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# Determining optimal parasitoid release timing for the biological control of emerald ash borer (Coleoptera: Buprestidae)

Kristopher J. Abell<sup>1,\*</sup>, Jian J. Duan<sup>2</sup>, and Paula M. Shrewsbury<sup>1</sup>

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## Abstract

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), quickly established itself as an invasive species in North America after it was first detected near Detroit, Michigan, USA, in 2002. Just 1 yr later, emerald ash borer was introduced accidentally into Maryland, USA, on imported ash nursery stock. After quarantine and eradication efforts failed, a classical biological control program was initiated in Maryland in 2009 with the release of 2 larval parasitoids: *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae), and 1 egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae). Timing is critical to the success, efficiency, and cost-effectiveness of classical biological control programs where release of parasitoids must coincide temporally with that of susceptible stages of the host. In 2017, periodic field surveys at 2 sites in central Maryland were conducted to assess emerald ash borer phenology using green funnel traps to sample adults, and debarking infested ash trees to sample larval stages. Adult emerald ash borer first appeared in traps on 18 May 2017 (479 growing degree d base 10 [GDD10]), peaked on 15 Jun (823 GDD10), and were absent from 13 Jul (1,301 GDD10) through 12 Oct (2,598 GDD10) when surveys were terminated. Larval sampling in early Aug (1,698 GDD10) found all larval instars present, the most common being the second instar (46%), followed by the third instar (28%), fourth instar (20%), first instar (5%), and prepupal J-shaped larvae (J-larva) (0.5%). J-larvae are so called because after excavating a pupal chamber, the head is bent down 180 degrees to the rest of the body, resembling the shape of the letter J. Larval sampling in late Oct (2,710 GDD10) found J-larvae to be the dominant stage present (92.2%), followed by fourth instar (4.8%), third instar (2.4%), and second instar (0.6%). We found that emerald ash borer was univoltine, and that nearly 50% of emerald ash borer larvae had developed to stages susceptible to parasitism (third and fourth instar) by early Aug (1,698 GDD10). By late Oct (2,710 GDD10), 92% had developed beyond parasitoid susceptible stages (J-larvae). These findings suggest that egg parasitoid releases are best targeted from early May to late Jun at an approximate GDD10 range of 300 and 1,100 with larval parasitoid releases best targeted between 1,400 and 2,500 GDD10.

Key Words: invasive species; phenology; growing degree day; forest pest

## Resumen

El barrenador esmeralda del fresno, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), se estableció rápidamente como una especie invasora en América del Norte desde que fue detectado por primera vez cerca de Detroit, Michigan, EE. UU., en el 2002. Apenas 1 año después, el barrenador esmeralda del fresno fue accidentalmente introducido en Maryland, EE. UU., sobre plantas de fresno para viveros importadas. Después de que fracasaron los esfuerzos de cuarentena y erradicación, se inició un programa de control biológico clásico en Maryland en el 2009 con la liberación de 2 parasitoides larvales *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) y *Spathius agrili* Yang (Hymenoptera: Braconidae), y un parasitoide de huevos, *Oobius agrili* Zhang y Huang (Hymenoptera: Encyrtidae). El tiempo es crítico para el éxito, la eficiencia y la rentabilidad de los programas de control biológico clásico donde la liberación de parasitoides debe coincidir temporalmente con la de los estadios susceptibles del hospedero. En el 2017, se realizaron estudios de campo periódicos en 2 sitios en el centro de Maryland para evaluar la fenología del barrenador esmeralda del fresno utilizando trampas de embudo verde para muestrear adultos, y quitando la corteza de los fresnos infestados para muestrear los estadios larvales. Los adultos del barrenador esmeralda de fresno aparecieron por primera vez en las trampas el 18 de mayo (479 grados-día de crecimiento de base 10), alcanzaron su punto máximo el 15 de junio (823 grados-día de crecimiento de base 10) y estuvieron ausentes desde el 13 de julio (1,301 grados-día de crecimiento base 10) hasta el 12 de octubre (2,598 grados-día de crecimiento de base 10) cuando se terminó el sondeo. El muestreo de larvas a principios de agosto (1,698 grados-día de crecimiento de base 10) encontró todos los estadios larvales presentes, el más común fue el segundo estadio (46%), seguido del tercer estadio (28%), cuarto estadio (20%), primer estadio (5%), y larvas-J (0.5%). El muestreo de larvas a fines de octubre (2,710 grados-día de crecimiento de base 10) encontró que las larvas-J eran el estadio mas dominante presente (92.2%), seguidas por el cuarto estadio (4.8%), el tercer estadio (2.4%) y el segundo estadio (0.6%). Descubrimos que el barrenador esmeralda del fresno es univoltina, y que casi el 50% de las larvas del barrenador esmeralda se habían desarrollado en estadios susceptibles al parasitismo (tercer y cuarto estadio) a principios de agosto (1,698 grados-día de crecimiento de base 10). A finales de octubre (2,710 grados-día de crecimiento de base 10), el 92% se había desarrollado más allá de los estadios susceptibles a los parasitoides (larvas-J). Estos hallazgos sugieren que las liberaciones de parasitoides de huevos se enfocan mejor desde el principio de mayo hasta el final de junio en un rango de grados-día de base 10 de aproximadamente 300 a 1,100, mientras que las liberaciones de parasitoides larvales se enfocan mejor entre 1,400 y 2,500 grados-días de crecimiento de base 10.

Palabras Clave: especies invasoras; control biológico; fenología días de grado creciente; plaga forestal

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Emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is an invasive species in North America, where it has killed millions of ash trees (*Fraxinus* spp.; Oleaceae) since its accidental introduction (Haack et al. 2002; Cappaert et al. 2005; Poland & McCullough 2006; Bray et al. 2011; Herms & McCullough 2014; Emerald Ash Borer Information 2018). Endemic to Asia, emerald ash borer was detected first near Detroit, Michigan, USA, in 2002 and has since spread to 34 US states. Emerald ash borer reached Maryland, USA, in 2003, much sooner than possible from natural dispersal, due to the accidental importation of infested nursery stock (Sargent et al. 2010, 2013). In an effort to control emerald ash borer, a classical biological control program was initiated in 2007 in Michigan (Bauer et al. 2008, 2015) and 2009 in Maryland (Gould et al. 2015). Initial surveys in Asia for emerald ash borer natural enemies found 2 larval parasitoids, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Liu et al. 2003, 2007; Yang et al. 2006), and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al. 2005), and 1 egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al. 2005). All 3 parasitoids were approved for release, and introductions began in 2007 (Federal Register 2007). A third larval parasitoid, *Spathius galinae* Belokobyl'skij & Strazanac (Hymenoptera: Braconidae), was later discovered from the Russian Far East and approved for release in 2015 (Belokobyl'skij et al. 2012; Duan et al. 2012, Federal Register 2015). All 3 larval parasitoids attack late third and fourth instar emerald ash borer larvae. Knowing when the susceptible emerald ash borer stages are present in the environment can greatly impact the establishment and effectiveness of released natural enemies. If susceptible host stages are not present, then the investment associated with releasing a given natural enemy is lost. To maximize the temporal and monetary investment of classical biological control programs, robust data delineating the phenology of susceptible host stages is needed. Here we report the results of a 1-yr study in Maryland to determine the emergence and flight phenology of adult emerald ash borer, as well as the developmental phenology of the larval stages of emerald ash borer under field conditions.

## Materials and Methods

### PHENOLOGY OF ADULT *AGRILUS PLANIPENNIS* USING GREEN FUNNEL TRAPS

Four green multi-funnel traps (AgBio, Westminster, Colorado, USA) were deployed at 2 sites with emerald ash borer infested ash trees: United States National Arboretum, Washington, DC, USA (38.90627°N, 76.97324°W), and Montgomery County, Maryland, USA (39.15586°N, 77.15482°W). Each trap was baited with a green leaf alcohol pouch (Synergy Semiochemicals Inc., Burnaby, British Columbia, Canada) that was replaced every 4 wk. Each trap was suspended from the branch of a living ash tree that exhibited signs of emerald ash borer infestation (woodpecker holes, emerald ash borer emergence holes, epicormic shoots, or poor canopy condition) according to methods described by Francese et al. (2013). Traps were deployed beginning on 11 May 2017, and checked every wk until 12 Oct 2017. Emerald ash borers captured in each trap were identified, counted, and totaled for each site. Growing degree d base 10 °C for each collection date were obtained using the Ronald Reagan National Airport weather station in Washington, DC, USA.

### PHENOLOGY OF LARVAL *AGRILUS PLANIPENNIS*

Phenology of emerald ash borer larvae was determined by debarking ash trees with visible signs of emerald ash borer infestation

(woodpecker holes, emerald ash borer emergence holes, epicormic shoots, or poor canopy condition) at the same 2 sites that green funnel traps were deployed. Trees were debarked until 2 criteria were met for each site: (1) at least 2 trees were completely debarked, and (2) at least 25 living emerald ash borer larvae were found. Debarked trees ranged in size from 6.4 to 10.6 cm diam at breast height. Trees were debarked twice in 2017, once at the approximate midpoint of larval development (2–9 Aug), and once near the end (26–31 Oct). All living emerald ash borer larvae recovered were classified visually in the field as first, second, third, fourth instar, J-shaped larvae, pre-pupa, or pupa. Degree d base 10 °C were obtained using the Ronald Reagan National Airport weather station in Washington, DC, USA.

## Results

### PHENOLOGY OF ADULT *AGRILUS PLANIPENNIS* USING GREEN FUNNEL TRAPS

A total of 7 adult emerald ash borers were captured in our traps in the first sample period (11–18 May, 479 GDD10) and continued to be present in traps until 6 Jul (1,174 GDD10) (Fig. 1). Trap captures of emerald ash borer peaked at a total of 37 during the 9 to 15 Jun sample (823 GDD10). The 4-wk period from 26 May through 22 Jun (556–941 GDD10) represented 84% of the total emerald ash borer captures for the entire season. No emerald ash borers were captured in traps from 6 Jul to 12 Oct (end of sampling period) (1,174–2,598 GDD10).

### PHENOLOGY OF LARVAL *AGRILUS PLANIPENNIS*

The first larval sampling took place from 2 to 9 Aug (about 1,750 GDD10). A total of 186 emerald ash borer larvae were collected, and the composition of larval stages were as follows: first instar = 5.4% ( $n = 10$ ), second instar = 45.7% ( $n = 85$ ), third instar = 28.5% ( $n = 37$ ), fourth instar = 19.9% ( $n = 37$ ), and J-larvae = 0.5% ( $n = 1$ ) (Fig. 2). The second larval sampling took place from 26 to 31 Oct (about 2,700 GDD10). A total of 167 emerald ash borer larvae were collected, and the composition of larval stages were as follows: first instar = 0.0% ( $n = 0$ ), second instar = 0.6% ( $n = 1$ ), third instar = 2.4% ( $n = 4$ ), fourth instar = 4.8% ( $n = 8$ ), J-larvae = 92.2% ( $n = 154$ ) (Fig. 2).

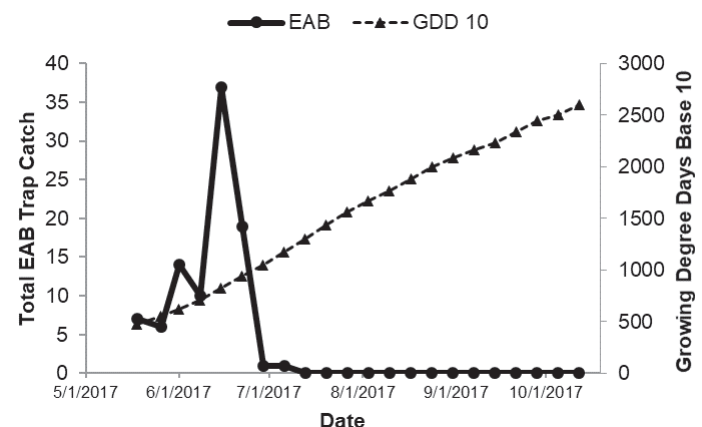
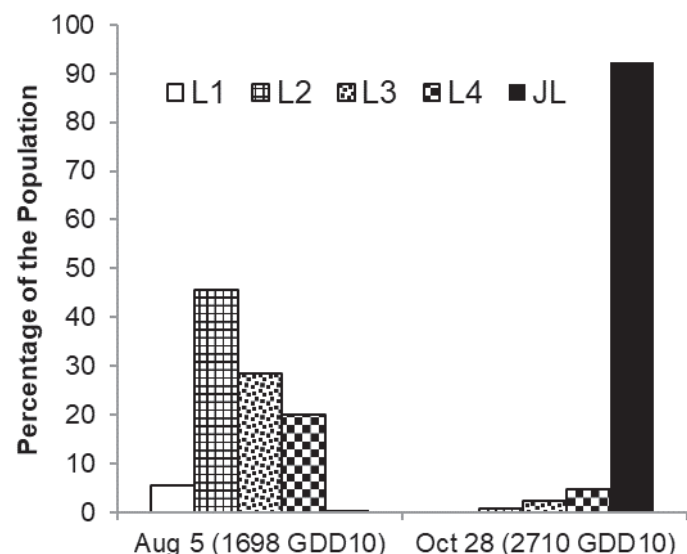


Fig. 1. Number of emerald ash borer adults captured in green funnel traps (pooled across sites for each sample date) in central Maryland and Washington, DC, in 2017. The secondary Y-axis is growing degree d base 10 °C (GDD10) obtained from Ronald Reagan National Airport weather station in Washington, DC, USA.



**Fig. 2.** Percentage of each immature stage of emerald ash borer at the approximate mid- and endpoint of the season collected in central Maryland and Washington, DC, in 2017. Growing degree d base 10 °C (GDD10) shown in parentheses for each date were obtained from Ronald Reagan National Airport weather station in Washington, DC, USA. L1 = first instar, L2 = second instar, L3 = third instar, L4 = fourth instar, and JL = J-larva.

## Discussion

Using green funnel traps, we found adult emerald ash borer flight phenology in central Maryland and Washington, DC, occurred primarily over a 2-mo period. The majority of captures (84%) occurred during just a 4-wk period from late May to late Jun (1,000–1,700 GDD10), where the optimal time period for egg parasitoid releases should occur. Emerald ash borer eggs are viable only for parasitism for about 7 to 10 d (Duan et al. 2014) under normal rearing temperature (25–30 °C); therefore, knowing when oviposition occurs and for how long is important for planning production and releases of the egg parasitoid *O. agrili*, as well as *O. primorskyensis* Yao & Duan (Larson & Duan 2016; Yao et al. 2016), which are being evaluated currently for introduction in the US.

Even though parasitoid susceptible larval stages (third and fourth instar) are present longer than eggs, it is still important to know when they are present for optimal parasitoid release timing. Our results showed that nearly 50% of emerald ash borer larvae had developed to third or fourth instar by early Aug (about 1,750 GDD10 since oviposition), and that by late Oct (2,700 GDD10 since oviposition) 92% had developed beyond the susceptible stage to J-larvae. These results suggest that larval parasitoid releases are best targeted at approximately 1,500 to 2,400 GDD10 after oviposition of the current yr (about 1 May).

Although our findings showed a clear univoltine pattern for emerald ash borer in central Maryland, variation in climatic conditions throughout North America may alter emerald ash borer population dynamics. Subsequently, the degree d recommendations in this work may not be appropriate in other regions that have overlapping or partial generations. Furthermore, natural environmental stochasticity, or the effects of climate change may affect the population dynamics of emerald ash borer and its parasitoids (Wetherington et al. 2017), further complicating efforts to accurately determine optimal parasitoid release timing. However, the findings we reported here provide a good baseline that together with regular monitoring for shifts in adult flight phenology should allow for reasonably accurate determination of egg and larval parasitoid release timing.

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