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Perspective

Photoperiodic modulation of diapause induction and termination in *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), an egg parasitoid of the invasive emerald ash borer

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ABSTRACT

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary and parthenogenetic egg parasitoid from China being introduced into North America (NA) as a biological control agent of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an extremely invasive and destructive pest of ash trees (*Fraxinus* spp.). *Oobius agrili* is being released over a broad geographical range in NA where photoperiod varies considerably during the season of parasitoid-host activity. We conducted laboratory studies to determine 1) if photoperiod-induced diapause is modulated maternally, grand-maternally, and/or directly in immature parasitoids; 2) interactions of maternal adult 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. We found that 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. However, older adults that experienced diapause as larvae produced more progeny that entered diapause when their progeny were exposed to short-day photoperiods. All progeny produced by adults that developed from nondiapaused larvae entered diapause when exposed to short-day photoperiods. 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 h of daylight (at 25 °C). Photoperiod and duration of chill affected diapause termination of diapausing *O. agrili* larvae. The cumulative number of degree days (base 10 °C) required for adult emergence was highest for the combination of 12 h light:12 h 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. We discuss the effects of photoperiod on parasitoid-host synchrony, population dynamics, and fitness of *O. agrili* across the geographical area where it is being released.

1. Introduction

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is an egg parasitoid of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), both from northeastern China. Emerald ash borer was inadvertently introduced into North America (NA) in the early 1990s from China, and as of March 2019, EAB has spread to 35 states and the District of Columbia in the U.S., and 5 provinces in Canada, 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). Given that all NA ash species EAB has encountered to date are highly susceptible to this invasive pest and ash tree mortality > 99% has occurred at many sites, EAB is considered a threat to most, if not all, ash species native to NA (Federal Register, 2007; Klooster et al., 2014; Knight et al., 2012). To suppress EAB populations and reduce ash mortality, research on the classical biological control of EAB began in the U.S. and China in 2003. This research led to the approval of environmental releases of three Chinese EAB parasitoids in Michigan in 2007, and subsequent development of the USDA EAB Biocontrol Program, which involves rearing and releasing the EAB biocontrol agents in the U.S. (Federal Register, 2007; Bauer et al., 2015). In addition, Canada began releasing classical biological control agents of EAB in 2014 (Bauer et al., 2015a; Duan et al., 2018). *Oobius agrili* is among the parasitoid species approved for environmental

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release, and as of 2018, almost 1.6 million *O. agrili* have been reared and shipped for release in EAB infestations in 27 states and the District of Columbia in the U.S., and 3 provinces in Canada (MapBioControl, 2019). To date, recovery of *O. agrili* has been confirmed at release sites in 11 U.S. states and 2 Canadian provinces (MapBioControl, 2019), while parasitism rates as high as 40% were recorded at some of the earliest release sites in Michigan (Abell et al., 2014). *Oobius agrili* continues to be released in EAB-infested areas of the U.S. and Canada.

Oobius agrili is a solitary, multivoltine parasitoid that is endemic to northeastern China (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 NA to date. During the winter, *O. agrili* diapauses as a mature larva inside the EAB egg (Liu et al., 2007; Bauer et al., 2015b). Development is completed in the spring, and adult females emerge and are active in spring and summer during EAB oviposition (Liu et al., 2007; Bauer et al., 2015b; Abell et al., 2016; 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 h light: 8 h 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. 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), and this proportion increased 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 photoperiods, 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 14–15 h for *Ooencyrtus ennomophagus* Yoshimoto (Hymenoptera: Encyrtidae) (Anderson and Kaya, 1974), 14–16 h for *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae) (Jackson, 1963), and 12–16 h 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 as well as its seasonal occurrence and that of 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 have 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 host 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 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).

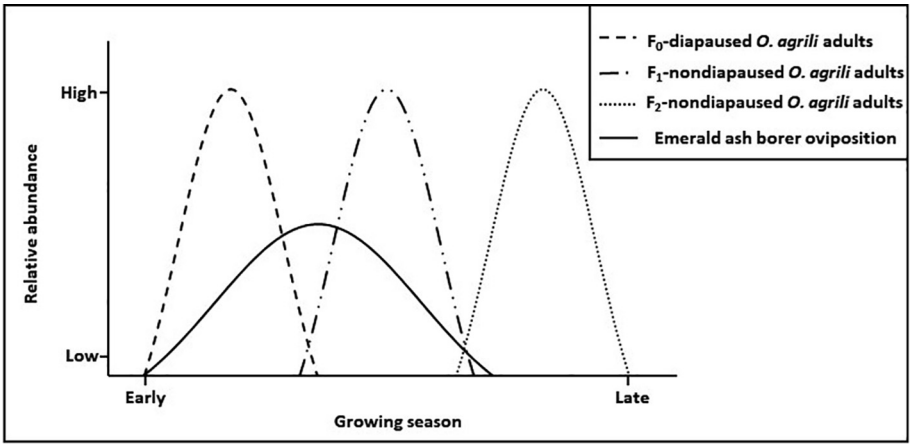

Fig. 1. Graphical depiction of the estimated seasonal phenology of *O. agrili* generations and their synchrony with emerald ash borer oviposition. *Oobius agrili* synchrony with emerald ash borer oviposition has not been confirmed in the field.

Oobius agrili is currently being released over a broad geographical range in efforts to manage expanding EAB populations (USDA–APHIS/ARS/FS, 2019; Bauer et al., 2015a; Duan et al., 2018). 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*, we conducted laboratory studies in 2016–2017 with specific objectives to determine 1) if photoperiod-induced diapause is modulated maternally, grand-maternally, and/or directly in developing parasitoids; 2) interactions of maternal adult 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 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, and thus provide insight into the likely geographical range across which *O. agrili* can establish and help control EAB.

2. 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 to 2014 for mass-rearing and releasing as EAB biocontrol agents.

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

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 min 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 lx. This variation in light intensities was considered unimportant for the current study given that photoperiod responses of egg parasitoids were shown to be induced by changes in light intensity rather than the absolute light intensity (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 the rearing 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 (Abell et al., 2012). Filter paper with eggs was removed every 2–3 days, and held at 25 ± 1 °C for an additional 24 h to allow embryogenesis to occur in eggs laid within the last 24 h. 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 we have previously 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). We also selected EAB eggs that were tan or brown for all 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.

2.1. Effects of maternal and progeny photoperiod exposures and maternal adult 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 1). For the first replicate, conducted October–December 2016, we selected 30 naïve F_0-diapaused adults (10 adults per photoperiod treatment) 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 a period of chill at 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 diapaused 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 in which they had developed from diapausing larvae to adults. Each adult was presented 9 EAB eggs for a 24-hr period once a week for 3 consecutive weeks. Maternal adult age for 1wk, 2 wk and 3 wk was 1–5, 9–12 and 16–19 days, respectively. After each 24 h exposure, the EAB eggs were removed from the adult parasitoids and randomly assigned to 16L:8D, 12L:12D, or 8L:16D progeny photoperiod treatments (3 eggs from each adult for each progeny photoperiod treatment), at which they were held for 8 wks (Table 1). After 8 wks (mean development time from egg to adult was ca. 4 wks at 25 °C) 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 larva inside EAB egg; c) parasitoid died inside EAB egg; or, d) EAB egg not parasitized. Percentage 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 larvae) by the number of eggs presented to each adult per adult photoperiod and maternal adult age treatments, and then multiplying by 100. The percentage of progeny that entered diapause (i.e., percentage diapaused) was calculated by dividing the number of diapausing larvae by the total number of successfully parasitized eggs by each female for each progeny and adult photoperiod treatment and each maternal adult age, 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

Table 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.

	No. ovipositing adults	Maternal photoperiod treatments	Maternal age when exposed to eggs	No. EAB eggs per adult	Developing progeny photoperiod	No. parasitized eggs per progeny photoperiod
Subsection/Experiment						
2.1. *Effects of maternal and progeny photoperiod exposures and maternal age of 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 per adult exposure
2.2. *Effect of grandmaternal, maternal, and progeny photoperiod exposures and maternal age on F_1-diapaused adults and their progeny.*	12–20 F_1 adults per maternal and grand-maternal 16L:8D photoperiod	1) F_1 16L:8D and F_0 grand-maternal 2) F_1 16L:8D and F_0 grand-maternal 8L:16D 3) F_1 8L:16D and F_0 grand-maternal 8L:16D	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 per adult exposure
2.3. *Critical day 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 adult per progeny photoperiod
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
2.5. *Effects of photoperiod and chill duration on diapause termination on diapausing larvae.*	NA	8L:16D	NA	NA	Diapausing 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 on diapausing larvae not subjected to chill.*	NA	1) 12L:12D, 2) 14L:10D, 3) 14.25L:9.75D, 4) 14.5L:9.5D,	NA	NA	Diapausing 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

linear mixed model (Proc GLIMMIX; SAS, 2008) was used with a repeated measures design and a binomial distribution to compare the percentage of successful parasitism and the percentage of progeny that entered diapause among maternal and progeny photoperiod treatments, maternal adult ages, 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.

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

This experiment was conducted from November 2016 to February 2017 to examine the effects of F_0-diapaused adult (grand-maternal), F_1-nondiapaused adult (maternal) and progeny photoperiod treatments, as well as maternal adult age of F_1-nondiapaused adults, on parasitism and diapause of their progeny (Table 1). For this experiment, we used adults that had developed from nondiapaused larvae and were the progeny of the F_0-diapaused adults from Section 2.1. The photoperiod exposures of F_0 and F_1 adults included: a) F_0-diapaused adults that developed in and were held at 8L:16D and F_1-nondiapaused adults that developed in and were held at 8L:16D; b) F_0-diapaused adults that developed in and were held at 16L:8D and F_1-nondiapaused adults developed in and held at 8L:16D; c) F_0-diapaused adults that developed in and were held at 16L:8D and F_1-nondiapaused adults that developed in and were held at 16L:8D. We did not include 12L:12D photoperiod treatments in this study because results for progeny of F_0-diapaused adults in Section 2.1 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.

To assess the effects of photoperiod and maternal adult 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 treatment. Maternal adult age for 1 wk, 2 wk and 3 wk was 1–5, 9–12 and 16–19 days, respectively (Table 1). After each 24-hr exposure period, EAB eggs were removed from adult parasitoids and randomly assigned to 16L:8D or 8L:16D progeny photoperiod treatments (5 eggs from each adult for each progeny photoperiod treatment), where they remained for 8 wks. After 8 wks, the fate of the progeny from each treatment was determined, and percentage parasitized and diapaused were calculated as described for Section 2.1. 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 was used to compare percentage successful parasitism and percentage of diapausing progeny produced by each adult among photoperiod treatments, maternal adult ages, and interactions between these variables. LSMs that were significantly different ($P < 0.05$) were compared using Tukey's HSD test.

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

To estimate the critical day length for diapause induction in the progeny of F_1- and F_2-nondiapaused adults, we used naïve adults that had developed and emerged in 16L:8D photoperiod and ranged in age from 1 to 7 days (Table 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. Each photoperiod treatment received 20–40 randomly selected F_1- and F_2-nondiapaused adults that remained with the EAB eggs for a minimum of 10 days (Table 1). Eight weeks after the photoperiod exposures began, the EAB eggs were examined to determine fate of the progeny with percentage of progeny that entered diapause per dish of 20 EAB eggs calculated as described in Section 2.1. Dishes with no successful parasitism were excluded from analyses. A generalized linear mixed model (Proc GLIMMIX) with binomial distribution was used to compare the percentage of progeny that entered diapause among photoperiod treatments, between progeny from F_1- and F_2-nondiapaused adult generations, and interactions between treatments and generations. LSMs that were significantly different were compared using Tukey's HSD test.

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

During March–May 2017, we examined the developmental age of immature *O. agrili* to determine when diapause response to photoperiod occurred for the progeny of F_1-nondiapaused adults (Table 1). We presented ca. 110 EAB eggs to 15 naïve, 1–10 day-old F_1-nondiapaused adults for a 24-hr period in 16L:8D photoperiod. Two days later, we presented another 110 EAB eggs 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 to 8L:16D or 14L:10D photoperiods. Ten parasitized eggs from each of the two exposures remained in 16L:8D to serve as controls. About 8 wks 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 X^2.

2.5. Effects of photoperiod and chill duration on diapause termination on diapausing O. agrili larvae

In this experiment, we examined the effects of photoperiod on diapause termination in diapausing larvae subjected to different chill durations (Table 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 and exposed to 8L:16D photoperiod to induce diapause. Six- to eight-weeks after parasitism, the diapausing 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; DD_{10}) 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.

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

To determine the effects of photoperiod on diapause termination in diapausing larvae not exposed to a period of chill, we used 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 h of day light as determined in Section 2.3 (Table 1). This experiment was

conducted from March to June 2017. Diapausing larvae were held for 3–4 months at 25 ± 1 °C after parasitism in their original photoperiod treatments of 12L:12D, 14L:10D, 14.25L:9.75D, or 14.5L:9.5D 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 that had not been exposed to a chill period. 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 if parasitoids emerged as adults or remained as diapausing larvae. Immature parasitoids that died were excluded from the analyses. Logistic regression (Proc LOGISTIC; SAS, 2008) 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.

2.7. 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 NA, we calculated the mean calendar day on which EAB adult emergence was estimated to begin (i.e., 250 DD_{10}) and peak (i.e., 560 DD_{10}), 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). We then determined the day length for the mean calendar day for the beginning and peak EAB emergence for each location using the following: http://aa.usno.navy.mil/data/docs/Dur_OneYear.php.

3. Results

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

Progeny photoperiod directly affected diapause induction (i.e., percentage diapaused) of developing progeny inside EAB eggs (Table 2). Specifically, we found no developing progeny from F_0-diapaused *O. agrili* adults entered diapause if exposed to the 16L:8D progeny photoperiod treatment regardless of maternal photoperiod treatment (Table 3). Because no developing progeny from F_0-diapaused adults entered diapause for the 16L:8D progeny photoperiod treatment, regardless of maternal photoperiod treatment, quasi-complete separation occurred for the generalized linear mixed model comparing percentage diapaused among all photoperiod treatments and maternal adult ages. Therefore, the 16L:8D progeny photoperiod treatment was excluded from the model to allow statistical comparison of the remaining treatments. No significant difference in percentage diapaused was found when 8L:16D and 12L:12D progeny-photoperiod treatments were compared ($F = 0.02$; df = 1, 78; $P < 0.8780$; Table 2). 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 the three maternal photoperiods combined (Table 3). Also, no significant differences were found among maternal photoperiod treatments or interactions between maternal and progeny photoperiod treatments (Table 2).

The maternal adult age of F_0-diapaused adults when presented EAB eggs had a significant effect on the percentage of their progeny that entered diapause for the 8L:16D and 12L:12D progeny photoperiod treatments ($F = 38.25$, df = 2, 114, $P < 0.0001$; Table 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 4). Interactions between maternal adult 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). In addition, maternal adult age when presented EAB eggs significantly affected percentage parasitized ($F = 11.25$; df = 2, 82; $P < 0.0001$; Table 2), with the highest rate for 2-wk-old adults (66.5 ± 4.8%) compared to 1-wk- (52.9 ± 4.5%) or 3-wk- (41.4 ± 5.2%) old adults (Table 5).

3.2. Effects of grand-maternal, maternal, and progeny photoperiod exposures and maternal adult 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 F_0 adult treatment (i.e., grand-maternal) or F_1 adult treatment (i.e., maternal) photoperiod treatments (Table 6). As with Section 3.1, this perfect correlation caused quasi-complete separation of our generalized linear mixed model. Therefore, the 8L:16D progeny-photoperiod treatment was excluded from the model. Percentage diapaused for developing progeny exposed to 16L:8D photoperiod ranged from 6.3 to 8.1% among the three F_0 and F_1 photoperiod treatments and did not differ significantly ($F = 0.57$; df = 2, 38; $P < 0.5723$; Tables 2, 6). Maternal adult age did not significantly affect the percentage diapaused for developing progeny reared in 16L:8D photoperiod ($F = 1.59$; df = 2, 47; $P < 0.2159$; Tables 2, 7). However, percentage diapaused for progeny produced by 1-wk-old adults was consistently lower compared to 2-wk- and 3-wk-old adults within each F_0 and F_1 photoperiod treatment, suggesting some effect of maternal adult age. Quasi-complete separation also prevented statistical comparison of interactions between adult photoperiod treatments and maternal adult ages on percentage of progeny that entered diapause. Maternal adult age did significantly affect percentage parasitized ($F = 11.69$; df = 2, 74; $P < 0.0001$, Table 2), in which 1wk- and 2wk- old-adults had higher percentages parasitized compared to 3-wk-old adults (Table 8). Percentage parasitized was not significantly affected by F_0- and F_1- photoperiod treatments (Table 2). Also, no interaction was found between maternal adult age and F_0 and F_1 photoperiod treatments for percentage parasitized (Table 2).

3.3. 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 ≥14 h daylight, the percentage of progeny that entered diapause differed significantly among photoperiods ($F = 22.97$; df = 1, 118; $P < 0.0001$), between generations ($F = 89.31$; df = 5, 118; $P < 0.0001$), and for the interaction between these treatments ($F = 2.69$; df = 5, 118; $P = 0.0245$; Table 2). Also for photoperiods ≥14 h daylight, the highest diapause rates were found for progeny from both F_1- and F_2-nondiapaused adults held at 14L:10D, and for progeny from F_2-nondiapaused adults, held at 14.25L:9.75D (Fig. 2). Diapause rates were lowest for progeny from both generations held at day lengths ≥14.5 h, with the exception of progeny from F_2-nondiapaused adults held at 14.5L:9.5D and 16L:8D (Fig. 2). Over 50% of progeny from F_1- and F_2-diapaused adults entered diapause at day lengths ≤14.25 h and less than 50% entered diapause at photoperiods with ≥14.5 h of day length. Therefore, the critical day length at which 50% of the progeny entered diapause was between 14.25 and 14.5 h of daylight (Fig. 2).

Table 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.*	Percentage diapaused	Maternal photoperiod[2]	2, 78	$F = 0.62$	0.5397
		Maternal adult 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[4] (8L:16D vs. 12L:12D only) × Maternal adult age[3]	2, 114	$F = 1.15$	0.3183
		Progeny's photoperiod[4] (8L:16D vs. 12L:12D only) × Maternal photoperiod[2]	2, 78	$F = 1.69$	0.1893
	Percentage parasitized	Maternal photoperiod[2]	2, 41	$F = 0.78$	0.4627
		Maternal adult age[3]	2, 82	$F = 11.25$	< 0.0001
		Maternal photoperiod[2] × Maternal adult age[3]	4, 82	$F = 1.50$	0.2129
3.2. *Effect of grand-maternal, maternal, and progeny photoperiod exposures and maternal age on F_1-nondiapaused adults and their progeny.*	Percentage diapaused	Maternal and grand-maternal photoperiod[5]	2, 38	$F = 0.57$	0.5723
		Maternal adult age	2, 47	$F = 1.59$	0.2159
		Maternal and grand-maternal photoperiod[5] × Maternal adult age[3]	4, 47	$F = 0.22$	0.9267
	Percentage parasitized	Maternal and grand-maternal photoperiod[5]	2, 37	$F = 0.40$	0.6743
		Maternal adult age[3]	2, 74	$F = 11.69$	< 0.0001
		Maternal and grand-maternal photoperiod[5] × Maternal adult age[3]	4, 74	$F = 0.64$	0.6368
3.3 *Critical day length length for diapause induction.*	Percentage diapaused	Progeny's photoperiod[6]	1, 118	$F = 22.97$	< 0.0001
		Generation post diapause[7]	5, 118	$F = 89.31$	< 0.0001
		Progeny's photoperiod[6] × Generation post diapause[7]	5, 118	$F = 2.69$	0.0245
3.4. *Critical age of developing parasitoids for photoperiod induced diapause in progeny of F1-diapaused adults.*	Percentage diapaused	8L:16D photoperiod	8	$\chi^2 = 28.6523$	< 0.0004
		14L:10D photoperiod	7	$\chi^2 = 31.6258$	< 0.0001
3.5. *Effects of photoperiod and chill duration on diapause termination on diapausing larvae.*	Development time (DD_{10})	Post diapause photoperiod[8]	3, 332	$F = 932.60$	< 0.0001
		Chill period[9]	2, 332	$F = 1089.03$	< 0.0001
		Post diapause photoperiod[8] × Chill period[9]	6, 332	$F = 137.04$	< 0.0001
3.6. *Effects of photoperiod on diapause termination on diapausing larvae not subjected to a chill.*	Percentage emerged	12L:12D photoperiod	5	$\chi^2 = 48.6862$	< 0.0001
		14L:10D photoperiod	4	$\chi^2 = 67.6677$	< 0.0001
		14.25L:9.75D photoperiod	3	$\chi^2 = 39.8459$	< 0.0001
		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 adult age treatments were 1 wk, 2 wk, or 3 wk 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.

Table 3
Least squared mean (LSM ± SE) percentage of *O. agrili* progeny entering diapause (percentage diapaused) from F_0-diapaused adults by maternal and progeny photoperiods (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	Percentage (LSM ± SE) of progeny that entered diapause by progeny 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 (females) with successful parasitism. Each replicate received 3 eggs.

3.4. 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 that they would enter 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). All developing progeny that were ≤4-days old or younger when they were transferred to 14L:10D entered diapause, and all developing progeny that were ≤5 days old or younger when they were transferred to 8L:16D entered diapause (Fig. 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. 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. 3).

3.5. 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 larvae subjected to a cold period varied significantly among photoperiods ($F = 932.6$; df = 3, 332; $P < 0.0001$) (Table 2). Mean DD_{10} for adult emergence was lowest for 16L:8D and 14.5L:9.5D, followed by 8L:16D and 12L:12D ($F = 1089$; df = 2, 332; $P < 0.0001$; Table 2, Fig. 4). Mean DD_{10} for emergence also varied significantly among chill periods, with DD_{10} increasing as chill times

Table 4
Least squared mean (LSM ± SE) percentage of *O. agrili* progeny that entered diapause that were produced by F_0-diapaused adults by maternal adult age and progeny photoperiod treatment (Experiment 2.1; 16L:8D progeny photoperiod treatment was 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 percentage diapaused 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	Percentage (LSM ± SE) of progeny that entered diapause 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 (females) with successful parasitism. Each replicate received 3 eggs.

Table 5
Least squared mean (LSM ± SE) percentage successful parasitism (i.e., progeny emerged or entered diapause) for *O. agrili* F_0-diapaused adults by maternal photoperiod treatment and maternal adult age (Experiment 2.1). EAB eggs were pooled from three progeny photoperiod treatments (8L:16D, 12L:12D, and 16L:8D). LSMs for overall percentage parasitized with the same letter were not significantly different ($P < 0.05$; Tukey's HSD) among F_0-diapaused maternal adult ages.

Maternal photoperiod treatment	Percentage parasitized (LSM ± SE) by maternal adult 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 (females) with successful parasitism. Each replicate received 9 eggs.

Table 6
Least squared mean (LSM ± SE) for percentage of progeny that entered diapause from *O. agrili* F_1-nondiapaused adults by F_0 (grand-maternal)/F_1 (maternal) photoperiod treatment and progeny photoperiod treatment (Experiment 2.2). 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 /F_1 maternal photoperiod treatments	Percentage (LSM ± SE) of progeny that entered diapause 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 (females) with successful parasitism. Each replicate received 5 eggs.

Table 7
Least squared mean (LSM ± SE) percentage of progeny that entered diapause from *O. agrili* F_1-nondiapaused adults by F_0 (grand-maternal)/ F_1 (maternal) photoperiod treatments and maternal adult age (Experiment 2.2). The eggs were then transferred to 16L:8D photoperiod 24hr after oviposition. Progeny transferred to 8L:16D photoperiod were not included because 100% entered diapause (see Table 6). Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD).

F_0/F_1 maternal photoperiod treatment	Percentage (LSM ± SE) of progeny that entered diapaused when placed in 16L:8D light treatments by maternal adult 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 (females) with successful parasitism. Each replicate received 5 eggs.

Table 8
Least squared mean (LSM ± SE) percentage successful parasitism (i.e., progeny emerged or entered diapause) of EAB eggs for *O. agrili* F_1-nondiapaused adults F_0 (grand-maternal)/ F_1 (maternal) photoperiod treatments and maternal adult age (Experiment 2.2). Data were pooled for 8L:16D and 16L:8D progeny photoperiod treatments. Overall means with the same letter were not significantly different ($P < 0.05$; Tukey's HSD).

F_0/F_1 maternal photoperiod treatment	Percent parasitism (LSM ± SE) by maternal adult age		
	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 (females) with successful parasitism. Each replicate received 10 eggs.

decreased (Fig. 4). There was also a significant interaction between photoperiod and length of chill ($F = 137$; df = 6, 332; $P < 0.0001$; Table 2). Diapausing *O. agrili* 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 larvae held at 16L:8D and 14.5L:9.5D photoperiods, for which emergence times were similar among the three different chill durations (Fig. 4).

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

Almost all diapausing 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 ≥14.5 h (Fig. 5). Significantly fewer larvae 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, Fig. 5). No diapausing 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. 5). One individual that originated and remained in 14L:10D (i.e., control) emerged, as did a few individuals that originated and remained in 14.25L:9.75D. 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. 5).

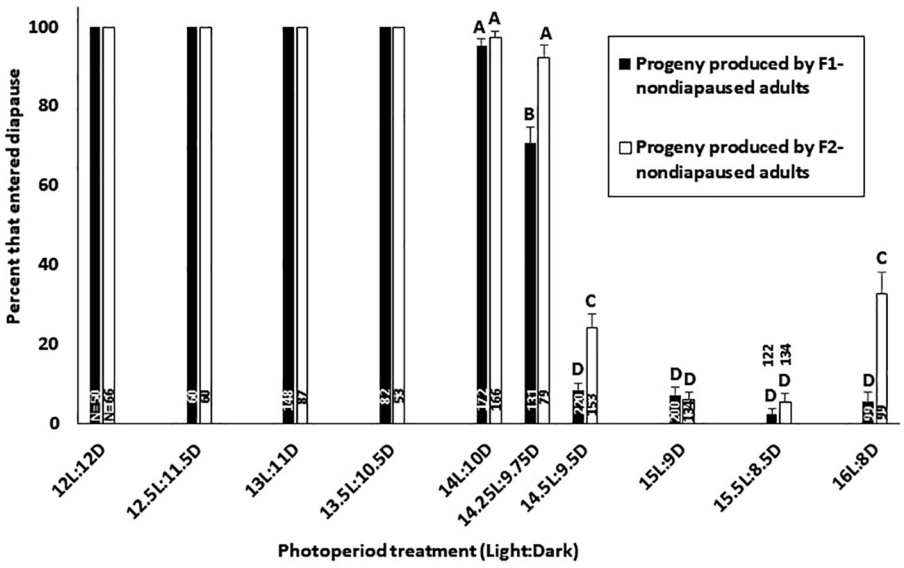

Fig. 2. Least-squared mean (LSM ± SE) percentage of *O. agrili* progeny entering diapause from F_1- and F_2-nondiapaused adults that devloped in different photoperiods (Experiment 2.3). LSMs with the same letter were not significantly different ($P < 0.05$; Tukey's HSD). ^{1}N = total number of successfully parasitized EAB eggs (i.e., emerged as adults or became diapausing larvae).

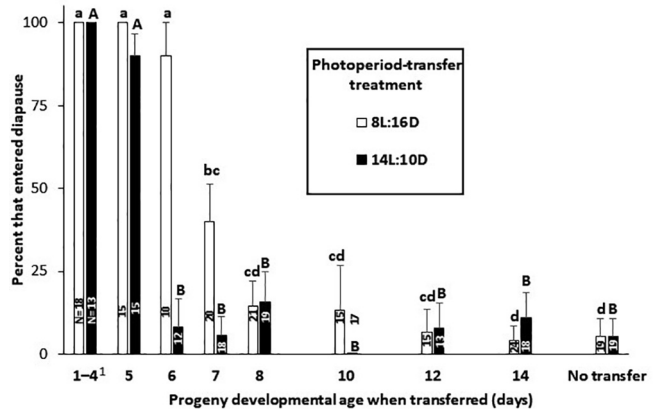

Fig. 3. Mean (± SE) percentage of individuals that entered diapause (percentage diapaused) when developing progeny were transferred from 16:8 to 8:16 or 14:10 photoperiod at different developmental ages (Experiment 2.4). Developmental ages with the same letter within each photoperiod-transfer treatment are not significantly different ($P < 0.05$; Wald's χ^2 test; Table 2). ^{1}N = number of diapausing larvae that were transferred.

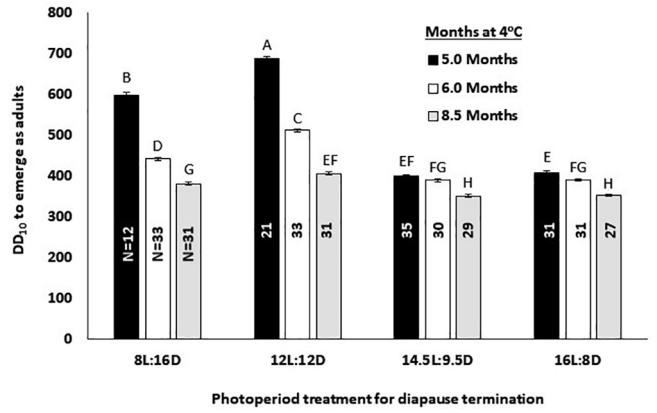

Fig. 4. Least-squared mean (LSM ± SE) degree days base 10 °C (DD_{10}) required for *O. agrili* to develop from diapausing larvae to adults after exposure to different chill durations at 4 ± 1 °C, followed by exposure to different photoperiods at 25 ± 1 °C (Experiment 2.5). LSMs with the same letter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

3.7. 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* was released (Table 9). At the most northern site (Houghton, Michigan), EAB emergence began in early July (ca., calendar day 190), whereas EAB emergence began in late January (ca., calendar day 22) at the most southern site (Minden, Louisiana). 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 9). The hours of daylight varied across the five sites at the estimated dates for both initial and peak emergence of EAB adults (Table 9). At the beginning of EAB emergence there would be 15:55 h of daylight at the most northern site and 10:24 h of daylight at the most southern site. During peak EAB emergence, there would be 14:40 h of daylight at the most northern site and 11:10 at the most southern site (Table 9).

4. 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 geographical 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. We found the critical day length for diapause induction in *O. agrili* fell within a narrow range (c.a., 15 min between 14.25 and 14.5 h of daylight at 25 °C), and diapause induction was directly determined by the photoperiod to which larvae were exposed prior to maturation. We also found that adult emergence was 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 3-wk-old, while parasitism

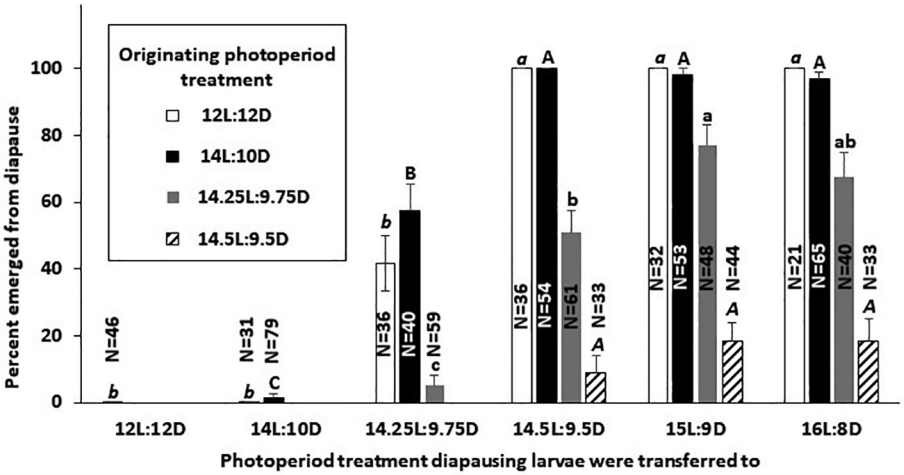

Fig. 5. Mean (± SE) percentage of *O. agrili* that developed to adults from diapausing larvae that were in photoperiod-induced diapause and not subjected to a chill period before transfer to photoperiods with longer day lengths (Experiment 2.6). Means with the same letter were not significantly different ($P < 0.05$; Wald X^2 test). [1]N = number of diapausing larvae that were transferred and either developed and emerged as adults or remained as diapausing larvae.

rates declined with increasing maternal adult age. This suggests an interaction between maternal adult age and progeny photoperiod (i.e., 16L:8D vs. 8L:16D and 12L:12D) for the induction of diapause, even though we could not statistically compare this interaction due to complete separation in the model as a result of the 16L:8D progeny photoperiod perfectly predicting the percentage of progeny that entered diapause (i.e., no larval 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 adult age and photoperiod on diapause induction was also found, as well as a similar decline in parasitism with increasing maternal adult age (Hoban et al., 2016).

The increase in photoperiod-induced diapause rates for progeny of F_0-diapaused adults as maternal adult age increased is likely attributed to an "interval timer" (Lees, 1958; 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 3 wk for *O. agrili*), or in subsequent post-diapause generations.

We found that the diapause response of *O. agrili* to short-day photoperiods is fully reinstated in the progeny of F_1- and F_2-nondiapaused adults; similar to other *O. agrili* studies in which both the adults and their developing progeny were reared at the same photoperiods (Hoban et al., 2016; Larson and Duan, 2016). In our 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 ≤13.5 h long. The percentage of progeny entering diapause was also very high at 14 h and 14.25 h day lengths but decreased to much lower rates for ≥14.5 h day lengths. As with progeny of F_0-diapaused adults, the photoperiod-diapause response occurred in the immature larval stages of progeny produced by F_1-nondiapaused adults, and maternal or grand-maternal photoperiod treatments did not affect diapause rates of their progeny (Tables 2, 6, 7). The response of developing larvae to photoperiod-induced diapause declined dramatically when developing larval progeny of the F_1-nondiapaused adults were transferred from 16L:8D to 8L:16D or 14L:10D photoperiods when > 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 a significant interaction between photoperiod and the nondiapaused generation of *O. agrili*. We found that when the developing 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 percentage entered diapause compared to the progeny of F_1-nondiapaused adults. Interestingly, increasing diapause rates with increasing post-diapause generation have also been reported in *O. ennomophagus* (Anderson and Kaya, 1974). This interaction between photoperiod and the nondiapaused generation may 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. However, photoperiods 14L:10D, 15L:9D or 15.5L:8.5D did not vary significantly among generations. 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. It is possible that excessively long day lengths (i.e., 16L:8D) may induce diapause or be so unnatural as to disrupt the pathway for photoperiodism in *O. agrili*, with this response being

Table 9
The mean calendar day for a 38-yr period (1978–2016) estimating when EAB adult emergence began (DD_{10} = 250) and peaked (DD_{10} = 560), and hours and minutes of daylight on this day, for five different locations where *O. agrili* was released in the U.S.

			EAB emergence begins (250 DD_{10})		EAB emergence peaks (560 DD_{10})	
Nearby city, State	Latitude	Longitude	Calendar day	Hours of daylight	Calendar day	Hours of daylight
Houghton, Michigan	47.2253	−88.4578	190	15:55[1]	219	14:40
Shepherd, Michigan	43.5330	−84.5050	165	15:23	196	15:08
Osborn, Missouri	39.8015	−94.3763	51	10:57	126	14:04
Indian Creek, Tennessee	36.2341	−85.7905	51	11:05	123	13:44
Minden, Louisiana	32.6625	−93.3693	22	10:24	50	11:10

[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).

stronger for progeny from F_2-nondiapaused adults. The molecular mechanisms and pathways for day-length determination 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).

Our 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 and 14.5 h 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. 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 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 in the season, they may have difficulty finding eggs to parasitize because the EAB oviposition period would be near completion (Fig. 1). In more southern latitudes, day lengths would be shorter than the critical day length for inducing diapause during EAB peak activity. This could lead to higher diapause rates for the progeny from older F_0-diapaused adults and 100% of progeny from F_1-nondiapaused adults entering diapause, thereby limiting *O. agrili* to a maximum of two generations. Although fewer generations of *O. agrili* may result in lower parasitism rates, a larger population of overwintering progeny would provide more F_0 adults 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* or different EAB egg parasitoid species from Asia with different critical day lengths may needed for EAB biocontrol.

The response of *O. agrili* to photoperiod may differ in natural settings compared to what we found 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, perceived day length may be reduced or undetectable by developing *O. agrili* larvae. Tree-bark texture, canopy cover, and topography may also affect diapause rates by reducing perceived day lengths. Alternatively, *O. 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. (2016) 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-diapaused adults (i.e. adults emerging from diapausing larvae after a chill period) was significantly affected by photoperiod and length of chill, with a significant interaction between these variables. Differences among treatments were small for all but the shortest chill period. However, we were able to detect a significant difference 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. Duan and Larson (2019) also found emergence times for *O. agrili* to be longer when diapausing larvae were chilled for shorter periods compared to longer periods. Although the differences among treatments in our study were small, these variables 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 diapausing larvae were exposed to 25 °C after the diapause- chill period. However, we did not monitor the developmental rate of post-diapause stages. 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 the diapause-chill period appear to override the lack of critical day length and allow morphogenesis to occur, albeit slightly delayed. Also, photoperiod may have directly affected development rates of *O. agrili*, as is reported for several insect species (Beck, 1980; Tauber et al., 1986). Emergence differences among photoperiods may also be attributed to a circadian basis for adult eclosion (Fanitinou 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 delayed compared to 8L:16D. From our results, it appears that *O. agrili* emergence from diapause follows a Type III photoperiod response curve as described by Beck (1980), with response to photoperiod occurring 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 is documented for 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, we do not know if lengthening 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 for spring emergence of the F_0-diapaused adults.

We found that photoperiod-induced diapause can be terminated for most individuals by increasing the day length to $\geq$14.5 h without subjecting diapausing larvae to a chill period. A moderate number of individuals also terminated diapause when transferred to 14.25 h day length, while only one individual developed to adult that originated and remained in 14.0 h 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 further south in NA. However, survival of diapausing *O. agrili* larvae may be negatively affected if subjected to long periods at temperatures above minimum developmental thresholds due to 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.

5. Conclusion

Our 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 and 14.5 h of day length for progeny of F_1- and F_2-nondiapaused adults. Sensitivity to photoperiod occurred only during the larval stage of *O. agrili* progeny, and the likelihood of photoperiodic induced diapause declined abruptly after the first five to six days of *O. agrili* progeny development. During the course of this study, we found no evidence that adult (maternal or grand-maternal) photoperiod history affected diapause induction in progeny. However, the age of F_0-diapaused adults does play a role in sensitivity of their larval 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 NA.

CRediT authorship contribution statement

Toby R. Petrice: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Deborah L. Miller:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Leah S. Bauer:** Conceptualization, Investigation, Methodology, Resources, Funding acquisition, Writing - original draft, Writing - review & editing. **Therese M. Poland:** Conceptualization, Resources, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Forrest W. Ravlin:** Conceptualization, Funding acquisition, Resources, Project administration, Supervision, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2019.104047.

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