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# Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae

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## Abstract

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an important invasive pest of ash (*Fraxinus*) trees in North America. Two larval parasitoid species, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae), and 1 egg parasitoid species, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), were introduced into the United States in 2007 as part of a classical biological control program. We conducted field studies to assess the flight phenology of introduced and native parasitoids of emerald ash borer in central Michigan from 2011 to 2013 by using sentinel logs. Parasitism rates of sentinel *A. planipennis* eggs by *O. agrili* fluctuated throughout the season from 0 to 22% in 2011 and 0 to 6% in 2012. Flight phenology of *O. agrili* adults varied between years, and discrete generations were not apparent. Rather, *O. agrili* adults were generally continually present over a 3 mo period each year. Parasitism rates of sentinel *A. planipennis* larvae by *T. planipennisi* and the North American native *Atanycolus* spp. (Hymenoptera: Braconidae), respectively, ranged from 0 to 5% and 33 to 77% in 2011, from 0 to 69% and 0 to 27% in 2012, and from 0 to 53% and 0 to 46% in 2013. Phenology of adult flight of both *T. planipennisi* and *Atanycolus* spp. was inconsistent between years. Development of nondestructive methods to determine when stages of *A. planipennis* suitable for parasitism are present in combination with the use of sentinel logs to observe parasitoid phenology as described here will enhance the ability to evaluate the impacts of parasitoids on emerald ash borer.

Key Words: invasive species; biological control; *Tetrastichus planipennisi*; *Oobius agrili*; *Atanycolus*

## Resumen

El barrenador esmeralda del fresno, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), es una plaga invasora importante de fresnos (*Fraxinus*) en América del Norte. Dos especies de parasitoides de larvas, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) y *Spathius agrili* Yang (Hymenoptera: Braconidae), y 1 especie parasitoide de huevos, *Oobius agrili* Zhang y Huang (Hymenoptera: Encyrtidae), se introdujeron en los Estados Unidos en 2007 como parte de un programa de control biológico clásico. Se realizó un estudio de campo para evaluar la fenología del vuelo de parasitoides introducidos y nativos del barrenador esmeralda del fresno en el centro del estado de Michigan desde el 2011 hasta el 2013 utilizando troncos centinelas. La tasa de parasitismo de huevos centinelas de *A. planipennis* de *O. agrili* fluctuaron a lo largo del la estación del 0 a 22% en 2011 y del 0 al 6% en 2012. La fenología del vuelo de adultos de *O. agrili* variaron entre años, y las generaciones discretas no fueron evidentes. Por el contrario, adultos de *O. agrili* estaban en general continuamente presentes durante un período de 3 meses cada año. La tasa de parasitismo de larvas centinelas de *A. planipennis* por *T. planipennisi* y el norteamericano nativo *Atanycolus* spp. (Hymenoptera: Braconidae), respectivamente, varió del 0 a 5% y un 33 a un 77% en 2011, de 0 a 69% y del 0 al 27% en 2012, y del 0 a 53% y del 0 a 46% en 2013. La fenología de vuelo de adultos de ambos *T. planipennisi* y *Atanycolus* spp. fue inconsistente entre años. El desarrollo de métodos no destructivos para determinar cuándo los estadios de *A. planipennis* adecuados para el parasitismo están presentes en combinación con el uso de troncos centinelas para observar la fenología del parasitoide como se describe aquí mejorará la capacidad de evaluación de los impactos de los parasitoides en el barrenador esmeralda del fresno.

Palabras Clave: especies invasoras; control biológico; *Tetrastichus planipennisi*; *Oobius agrili*; *Atanycolus*

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The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive beetle from China that has killed hundreds of millions of ash trees (*Fraxinus* species; Oleaceae) in North America since it was detected in 2002 (Haack et al. 2002; Cappaert et al. 2005; Poland & McCullough 2006; Bray et al. 2011; Herms & McCullough 2014; Emerald Ash Borer Information Network 2016). Three emerald ash borer parasitoids from China were first released in Michigan,

USA, in 2007 as part of a classical biological control program (Bauer et al. 2007, 2008, 2014, 2015a; USDA APHIS 2007): 2 gregarious larval parasitoid species – *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Liu et al. 2003; Yang et al. 2005) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Liu et al. 2003; Yang et al. 2006) – and 1 egg parasitoid species, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al. 2005). Since their introduction, efforts have

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been made to determine establishment and prevalence of these parasitoids (Duan et al. 2011, 2012a, 2012b, 2013a, 2014a, 2015; Abell et al. 2014). Although the phenology of the native parasitoid *Atanycolus cappaerti* Marsh & Strazanac (Hymenoptera: Braconidae) attacking emerald ash borer has been described (Cappaert & McCullough 2009), no work to date has described the flight phenology of these parasitoids. Flight phenology of parasitoids and its synchrony with the susceptible stage of the host have important implications for parasitoid effectiveness (McClure 1986; Godfray et al. 1994; Van Nouhuys & Lei 2004; Abell & Van Driesche 2012). In this study, we evaluated the flight phenology of egg and larval parasitoids of emerald ash borer at sites in central Michigan using sentinel logs.

## Materials and Methods

### PHENOLOGY OF *O. AGRILI* DETERMINED USING SENTINEL *A. PLANIPENNIS* EGGS ON ASH LOGS

Egg sentinel logs (ESLs) were prepared in the laboratory following procedures similar to those described in Duan et al. (2012b) and Abell et al. (2015). Logs were cut 18 cm long from the bole of 5 to 10 cm diameter at breast height (DBH) green ash (*Fraxinus pennsylvanica* Marshall; Oleaceae) trees harvested from non-study sites not yet infested with emerald ash borer. Once cut to size, each ash log was washed in warm tap water, scrubbed clean of moss and lichen, air dried at room temperature, and then sealed on each end with paraffin wax to prevent desiccation. Each log was then wrapped along its entire length 7 to 8 times with 0.5 cm wide curling ribbon (Berwick Industries, Berwick, Pennsylvania) held in place at each end with a thumb tack. The ribbon was wrapped tightly enough to prevent it from sliding down, but loosely enough to allow *A. planipennis* females to oviposit on the bark under the ribbon. Using the ribbon in this manner effectively simulated tight crevices that *A. planipennis* females prefer for oviposition and provided protection from predators. The ribbon-wrapped log was next placed inside a 3.8 L clear plastic container with fresh green ash foliage, 5 to 7 gravid adult *A. planipennis* females, and 2 to 3 adult *A. planipennis* males and then held in growth chambers (16:8 h L:D photoperiod, 25 °C day and 20 °C night temperature). Logs were removed after 24 h, and the ribbon was carefully removed to count the number of eggs present. The ribbon was then replaced and the log returned. This process was repeated until >50 eggs were present (2 to 3 d). Emerald ash borer adults used in this process were reared according to the methods described by Duan et al. (2013b).

ESLs were deployed at Central Park in Meridian Township, Ingham County, Michigan (42.731°N, 84.423°W), where *O. agrili* had been introduced from 2007 to 2009 (Abell et al. 2014). For these results to be applicable to other climate zones of North America, we converted sample dates to growing degree day base 10 °C (GDD10) with weather station data collected nearby at Michigan State University's Horticulture Teaching & Research Center, East Lansing, Michigan (42.673°N, 84.487°W) using the Baskerville–Emin method (EnviroWeather 2016). One ESL was placed on each of 6 living ash trees (DBH 9–28 cm), and each ESL was replaced on the same tree with a new one every 14 d, from 20 May to 7 Oct in 2011 (119–1,481 GDD10). This was repeated in 2012 on each of 12 trees from 18 May to 19 Oct (trees 1–6) and 25 May to 26 Oct (trees 7–12) (236–1,699 GDD10). The 6 extra trees in 2012 were added because only ESLs from 2 trees had substantial parasitism in 2011. ESLs on trees 7 to 12 were staggered 1 wk later than trees 1 to 6 to allow for a more fine-grained description of phenology and because producing 6 ESL per wk was more feasible than 12. ESLs were deployed in the field by hammering

an aluminum nail into a living ash tree through an eye hook screwed into the top of the ESL. After each 14 d period, ESLs were collected and returned to the laboratory.

In the laboratory, each egg along with a small portion of bark was cut off using a razor blade and placed in a 50 × 9 mm Petri dish with friction-fit lids (BD Falcon #08757105, ThermoFisher Scientific, Hanover Park, Illinois), and held in a growth chamber (16:8 h L:D photoperiod, 24 °C day and 20 °C night temperature) for 7 to 10 d to allow further development for easier determination of parasitism. Once all eggs were removed, the sentinel log was disposed of. Following the incubation period, each egg was examined using a dissecting microscope and categorized as (1) hatched: oval hole on the ventral surface with small light-colored frass; (2) unhatched: no exit hole and no sign of parasitism; or (3) parasitized: parasitoid egg, larva, or pupa present, or round exit hole on the dorsal surface with dark meconium and the presence of an adult of *O. agrili* in the Petri dish. Percentage of parasitism was calculated for each ESL as the number of parasitized eggs divided by the sum of hatched, unhatched, and parasitized eggs. Changes in parasitism rates in ESLs for each 14 d sample period were assumed to imply changes in the number of parasitoids present in the field during that period, thus allowing an inference of flight phenology.

### PHENOLOGY OF *T. PLANIPENNISI* DETERMINED USING SENTINEL *A. PLANIPENNIS* LARVAE IN ASH LOGS

Larval sentinel logs (LSLs) were prepared in the laboratory following procedures similar to those described in Abell et al. (2015). Briefly, LSLs were prepared by cutting, washing, and sealing as already described for ESLs. Next, a small strip of bark ~2 cm wide × 6 cm long was cut vertically at the top of each log using a razor blade or utility knife and peeled back to expose the wood. A groove large enough to fit a 3rd or 4th instar *A. planipennis* larva was carved into the exposed wood using a Speedball linoleum knife (Speedball Art Products Co., North Carolina). Three such grooves were made around the top of each LSL, and a 3rd or 4th instar *A. planipennis* larva was placed head down and ventral surface out into each groove. The top of each LSL was then wrapped with a layer of Parafilm®, sealing the cut bark strips over the grooves to prevent desiccation and entry by other invertebrates or pathogens.

The *A. planipennis* larvae placed into each groove to produce LSLs were reared in the laboratory from eggs laid on coffee filter paper by using the rearing procedure described in Duan et al. (2013a). Eggs obtained using this procedure were allowed to hatch, and within 24 h, each neonate was placed into an ash log using the aforementioned procedure (except that ash logs were not sealed with paraffin wax). Each log was placed on a substrate of water-saturated Grodan rock-wool growcubes (ROXUL Inc., Milton, Ontario, Canada) and held in growth chambers (16:8 h L:D photoperiod, constant 28 °C) for 20 to 22 d.

LSLs were deployed from 27 May to 14 Oct 2011 (164–1,521 GDD10), 29 Jun to 2 Nov 2012 (641–1,699 GDD10), and 30 Apr to 16 Oct 2013 (26–1,461 GDD10) in Nancy Moore Park in Meridian Township, Ingham County, Michigan (42.738°N, 84.418°W), and William M. Burchfield County Park, Holt, Ingham County, Michigan (42.589°N, 84.586°W) (2012–2013 only). Although releases of *T. planipennisi* were never made at Nancy Moore Park, this parasitoid had dispersed and established there from releases done in 2007 to 2009 at nearby Central Park (Duan et al. 2013a). Releases of *T. planipennisi* at Burchfield County Park had been done in 2008 to 2009 (Duan et al. 2013a). One LSL was placed on each of 6 living ash trees (DBH 8–29 cm) at each site and was replaced on the same tree with a new one every 14 d during the sample period for all 3 yr of the study. LSLs were hung from trees in the same manner as ESLs.

After each 14 d period, LSLs were collected and returned to the laboratory, where they were debarked to recover the *A. planipennis* larvae. Once all larvae were removed, the sentinel logs were discarded. Each larva was examined visually and categorized as (1) alive, (2) dead from causes other than parasitism (desiccation, fungus, or unknown), or (3) parasitized (parasitoid larvae or pupae present). Larvae categorized as alive were individually isolated in 12-well Falcon tissue culture plates (Corning Inc. #353225, ThermoFisher Scientific, Hanover Park, Illinois) and held in growth chambers (16:8 h L:D photoperiod, constant 25 °C) for 14 d to allow parasitoids potentially present to develop and become visually apparent. Percentage of parasitism was calculated for each site as the number of host larvae parasitized by a particular species, divided by the sum of alive and parasitized larvae pooled for all LSLs. Changes in parasitism rates in LSLs for each 14 d sample period were assumed to imply changes in the number of parasitoids present in the field during that period, thus allowing an inference of flight phenology.

## Results

### PHENOLOGY AND PREVALENCE OF *O. AGRILI* DETERMINED USING SENTINEL EGGS

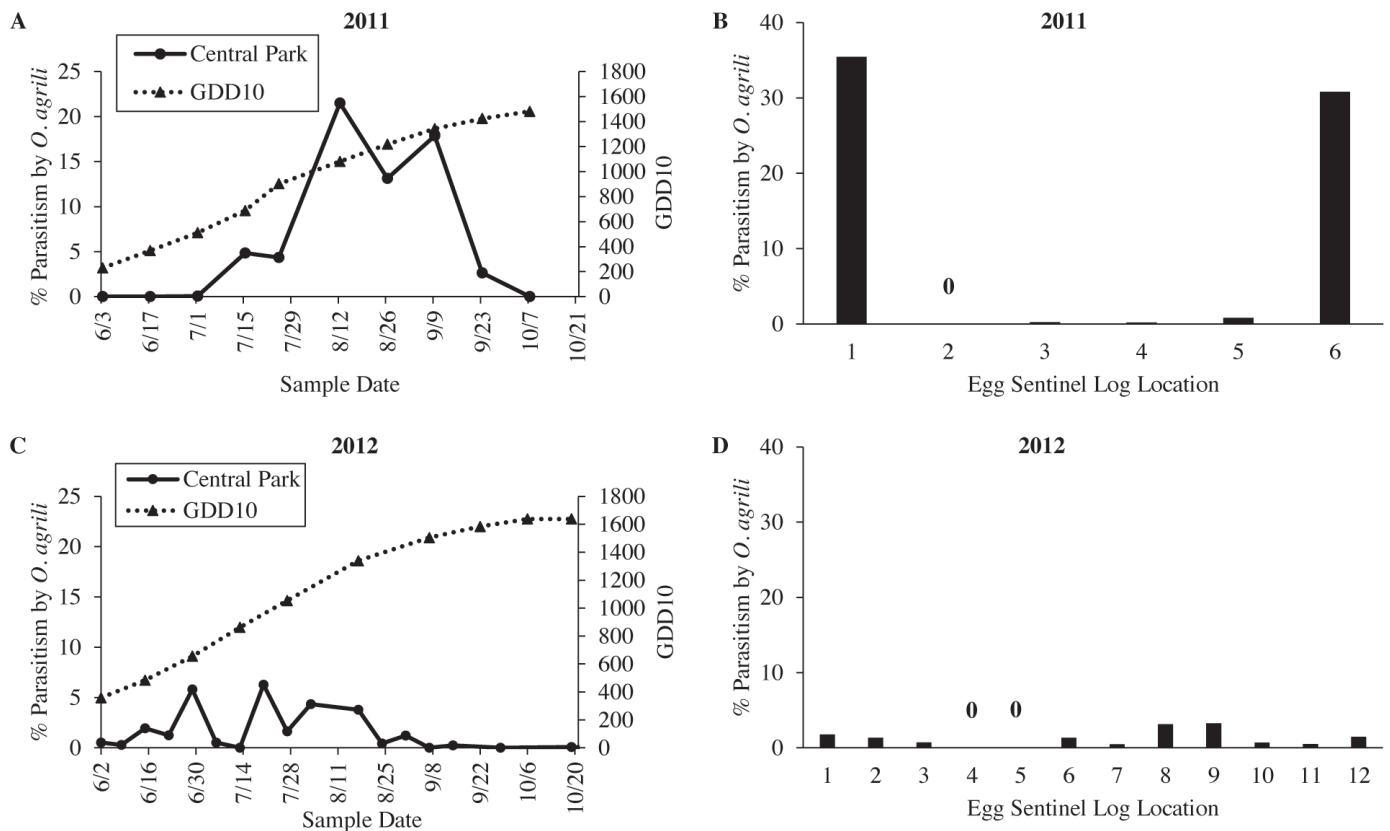
In 2011 at the Central Park site, emerald ash borer egg parasitism rates in ESLs by *O. agrili* ranged from 0 to 22% among sample periods (Fig. 1A). Eggs parasitized by *O. agrili* were first seen in mid-Jul (511–688 GDD10), peaked about 4 wk later in mid-Aug (1,081 GDD10), and the last *O. agrili* individuals were recovered from ESLs deployed from late Sep to early Oct (1,424–1,481 GDD10) (Fig. 1A). Parasitism rates of 30

to 40% were seen in ESLs from trees 1 and 6, whereas ESLs from trees 2 to 5 had 0 to 1% parasitism pooled over all sample periods (Fig. 1B). In 2012, egg parasitism rates ranged from 0 to 6% among sample periods (Fig. 1C). Parasitized eggs were seen in the first sample in early Jun (234–358 GDD10), and remained steady at ~5% egg parasitism from late Jun through mid-Aug (656–1,339 GDD10) (Fig. 1C). Variance among trees was lower in 2012, with 10 of 12 ESLs yielding parasitized eggs, ranging from 0.5 to 3.3% when pooled over all sample dates (Fig. 1D).

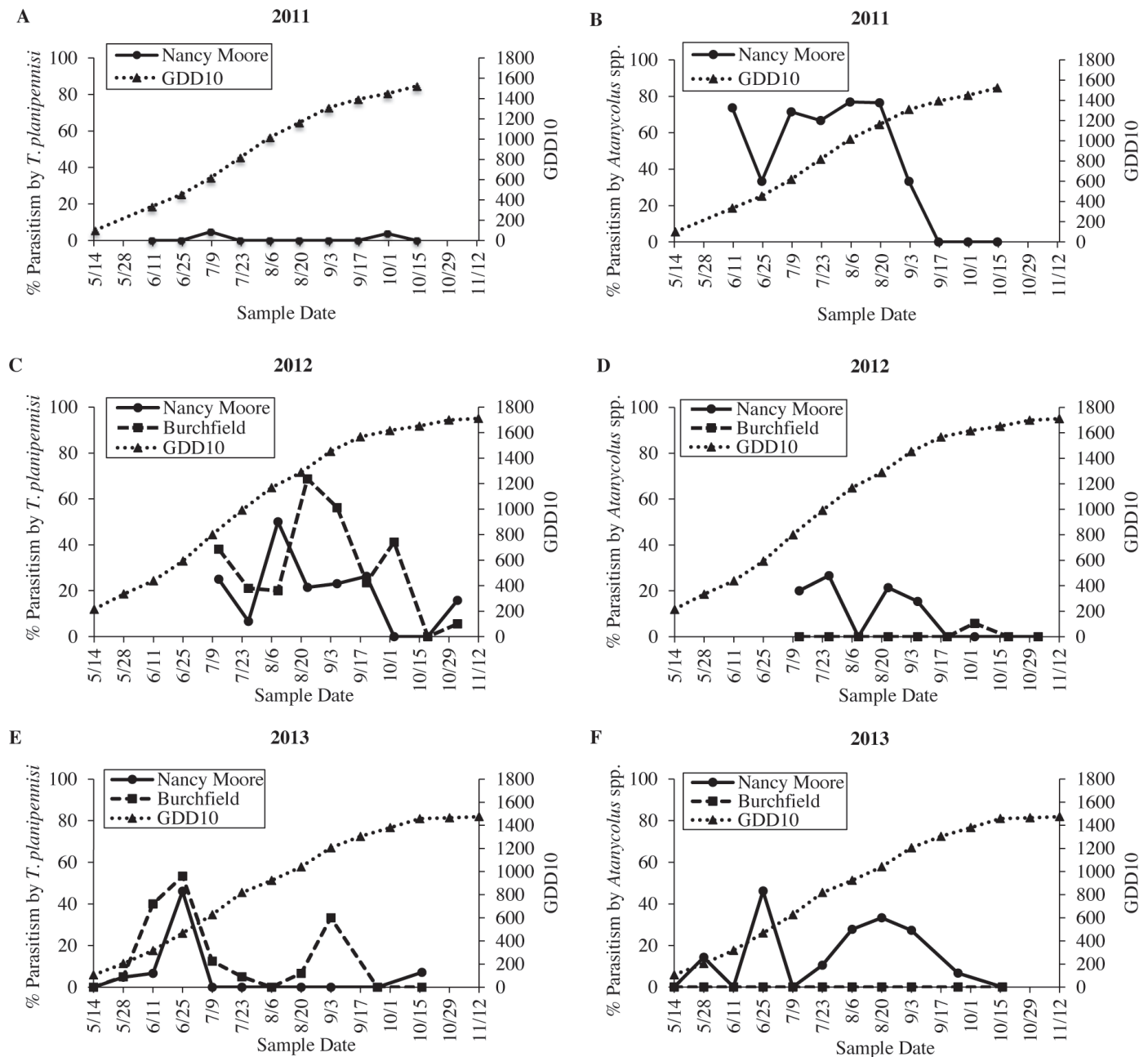
### PHENOLOGY AND PREVALENCE OF LARVAL PARASITIDS DETERMINED USING SENTINEL LARVAE

In 2011, the Nancy Moore site had only 2 sentinel larvae parasitized by *T. planipennisi*, resulting in 4% parasitism in Jul (618–818 GDD10) and 5% in Oct (1,449–1,521 GDD10) (Fig. 2A). In contrast, *Atanycolus* spp. parasitized over 70% of sentinel larvae in 5 of the 7 sample periods that it was detected (Fig. 2B). *Atanycolus* spp. parasitoids were present in the first sample in early Jun (334–453 GDD10) until early Sep (1,309–1,393 GDD10) (Fig. 2B). The Burchfield site was not sampled in 2011.

In 2012, parasitism of sentinel larvae by *T. planipennisi* ranged from 0 to 50% and from 0 to 69% at Nancy Moore and Burchfield, respectively (Fig. 2C). The phenological pattern was similar between the 2 sites, except that Burchfield was delayed by 1 sample period (Fig. 2C). *Tetrastichus planipennisi* was present in our first sample in early Jul, peaked in Aug (1,169–1,288 GDD10), declined to 0 in Oct, but was present again in the last sample in early Nov (1,699–1,711 GDD10). In 2012, parasitism of ESL larvae by *Atanycolus* spp. was lower than the previous year, being 0 to 27% and 0 to 6% in Nancy Moore and Burchfield, respectively (Fig. 2D). At the Burchfield site, parasitism by *Atanycolus*



**Fig. 1.** Percentage of parasitism by *Oobius agrili* of emerald ash borer eggs on all egg sentinel logs (pooled by sample date, i.e., the date that egg sentinel logs were collected) in Central Park, Michigan, in (A) 2011 and (C) 2012, and on individual egg sentinel logs pooled over all sample dates in (B) 2011 and (D) 2012. The secondary Y-axis is growing degree day base 10 °C (GDD10) using the Baskerville–Emin method.



**Fig. 2.** Percentage of parasitism by *Tetrastichus planipennis* of emerald ash borer larvae in larval sentinel logs (pooled by sample date, i.e., the date that larval sentinel logs were collected) in Nancy Moore and Burchfield Parks, Michigan, in (A) 2011, (C) 2012, and (E) 2013, and by *Atanycolus* spp. in (B) 2011, (D) 2012, and (F) 2013. The secondary Y-axis is growing degree day base 10 °C (GDD10) using the Baskerville–Emin method.

spp. was only found in the early Oct sample (1,616–1,651 GDD10), but was present at Nancy Moore from the first sample in early Jul through early Sep (1,453–1,567 GDD10) (Fig. 2D).

In 2013, LSLs were deployed earlier (May) to detect the first flight of *T. planipennis*. This was apparently accomplished as the first sample had no parasitized larvae and thus presumably preceded parasitoid flight. Sentinel larvae were parasitized in the second sample (late May) (106–204 GDD10), and parasitism peaked in late Jun (467–627 GDD10), declined in Jul, reached 0 in early Aug, then peaked again in early Sep (1,206–1,304 GDD10) before declining again to 0 in late Sep (Fig. 2E). The second peak seen in Sep only occurred at Burchfield, whereas *T. planipennis* was not detected at Nancy Moore from Jul through Sep, but was detected in the last sample in mid-Oct (Fig. 2E).

Parasitism by *T. planipennis* ranged from 0 to 46% at Nancy Moore and from 0 to 53% at Burchfield (Fig. 2E). In contrast, in 2013, no parasitism by *Atanycolus* spp. was detected in samples from Burchfield (Fig. 2F). At Nancy Moore, parasitism by *Atanycolus* spp. peaked twice, once in Jun (467–627 GDD10) and again in Aug (943–1,041 GDD10), and rates ranged from 0 to 46% (Fig. 2F).

## Discussion

Using ESLs, we consistently detected the egg parasitoid *O. agrili* in the field over a 3 mo period in each of the 2 yr of this study; however, parasitism rates and flight phenology were inconsistent between

years. Relatively high parasitism rates were seen on only 2 ESLs in the 1st year, whereas relatively low parasitism rates were seen on nearly all ESL in the 2nd year. The 1st year of the study showed a peak in *O. agrili* parasitism in late summer, whereas the 2nd year showed a nearly constant low level of parasitism from Jun to Aug. Additionally, our results did not detect discrete peaks of *O. agrili* parasitism corresponding to discrete parasitoid generations, but rather recorded the continued presence of *O. agrili* over a 3 mo period, suggesting the occurrence of at least 2 overlapping generations. It should be noted that patterns of diapause in *O. agrili* are not fully understood (Bauer et al. 2015b), complicating interpretation of its phenology in the field.

Because ESLs are non-destructive to the tree on which they are placed to detect emerald ash borer egg parasitism, the same tree can be used repeatedly with a new ESL, unlike sampling methods that require the removal of bark from the sample tree (Duan et al. 2011, 2012b; Abell et al. 2012, 2015). The ability to use the same tree repeatedly eliminates variability that could exist when taking serial samples from different trees. Despite this advantage, there are still several disadvantages that warrant consideration given that *O. agrili* adults seem to be relatively poor dispersers (Duan et al. 2012b; Abell et al. 2014). First, as the health of a particular tree declines and it becomes less attractive to egg-laying emerald ash borer females, the local *O. agrili* population may decrease sharply. Second, given that *O. agrili* populations are still low, repeatedly sampling throughout the year at the same location may diminish the localized population enough to significantly affect samples taken in subsequent years. Either or both of these possibilities may explain the order of magnitude greater parasitism seen on particular trees and the inconsistent phenological pattern seen from one year to the next in this study.

Alternatively, variation in phenological synchrony between *O. agrili* and *A. planipennis* oviposition (due to annual variation in climate) may play a role. Because of the short window of time that *A. planipennis* eggs are suitable for parasitism (Duan et al. 2014b), changes in timing of *A. planipennis* oviposition or *O. agrili* phenology by as little as 7 d could have major effects on the population size of subsequent generations of *O. agrili* that in turn may result in highly variable levels of parasitism from year to year. Development of methods to determine the timing and duration of *A. planipennis* oviposition would be of great benefit when combined with the use of ESLs for *O. agrili* phenology described in this study for evaluation of *O. agrili*, particularly as it is introduced to other parts of North America where differences in climate may significantly affect host–parasitoid synchrony. One final consideration regarding ESLs is that they only provide a relative estimate of parasitism rates. This is because ESLs do not represent the actual density or availability of susceptible *A. planipennis* eggs in the field. However, as long as the numbers of eggs are relatively constant on ESLs and in the field, ESLs could potentially provide a measure of *O. agrili* parasitism that correlates with actual parasitism in the field.

Our results show that LSLs can readily detect parasitoids of *A. planipennis* larvae. However, because of the presence of native *Atanycolus* spp., monitoring of *T. planipennis* is somewhat complicated. Larvae in LSLs deployed in the 1st year of this study were mostly parasitized by *Atanycolus* spp., but this was not the case in the 2nd and 3rd years of our study. Indeed, at the Burchfield Park site (added in the 2nd and 3rd years) parasitism by *Atanycolus* spp. was virtually absent from samples. This allowed for a clearer picture of *T. planipennis* phenology. Despite the virtual absence of *T. planipennis* at Nancy Moore Park in the 1st year, parasitism rates there in the 2nd and 3rd years were comparable to those seen at Burchfield Park in those years. However, based on the continued presence of both parasitoid species at Nancy Moore Park in the final 2 yr, it seems unlikely that either has a competitive advantage over the other, but further study is needed to discern this interaction.

We did not see a consistent phenological pattern of either *T. planipennis* or *Atanycolus* spp., but this may be an accurate reflection of conditions in the field. In Michigan, emerald ash borer development rates vary from 1 to 2 yr depending on tree species, tree health, environmental conditions, and time of oviposition (Cappaert et al. 2005; Duan et al. 2010; Siegert et al. 2010; Tluczek et al. 2011; Herms & McCullough 2014). Therefore, emerging parasitoids encounter a great deal of variation in the presence and abundance of suitable hosts throughout the year. In addition, as native parasitoids, *Atanycolus* species have alternate hosts besides emerald ash borer, which further complicates the possibility of observing or predicting a regular pattern of adult flight from year to year. Data on emerald ash borer life stages present while deploying LSLs would be valuable in helping to clarify the variability in phenology seen in this study.

Although LSLs appear to be a viable tool for monitoring and evaluating emerald ash borer larval parasitoids, it must be noted that their production requires substantial time and resources. A source of unfested, appropriately sized ash trees is needed, as well as laboratory-reared 3rd and 4th instar *A. planipennis* larvae. Care must be taken to prevent the introduction of pathogens when cutting bark strips and grooves for larvae. Unlike *O. agrili*, *T. planipennis* is capable of dispersing throughout the environment quickly (Duan et al. 2013a,b; Fahrner et al. 2014, 2015), which eliminates many of the disadvantages discussed for ESLs. Additionally, because *T. planipennis* is gregarious, there is little concern that repeated sampling with LSLs would significantly affect the population. Overall, LSLs are a valuable tool for monitoring introduced parasitoids of *A. planipennis*. LSLs should be particularly valuable for evaluating *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae), a larval parasitoid of *A. planipennis* recently approved for release in the United States, for its establishment as well as its interaction with *T. planipennis* and *Atanycolus* spp. However, as already noted, knowing when susceptible stages of *A. planipennis* are present will be a critical component in such an evaluation.

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