Coleoptera: Buprestidae) egg parasitoid *Oobius agrili* (Hymenoptera: Encyrtidae) in Maryland, USA. Forests 9, 1–9. https://doi.org/10.3390/F9100659

Jones, M.I., Gould, J.R., Warden, M.L., Fierke, M.K., 2019. Dispersal of emerald ash borer (Coleoptera: Buprestidae) parasitoids along an ash corridor in western New York. Biol. Control 128, 94–101. https://doi.org/10.1016/j.biocontrol.2018.09.004

Kashian, D.M., Bauer, L.S., Spei, B.A., Duan, J.J., Gould, J.R., 2018. Potential impacts of emerald ash borer biocontrol on ash health and recovery in southern Michigan. Forests 9, 1–16. https://doi.org/10.3390/f9060296

Keever, C.C., Nieman, C., Ramsay, L., Ritland, C.E., Bauer, L.S., Lyons, D.B., Cory, J.S., 2013.

Microsatellite population genetics of the emerald ash borer (*Agrilus planipennis* Fairmaire): comparisons between Asian and North American populations. Biol. Invasions 15, 1537–1559. https://doi.org/10.1007/s10530-012-0389-4

Kim, D.S., Lee, J.H., 2003. Oviposition model of *Carposina sasakii* (Lepidoptera: Carposinidae). Ecol. Modell. 162, 145–153. https://doi.org/10.1016/S0304-3800(02)00402-7

Klooster, W.S., Herms, D.A., Knight, K.S., Herms, C.P., McCullough, D.G., Smith, A., Gandhi, K.J.K., Cardina, J., 2014. Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (*Agrilus planipennis*). Biol. Invasions 16, 859–873. https://doi.org/10.1007/s10530-013-0543-7

Knight, K.S., Brown, J.P., Long, R.P., 2013. Factors affecting the survival of ash (*Fraxinus* spp.) trees infested by emerald ash borer (*Agrilus planipennis*). Biol. Invasions 15, 371–383. https://doi.org/10.1007/s10530-012-0292-z

Koch, J.L., Carey, D.W., Knight, K.S., Poland, T., Herms, D.A., Mason, M.E., 2011. Breeding strategies for the development of emerald ash borer-resistant North American ash, in: Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. pp. 235–239.

Koch, J.L., Carey, D.W., Mason, M.E., Poland, T.M., Knight, K.S., 2015. Intraspecific variation in *Fraxinus pennsylvanica* responses to emerald ash borer (*Agrilus planipennis*). New For. 46, 995–1011. https://doi.org/10.1007/s11056-015-9494-4

Larson, K.M., Duan, J.J., 2016. Differences in the reproductive biology and diapause of two congeneric species of egg parasitoids (Hymenoptera: Encyrtidae) from northeast Asia: Implications for biological control of the invasive emerald ash borer (Coleoptera: Buprestidae). Biol. Control 103, 39–45. https://doi.org/10.1016/j.biocontrol.2016.08.001

Lees, A.D., 1960. The role of photoperiod and temperature in the determination of parthogenetic and sexual forms in the aphid *Megoura viciae* Buckton-II. The operation of the "interval timer" in young clones. J. Insect Physiol. 4, 154–175. https://doi.org/10.1016/0022-1910(60)90078-0

Lelito, J., Lavallée, R., Lomeli, J.R., Lyons, B., Marcotte, M., 2013. NAPPO Science and Technology Documents - ST 03: Application of biological control of the emerald ash borer (EAB) in North America 38 p.

Li, W., Li, J., Coudron, T.A., Lu, Z., Pan, W., Liu, X., Zhang, Q., 2008. Role of photoperiod and temperature in diapause induction of endoparasitoid wasp *Microplitis mediator* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 101, 613–618. https://doi.org/10.1603/0013-8746(2008)101[613:ropati]2.0.co;2

Liebhold, A.M., Berec, L., Brockerhoff, E.G., Epanchin-Niell, R.S., Hastings, A., Herms, D.A., Kean, J.M., McCullough, D.G., Suckling, D.M., Tobin, P.C., Yamanaka, T., 2016. Eradication of invading insect populations: from concepts to applications. Annu. Rev.

Entomol. 61, 335–352. https://doi.org/10.1146/annurev-ento-010715-023809

Liu, H., Bauer, L.S., Gao, R., Zhao, T., Petrice, T.R., Haack, R.A., 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. Gt. Lakes Entomol. 36, 191–204.

Liu, H., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., Song, L., Luan, Q., Jin, R., Gao, C., 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biol. Control 42, 61–71. https://doi.org/10.1016/j.biocontrol.2007.03.011

Logan, J.A., Wollkind, D.J., Hoyt, S.C., Tanigoshi, L.K., 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5, 1133–1140. https://doi.org/10.1093/ee/5.6.1133

MapBioControl, 2020. Agent release tracking and data management for federal, state, and researchers releasing four biocontrol agents released against emerald ash borer. [WWW Document]. URL http://www.mapbiocontrol.org/ (accessed 10.1.19).

Margulies, E., Bauer, L., Ibáñez, I., 2017. Buying time: Preliminary assessment of biocontrol in the recovery of native forest vegetation in the aftermath of the invasive emerald ash borer. Forests 8, 1–14. https://doi.org/10.3390/f8100369

Marsh, P.M., Strazanac, J.S., Laurusonis, S.Y., 2009. Description of a new species of *Atanycolus* (Hymenoptera: Braconidae) from Michigan reared from the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae: Agrilinae). Gt. Lakes Entomol. 42, 8–15.

McCullough, D.G., Mercader, R.J., 2012. Evaluation of potential strategies to slow ash mortality (slam) caused by emerald ash borer (*Agrilus planipennis*): Slam in an urban forest. Int. J. Pest Manag. 58, 9–23. https://doi.org/10.1080/09670874.2011.637138

McCullough, D.G., Mercader, R.J., Siegert, N.W., 2015. Developing and integrating tactics to slow ash (Oleaceae) mortality caused by emerald ash borer (Coleoptera: Buprestidae). Can. Entomol. 147, 349–358. https://doi.org/10.4039/tce.2015.3

McCullough, D.G., Poland, T.M., Anulewicz, A.C., Lewis, P., Cappaert, D., 2011. Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) control provided by emamectin benzoate and two neonicotinoid insecticides, one and two seasons after treatment. J. Econ. Entomol. 104, 1599–1612. https://doi.org/10.1603/ec11101

McCullough, D.G., Poland, T.M., Lewis, P.A., 2016. Lethal trap trees: A potential option for emerald ash borer (*Agrilus planipennis* Fairmaire) management. Pest Manag. Sci. 72, 1023–1030. https://doi.org/10.1002/ps.4083

McCullough, D.G., Poland, T.M., Tluczek, A.R., Anulewicz, A., Wieferich, J., Siegert, N.W., 2019. Emerald Ash Borer (Coleoptera: Buprestidae) densities over a 6-yr period on untreated trees and trees treated with systemic insecticides at 1-, 2-, and 3-yr intervals in a

Central Michigan forest. J. Econ. Entomol. 112, 201–212. https://doi.org/10.1093/jee/toy282

Mercader, R.J., Siegert, N.W., Liebhold, A.M., McCullough, D.G., 2011. Simulating the effectiveness of three potential management options to slow the spread of emerald ash borer (*Agrilus planipennis*) populations in localized outlier sites. Can. J. For. Res. 41, 254–264. https://doi.org/10.1139/X10-201

Meuti, M.E., Denlinger, D.L., 2013. Evolutionary links between circadian clocks and photoperiodic diapause in insects. Integr. Comp. Biol. 53, 131–143. https://doi.org/10.1093/icb/ict023

Michereff, M.F.F., Borges, M., Aquino, M.F.S., Laumann, R.A., Mendes Gomes, A.C.M., Blassioli-Moraes, M.C., 2016. The influence of volatile semiochemicals from stink bug eggs and oviposition-damaged plants on the foraging behaviour of the egg parasitoid *Telenomus podisi*. Bull. Entomol. Res. 106, 663–671. https://doi.org/10.1017/S0007485316000419

Michigan State University Enviroweather, 2019. Weather-based pest, natural resources, and production tools. Michigan State University, East Lansing, Michigan [WWW Document]. URL https:://www.enviroweather.msu.edu/ (accessed 1.1.20).

Minnesota Department of Agriculture, 2019. Biological control of emerald ash borer: bark sifting [WWW Document]. URL https://www.mda.state.mn.us/sites/default/files/inline-files/barksifting2016.pdf/ (accessed 12.14.19).

Moya-Raygoza, G., Albarracin, E.L., Virla, E.G., 2012. Diversity of egg parasitoids attacking Dalbulus maidis (Hemiptera: Cicadellidae) populations at low and high elevation sites in Mexico and Argentina. Florida Entomol. 95, 105–112. https://doi.org/10.1653/024.095.0117

Noyes, J.S., 1990. A new genus and species of encyrtid (Hymenoptera, Chalcidoidea) parasitoid of the eggs of the varicose borer, *Agrilus sexsignatus* (fisher) (Coleoptera, Buprestidae), a pest of bagras (*Eucalyptus deglupta* Blume) in the Philippines. J. Nat. Hist. 24, 21–25. https://doi.org/10.1080/00222939000770031

Parisio, M.S., Gould, J.R., Vandenberg, J.D., Bauer, L.S., Fierke, M.K., 2017. Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biol. Control 106, 45–53. https://doi.org/10.1016/j.biocontrol.2016.12.009

Peri, E., Sole, M.A., Wajnberg, E., Colazza, S., 2006. Effect of host kairomones and oviposition experience on the arrestment behavior of an egg parasitoid. J. Exp. Biol. 209, 3629–3635. https://doi.org/10.1242/jeb.02416

Petrice, T.R., Miller, D.L., Bauer, L.S., Poland, T.M., Ravlin, F.W., 2019. Photoperiodic modulation of diapause induction and termination in *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), an egg parasitoid of the invasive emerald ash borer. Biol. Control 138, 1–13. https://doi.org/10.1016/j.biocontrol.2019.104047

Pizzol, J., Pintureau, B., 2008. Effect of photoperiod experienced by parents on diapause induction in *Trichogramma cacoeciae*. Entomol. Exp. Appl. 127, 72–77. https://doi.org/10.1111/j.1570-7458.2008.00671.x

Poland, T.M., McCullough, D.G., 2010. SLAM: A multi-agency pilot project to SL.ow A.sh M.ortality caused by emerald ash borer in outlier sites. Newsl. Michigan Entomol. Soc. 55, 4–8.

Poland, T.M., Petrice, T.R., Ciaramitaro, T.M., 2019. Trap designs, colors, and lures for emerald ash borer detection. Front. For. Glob. Chang. 2, 1–11. https://doi.org/10.3389/ffgc.2019.00080

Potter, B.E., Andresen, J.A., 2002. A finite-difference model of temperatures and heat flow within a tree stem. Can. J. For. Res. 32, 548–555. https://doi.org/10.1139/x01-226

R Core Team, 2018. R: A language and environment for statistical computing [WWW Document]. URL http://www.r-project.org/

Régnière, J., 1984. A method of describing and using variability in development rates for the simulation of insect phenology. Can. Entomol. 116, 1367–1376.

Régnière, J., Powell, J., Bentz, B., Nealis, V., 2012. Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. J. Insect Physiol. 58, 634–647. https://doi.org/10.1016/j.jinsphys.2012.01.010

Reznik, S.Y., Vaghina, N.P., Voinovich, N.D., 2012. Multigenerational maternal effect on diapause induction in *Trichogramma* species (Hymenoptera: Trichogrammatidae). Biocontrol Sci. Technol. 22, 429–445. https://doi.org/10.1080/09583157.2012.662942

Reznik, S.Y., Vaghina, N.P., Voinovich, N.D., 2011. On the direct influence of photoperiod on diapause in *Trichogramma embryophagum* (Hymenoptera, Trichogrammatidae). Entomol. Rev. 91, 301–305. https://doi.org/10.1134/S0013873811030031

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, 1–13. https://doi.org/10.1371/journal.pone.0146021

Rutledge, C.E., Keena, M.A., 2012. Mating frequency and fecundity in the emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). Ann. Entomol. Soc. Am. 105, 66–72. https://doi.org/10.1603/an11037

SAS Institute, 2012. OCUser's Manual, Version 9.4. SAS Institute, Cary, NC.

Saunders, D.S., 2014. Insect photoperiodism: Effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. Entomol. Sci. 17, 25–40. https://doi.org/10.1111/ens.12059

Siegert, N.W., Mccullough, D.G., Liebhold, A.M., Telewski, F.W., 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America.

Divers. Distrib. 20, 847–858. https://doi.org/10.1111/ddi.12212

Simonoff, J., Efron, B., Tibshirani, R., Hjorth, J.S.U., 1994. An introduction to the bootstrap. J. Am. Stat. Assoc. 89, 1559. https://doi.org/10.2307/2291020

Smitley, D.R., Doccola, J.J., Cox, D.L., 2010. Multiple-year protection of ash trees from emerald ash borer with a single trunk injection of emamectin benzoate, and single-year protection with an imidacloprid basal drench. Arboric. Urban For. 36, 206–211.

Sobek-Swant, S., Crosthwaite, J.C., Lyons, D.B., Sinclair, B.J., 2012a. Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. Biol. Invasions 14, 115–125. https://doi.org/10.1007/s10530-011-9988-8

Sobek-Swant, S., Kluza, D.A., Cuddington, K., Lyons, D.B., 2012b. Potential distribution of emerald ash borer: what can we learn from ecological niche models using Maxent and GARP? For. Ecol. Manage. 281, 23–31. https://doi.org/10.1016/j.foreco.2012.06.017

Stanovick, J.S., Zweifel, R.D., Wagner, B., 2002. Precision of statistics derived from Missouri statewide angler surveys. North Am. J. Fish. Manag. 22, 1266–1269. https://doi.org/10.1577/1548-8675(2002)022<1266:posdfm>2.0.co;2

Tauber, C.A., Tauber, M.J., Masaki, C.A., 1986. Seasonal Adaptations of Insects. Oxford University Press, New York.

Tobin, P.C., Kean, J.M., Suckling, D.M., McCullough, D.G., Herms, D.A., Stringer, L.D., 2014. Determinants of successful arthropod eradication programs. Biol. Invasions 16, 401–414. https://doi.org/10.1007/s10530-013-0529-5

Triapitsyn, S.V., Petrice, T.R., Gates, M.W., Bauer, L.S., 2015. Two new species of *Oobius* Trjapitzin (Hymenoptera, encyrtidae) egg parasitoids of *Agrilus* spp. (Coleoptera, buprestidae) from the USA, including a key and taxonomic notes on other congeneric Nearctic taxa. Zookeys 498, 29–50. https://doi.org/10.3897/zookeys.498.9357

USDA–APHIS/ARS/FS, 2019. Emerald ash borer biological control release and recovery guidelines, USDA–APHIS–ARS-FS, Riverdale, Maryland.

Vermunt, B., Cuddington, K., Sobek-Swant, S., Crosthwaite, J., 2012a. Cold temperature and emerald ash borer: modelling the minimum under-bark temperature of ash trees in Canada. Ecol. Modell. 235–236, 19–25. https://doi.org/10.1016/j.ecolmodel.2012.03.033

Vermunt, B., Cuddington, K., Sobek-Swant, S., Crosthwaite, J.C., Barry Lyons, D., Sinclair, B.J., 2012b. Temperatures experienced by wood-boring beetles in the under-bark microclimate. For. Ecol. Manage. 269, 149–157. https://doi.org/10.1016/j.foreco.2011.12.019

Vinson, S.B., 1998. The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol. Control 11, 79–96. https://doi.org/10.1006/bcon.1997.0601

Wagner, T.L., Olson, R., Willers, J.L., 1991. Modeling arthropod development time. J. Agric. Res. 8, 251–270.

Wagner, T.L., Wu, H.-I., Sharpe, P.J.H., Coulson, R.N., 1984. Modeling distributions of insect development time: a literature review and application of the Weibull function. Ann. Entomol. Soc. Am. 77, 475–483. https://doi.org/10.1093/aesa/77.5.475

Wang, T., Laing, J.E., 1989. Diapause termination and morphogenesis of *Holcothorax testaceipes* Ratzeburg (Hymenoptera: Encyrtidae), and introdued parasitoid of the spotted tentiform leafminer, *Phyllonorycter blancardella* (F.) (Lepidoptera: Gracillariidae). Can. Entomol. 121, 65–74.

Wang, X.-Y., Yang, Z.-Q., Gould, J.R., Zhang, Y.-N., Liu, G.-J., Liu, E., 2010. The biology and ecology of the emerald ash borer, Agrilus planipennis, in China. J. Insect Sci. 10, 1–23. https://doi.org/10.1673/031.010.12801

Wang, X.Y., Cao, L.M., Yang, Z.Q., Duan, J.J., Gould, J.R., Bauer, L.S., 2015. Natural enemies of emerald ash borer (Coleoptera: Buprestidae) in northeast China, with notes on two species of parasitic Coleoptera. Can. Entomol. 148, 329–342. https://doi.org/10.4039/tce.2015.57

Wei, X., Wu, Y., Reardon, R., Sun, T.H., Lu, M., Sun, J.H., 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. Insect Sci. 14, 367–373. https://doi.org/10.1111/j.1744-7917.2007.00163.x

Wertman, D.L., Bleiker, K.P., 2019. Shedding new light upon circadian emergence rhythmicity in the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). Can. Entomol. 151, 273–277. https://doi.org/10.4039/tce.2019.18

Wetherington, M.T., Jennings, D.E., Shrewsbury, P.M., Duan, J.J., 2017. Climate variation alters the synchrony of host–parasitoid interactions. Ecol. Evol. 7, 8578–8587. https://doi.org/10.1002/ece3.3384

Yang, Z.-Q., Strazanac, J.S., Marsh, P.M., Van Achterberg, C., Choi, W.-Y., 2006. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Ann. Entomol. Soc. Am. 98, 636–642. https://doi.org/10.1603/0013-8746(2005)098[0636:frpfco]2.0.co;2

Yao, Y.X., Duan, J.J., Hopper, K.P., Mottern, J.L., Gates, M.W., 2016. A new species of *Oobius* Trjapitzin (Hymenoptera: Encyrtidae) from the Russian Far East that parasitizes eggs of emerald ash borer (Coleoptera: Buprestidae). Ann. Entomol. Soc. Am. 109, 629–638. https://doi.org/10.1093/aesa/saw022

Zhang, Y.Z., Huang, D.W., Zhao, T.H., Liu, H.P., Bauer, L.S., 2005. Two new species of egg parasitoids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica 33, 253–260. https://doi.org/10.1007/BF02979863