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Differences in the reproductive biology and diapause of two congeneric species of egg parasitoids (Hymenoptera: Encyrtidae) from northeast Asia: implications for biological control of the invasive emerald ash borer (Coleoptera: Buprestidae)

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ABSTRACT The emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae) is a serious invasive pest that has killed millions of North American ash (*Fraxinus* spp.) trees in the United States and Canada since its first detection in 2002. *Oobius primorskyensis* Yao and Duan and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) constitute a cryptic species complex of egg parasitoids attacking *A. planipennis* in their native range of northeast Asia. While *O. primorskyensis* is currently being evaluated in quarantine for potential biocontrol introduction against *A. planipennis* to the U.S., *O. agrili* has established populations in some areas of the U.S. since its first introduction in 2007. In the present study, we quantify and compare the survivorship, fecundity and progeny diapause between *O. primorskyensis* and *O. agrili* for both diapaused and non-diapaused types. Both parasitoid species were reared in the laboratory with fresh *A. planipennis* eggs at $25 \pm 1^\circ\text{C}$, $\approx 65\%$ RH, and 16: 8 h L: D photoperiod. Results of the study demonstrate that there are distinctive differences in the reproductive biology and progeny diapause pattern between the two parasitoid species as well as between diapaused and non-diapaused types within each species. Adults of diapaused *O. primorskyensis* had significantly longer longevity and produced significantly more progeny over their lifetime than diapaused adult *O. agrili*. Although there was no significant difference in their longevity, adults of the non-diapaused *O. primorskyensis* produced significantly less progeny than those of non-diapaused *O. agrili*. While a majority ($>64\%$) of the progeny produced by diapaused *O. primorskyensis* and both diapaused and non-diapaused *O. agrili* continued development to adults, nearly 100% of the progeny from non-diapaused *O. primorskyensis* entered diapause. These findings suggest that the effectiveness of *O. primorskyensis* and *O. agrili* in classical biocontrol of *A. planipennis* may vary in different regions of North America, where the host phenology in adult emergence and oviposition may differ. Our study indicates that *O. primorskyensis* and *O. agrili* may be

complementary to each other in biocontrol of *A. planipennis* in regions where both species can be successfully established.

Keywords: Invasive, wood boring insects, risk-spreading, bet-hedging, *Agrilus planipennis*, *Oobius primorskyensis*, *Oobius agrili*.

1. Introduction

Knowledge of the biology and life history of arthropod natural enemies is important to the development of biological control programs against agricultural and forest pests.

Information on the life history attributes related to the success of natural enemies' reproduction such as longevity, fecundity and diapause is often required for developing effective rearing protocols to produce large quantities of the natural enemies, which would be needed for determining their host specificity and conducting field releases and other biological studies (Singh, 1982; Nordlund and Greenberg, 1994). In addition, these life history attributes can profoundly impact their establishment and prevalence in the new ecosystem where they are introduced for biological control of targeted pests (Kimberling, 2004).

The emerald ash borer, *Agrilus plannipennis* Fairmaire (Coleoptera: Buprestidae) was accidentally introduced from Northeast Asia to the United States and Canada probably in the 1990s, and has since become a serious invasive pest that threatens to decimate all species of North American ash (*Fraxinus* spp.) trees (Haack et al. 2002; Bauer et al., 2015b; Herms and McCullough 2014). As of April 2016, this invasive pest has been detected in 27 states in the U.S. and two provinces in Canada (EAB Information 2016). The encyrtid parasitoid *Oobius primorskyensis* Yao and Duan (Hymenoptera: Encyrtidae), is a recently described egg parasitoid of *A. plannipennis* in the Russian Far East (Yao et al., 2016). This new species and its congener *Oobius agrili* Zhang and Huang from northeast China constitute a cryptic species complex of *A. planipennis* egg parasitoids in northeast Asia (Zhang et al. 2005; Triapitsin et al., 2015; Yao et al., 2016). These egg parasitoids have the potential for effective biocontrol of *A. planipennis* because they kill their hosts prior to the larval damage to ash trees.

While *O. primorskyensis* is currently being evaluated in quarantine for potential introduction against *A. planipennis* in the U.S., *O. agrili* was introduced in 2007 to the U.S. for classical biological control of *A. planipennis* and has since been released in numerous natural ash stands infested with *A. planipennis* (USDA APHIS, 2007; Bauer et al., 2015a, b). Recent field studies in Michigan showed that *O. agrili* has established self-sustaining populations following field releases between 2007 and 2010 (Abell et al., 2014). However, successful establishment of *O. agrili* in many other release sites (e.g., in Maryland and New York) has yet to be determined and requires further evaluation as well as an improved sampling method (Duan et al., 2011; 2012, Jennings et al., 2014). Even in areas with established populations, such as Michigan, parasitism rates (<30%) by *O. agrili* are still not comparable to those (up to 67%) in its native range of northeast China (Liu et al., 2007; Abell et al., 2014; Wang et al., 2015).

Both *O. primorskyensis* and *O. agrili* are solitary egg parasitoids of *A. planipennis* and reproduce parthenogenetically. While males of *O. agrili* were reported in northeast China (Zhang et al., 2005; Liu et al., 2007), only females of both *O. primorskyensis* and *O. agrili* have been observed in laboratory colonies (Duan et al., 2014; Hoban et al., 2016) and no males of *O. primorskyensis* have been collected in the Russian Far East (Yao et al., 2016; KML and JJD unpublished data). Based on limited field observations in northeast China (Liu et al. 2007; Wang et al. 2010), the life cycle of *O. agrili* and its natural host *A. planipennis* is briefly described in Table 1. In their native range, both parasitoid species appear to aestivate and overwinter as diapausing mature larvae in parasitized *A. planipennis* eggs (Liu et al, 2007; JJD unpublished data). After overwintering, the diapaused parasitoid larvae resume development to pupae in the spring (April) and then to adults by late spring (May). Adult parasitoids of both species may

occur in late spring (May) through July to synchronize with their hosts' oviposition activity (Table 1).

Depending on the climatic condition and geographic distribution, however, *A. planipennis* may have both univoltine and semi-voltine life cycles in its native range (Wei et al. 2007; Wang et al. 2010). Currently, it is not known how the *A. planipennis* voltinism will affect the life cycle of the egg parasitoids. It is conceivable that *O. primorskyensis* and *O. agrili* can effectively attack both univoltine and semi-voltine host eggs, which only occur during the growing season from May to July. The univoltine and semi-voltine life cycles in *A. planipennis* mainly differ in the larval overwintering time, and not much in adult emergence and oviposition time (Table 1; also see reviews in Bauer et al. 2014).

Currently, there is no information available on the voltinism of *O. primorskyensis* in its native range, whereas *O. agrili* was anecdotally observed as having bivoltine and, in some cases, multivoltine generation cycles in northeast China (Bauer and Liu, 2006; Liu et al., 2007). In addition, there is little published information on the reproductive biology of this cryptic species complex except for two recent studies that have examined the impact of temperature and photoperiod on the reproduction and diapause of *O. agrili* (Duan et al., 2014; review in Bauer et al., 2015a; Hoban et al., 2016). This lack of information on their reproductive biology hinders the development of effective mass-production and field release strategies for these egg parasitoids against the invasive pest *A. planipennis* in North America.

In the present study, we quantify and compare the survivorship, fecundity and progeny diapause patterns of diapaused and non-diapaused parasitoids of both *O. primorskyensis* and *O. agrili*. Given the limited knowledge on the reproductive biology and diapause of this cryptic species complex, the present study provides useful information for the development of effective

rearing protocols and release strategies for both *O. primorskyensis* and *O. agrili* and thus may contribute to the development of effective biological control programs against *A. planipennis* in North America.

2. Materials and Methods

2.1. Parasitoids

Adults of *O. primorskyensis* used in the study were the F₂₋₃ progeny of a founder colony collected in 2010 in the Russian Far East region near Vladivostok, whereas adult *O. agrili* were the F₂₋₅ progeny of a founder colony collected from northeast China (Liaoning Provinces) in 2010 (Duan et al., 2014; Yao et al. 2016). Only naïve (never before exposed to host eggs), newly emerged (within 3 days old) adults of both species were used in this study. Two diapause types of both species, diapaused and non-diapaused groups, were investigated in this study.

Adult parasitoids of the diapaused group in each species originated from diapaused larvae that had been chilled at 1.7°C for 3–6 months prior to the start of the experiment. This group of adult parasitoids would be equivalent to the overwintered generation in nature, and is thereafter termed as “diapaused” parasitoids. Adult parasitoids of the non-diapaused group in each species originated from the non-diapaused larvae, which were reared at the normal rearing condition [25°C, 65% RH, 16:8 (L:D) h.] for one generation from diapaused parasitoids that had originally experienced 3–6 month storage at 1.7°C. This population of adult parasitoids is hereafter termed “non-diapaused” parasitoids, and would be comparable to the immediate (“daughter”) progeny produced by the diapaused parasitoids in late spring or early summer in nature, where they would not have experienced winter (cold temperature). Both diapaused or non-diapaused parasitoid

larvae (inside parasitized host eggs) were incubated in environmental chambers (Percival Scientific, Perry, Iowa, USA) at the same rearing condition described above for production of adult parasitoids used in the study.

2.2. Host Eggs

Host eggs (2–3 days old) used in this study were laid on coffee filter papers by gravid *A. planipennis* females reared with tropical ash [*Fraxinus uhdei* (Wenzig) Lingelsh] leaves freshly cut from greenhouse-grown trees according to procedures described in Duan et al. (2013, 2014). Briefly, adult *A. planipennis* beetles emerging from field-collected green ash (*F. pennsylvanica* Marsh) logs were provided with fresh tropical ash leaves every 2–3 days and held in 1-liter ventilated, clear plastic cups at 25°C, 65% RH, 16:8 (L:D) h. A mesh nylon screen (1000 micron opening, CS Component Supply, Fort Meade, FL) was placed over the cup opening followed by an un-bleached coffee filter paper (HomeLife, Eden Prairie, MN) secured by a rubber band, creating a facade mimicking bark cracks to stimulate gravid females to oviposit their eggs onto the coffee paper. Eggs laid on the coffee filter paper by gravid *A. planipennis* beetles were collected every 2–3 days and cut into 0.5–1 cm wide strips for exposure to test parasitoids.

2.3. Observation of Adult Longevity, Fecundity, and Diapause Pattern of the parasitoids.

Upon emergence from the parasitized host eggs, single females (within 3 days old) of both diapaused and non-diapaused *O. primorskyensis* and *O. agrili* were placed in clear 12 ml screw-cap vials (one parasitoid per vial; each vial 12 cm long x 1 cm in diameter, Tri-State Plastics, Latonia, KY, USA). Four or five thin streaks of non-diluted clover honey were applied to the inside of the vial wall with an insect pin as a food and water source for parasitoids. Three

times a week, each female parasitoid inside the test vial was provided with abundant host eggs freshly laid on two to three strips of coffee filter paper: 20 eggs per parasitoid each time (every 2 – 3 days) for the first two weeks (60 eggs per week), 15 eggs each time for the third and fourth weeks (45 eggs per week) and 10 eggs each time thereafter until the parasitoid died (30 eggs per week). Prior to the study, our observations indicate that both *O. primorskyensis* and *O. agrili* attack less than six host eggs per day even at the peak of oviposition (first week after emergence) and their host attack rates decrease sharply as the parasitoid age passes two weeks (Duan et al. 2014). Thus, the number of the host eggs provided to each test parasitoid should not have limited the parasitoid's oviposition or host attack rates (see Supplementary Data). Each time new host eggs were provided to the test parasitoid, parasitoid mortality was noted and the surviving parasitoid was transferred into a new, freshly honeyed vial.

Exposed *A. planipennis* from each exposure period (48 to 72 hr, three times a week) were then moved to clean vials and incubated in the growth chamber at the normal rearing condition described previously to allow parasitoid progeny development to adults, which typically takes approximately three to four weeks (Duan et al. 2014). Eight weeks after each exposure period, parasitized eggs (normally darkened in 5 - 7 days after parasitization) with or without adult exit holes were observed and counted under a microscope. Using sharp forceps, all parasitized host eggs were dissected and scored as emerged (presence of adults or adult exit holes), diapaused (no exit hole; mature, live larvae inside parasitized egg), or dead (no exit hole; dead cadavers within parasitized egg). Diapaused parasitoid larvae are whitish-gray, plump mature (last instar) larvae within the host egg. Dead parasitoid cadavers appear as either brittle, dry adults, or dessicated brown or black larvae. A total of 15 to 16 parental adult parasitoids (or replicates) of each species and diapaused (vs non-diapaused) type were used for quantifying and comparing their

longevity, progeny production (or fecundity) and diapause rates. Throughout the study, less than 3% of the parasitoid progeny died inside the host eggs as larvae or adults across different species and diapause types and were excluded from calculations of progeny production and diapause rates.

2.4. Data Analysis.

The Kaplan-Meier survival platform was used to estimate the median survival time or longevity (in weeks) and associated lower and upper limits of the 95% confidence interval (CI) for parental adults of each parasitoid species and diapause type. Log-rank χ^2 tests based on the survival analysis platform were used to detect statistical differences in survivorship (or longevity) of parental adult parasitoids between the two different species or different diapause types within each species. Each individual log-rank χ^2 test was conducted with the type I error rate corrected with the Bonferroni method. Lifetime-realized fecundity was estimated using the total number of viable or live progeny ($\pm$ SE) produced by each female parasitoid over her lifetime, and weekly oviposition rates were estimated using the mean numbers of progeny ($\pm$ SE) produced per week by each female parasitoid. Two-way analysis of variance (ANOVA) and multiple mean comparison procedures based on the student's t tests were used to detect statistical significance in differences of lifetime-realized fecundity between the two different species as well as between the two diapause types within each species.

Diapause rates of the progeny for each species and parental diapause type were calculated on a weekly basis as well as over the lifetime of each parental (adult) parasitoid. The weekly progeny diapause rate was calculated as the proportion of the diapaused larvae relative to the number of the progeny produced weekly by the parental parasitoid, whereas the lifetime diapause

rate of the progeny was calculated as the proportion of the diapaused larvae relative to the total number of the progeny produced by the parental parasitoid over her lifetime. Nominal logistic regression analysis was used to examine the relationship between the weekly diapause rate and the sampling time or parental age for each diapause type within each species. Likelihood ratio χ^2 tests based on nominal logistic regression analyses were used to detect statistical differences in the lifetime progeny diapause rate between species and diapauses type of the parental parasitoids. All statistical analyses and calculations were carried out with JMP Version 12.01 (SAS Institute 2015).

3. Results

3.1. Longevity of adult parasitoids.

The survivorship of adult (parental) parasitoids for both diapaused and non-diapaused *O. primorskyensis* and *O. agrili* appeared to be similar to the classical type II survivorship curve, with the probability of survivorship gradually decreasing as the adult age increases (Figure 1A). However, the median survival time for the diapaused *O. primorskyensis* (8 weeks) was significantly longer than for the diapaused *O. agrili* (5.5 weeks; Log Rank $\chi^2 = 12.75$, $df = 1$, $P = 0.0004$), but not significantly different from the non-diapaused *O. primorskyensis* (6 weeks) or the non-diapaused *O. agrili* (7 weeks) (Fig. 1B). There were no significant differences in the median survival time among non-diapaused *O. primorskyensis*, diapaused and non-diapaused *O. agrili*.

3.2. Weekly oviposition rate and lifetime realized fecundity of adult parasitoids.

Throughout the study, less than 50% of host eggs were attacked by the test parasitoids of both species, regardless of their diapause type and sampling time, indicating the number of host eggs provided to the test parasitoids was sufficiently abundant and would not have limited their progeny production throughout the study period (see Supplementary Data). Regardless of the diapause type, adult (parental) parasitoids of both species produced the most progeny in the first week of their lifetime, averaging 19 to 24 progeny produced per adult parasitoid (Fig. 2B). After the first week, the number of progeny produced weekly per surviving adult decreased gradually for both diapaused and non-diapaused *O. primorskyensis* and *O. agrili* (Fig. 2B). ANOVA detected significant interactive effects of parasitoid species and diapause type on the mean number of the progeny produced over the lifetime of the test parasitoids ($F_{1,57} = 14.97$, $P = 0.0003$). The mean ($\pm$ SE) number of the progeny produced over the lifetime of the test parasitoid was significantly higher for diapaused *O. primorskyensis* (77.3 ± 5.2) and non-diapaused *O. agrili* (70.6 ± 7.1) than for non-diapaused *O. primorskyensis* (48.8 ± 4.6) and diapaused *O. agrili* (52.1 ± 6.8) (Fig. 2B). There was no significant difference between diapaused *O. primorskyensis* and non-diapaused *O. agrili* ($P > 0.05$), nor was there any significant difference between non-diapaused *O. primorskyensis* and diapaused *O. agrili* ($P > 0.05$).

3.3. Diapause rates of the progeny produced weekly and over lifetime by parental parasitoids.

The pattern in the weekly rates of diapaused progeny was clearly different between diapaused and non-diapaused *O. primorskyensis*, though to a much lesser degree between diapaused and non-diapaused *O. agrili* (Fig. 3A). The percentage of diapaused progeny produced by the diapaused *O. primorskyensis* increased from <10% at the first week to nearly

100% at week five and after. The parental parasitoid age or sampling time was a significant predictor of the weekly diapause rate of the progeny produced by parental parasitoids in the diapaused *O. primorskyensis* (logistic regression model: likelihood ratio $\chi^2 = 834.26$, df = 4, $P < 0.0001$). In contrast, the non-diapaused *O. primorskyensis* consistently produced nearly 100% diapaused progeny throughout the sampling time. The weekly diapause rate of the progeny produced by diapaused *O. agrili* varied from 5 to 17%; the non-diapaused *O. agrili* population produced 20–50% diapaused progeny over different sampling times (Fig. 3A). Parental parasitoid age did not significantly affect the rate of weekly diapause rates of progeny in non-diapaused *O. primorskyensis* (likelihood ratio $\chi^2 = 8.62$, df = 4, $P = 0.0714$) as well as in diapaused *O. agrili* (likelihood ratio $\chi^2 = 9.72$, df = 4, $P = 0.0500$). However, there were significant differences in weekly diapause rates of the progeny produced by non-diapaused *O. agrili* (likelihood ratio $\chi^2 = 43.82$, df = 4, $P < 0.0001$).

The mean proportion ($\pm$ SE) of diapaused progeny produced over the lifetime of parental parasitoids was the highest ($99.7 \pm 0.3\%$) for the non-diapaused *O. primorskyensis* population and lowest ($7.5 \pm 2.3\%$) for the diapaused *O. agrili* population (likelihood ratio $\chi^2 = 1618.73$, df = 1, $P < 0.0001$; Figure 3B). There were no significant differences in the mean proportion ($\pm$ SE) of diapaused progeny produced over the lifetime of parental parasitoids between diapaused *O. primorskyensis* ($37.5 \pm 2.9\%$) and non-diapaused *O. agrili* ($33.9 \pm 3.7\%$) populations (likelihood ratio $\chi^2 = 1.11$, df = 1, $P = 0.2923$). Both diapaused *O. primorskyensis* and *O. agrili* populations produced significantly lower proportions of diapaused progeny than their respective non-diapaused populations (likelihood ratio $\chi^2 = 885.28$, df = 1, $P < 0.0001$ for *O. primorskyensis*; likelihood ratio $\chi^2 = 224.85$, df = 1, $P < 0.0001$ for *O. agrili*).

4. Discussion

Findings from our study demonstrate that there are distinctive differences in the reproductive biology and diapause pattern between the two parasitoid species *O. primorskyensis* and *O. agrili* as well as between diapause types (diapaused vs. non-diapaused parasitoids) within each species. Adults of the diapaused *O. primorskyensis* had a significantly longer longevity and produced significantly more progeny over their lifetime than those of diapaused *O. agrili*.

Although there was no significant difference in their longevity, adults of the non-diapaused *O. primorskyensis* produced significantly less progeny than those of non-diapaused *O. agrili*.

Adults of the diapaused *O. primorskyensis* population lived longer and produced a significantly higher number of progeny than those of non-diapaused *O. primorskyensis*; however, this trend in both longevity and progeny production rate was reversed between diapaused and non-diapaused *O. agrili* populations. While a majority (>64%) of progeny produced by adults of the diapaused *O. primorskyensis* as well as by those of both diapaused and non-diapaused *O. agrili* continued development to adults, nearly 100% of the progeny produced by adults of the non-diapaused *O. primorskyensis* diapaused. These differences in their reproductive biology and diapause pattern between *O. primorskyensis* and *O. agrili* as well as between the diapaused and non-diapaused types of each species may have profound impacts on the successful development of effective classical biocontrol programs against *A. planipennis* in North America.

Regardless of their diapause status, parental adults of both *O. primorskyensis* and *O. agrili* produced the most progeny in the first week after adult emergence followed by a gradual reduction in progeny production. This strongly indicates that the realized fecundity or oviposition rate for both *O. primorskyensis* and *O. agrili* largely depends on their longevity. In nature, diapaused *O. primorskyensis* and *O. agrili* used in this study would be equivalent to the

overwintered generation of the parasitoids, which emerge and reproduce from late spring (May) to early summer (July) when the host *A. planipennis* eggs are available (Table 1). Because adults of diapaused *O. primorskyensis* live significantly longer and produce significantly more progeny than those of diapaused *O. agrili*, adults of the overwintered *O. primorskyensis* would be expected to attack more host *A. planipennis* eggs in the growing season than overwintered *O. agrili*. However, the non-diapaused population from the subsequent generation of *O. primorskyensis* lived for a shorter period and reproduced significantly less progeny than non-diapaused *O. agrili* population. Thus, the shorter longevity and lower fecundity of overwintered *O. agrili* generations in the growing season would be compensated later by the continuous reproduction of non-diapaused parasitoids for at least one more generation through early summer (July) when viable *A. planipennis* eggs are still available.

Diapause has been a common strategy for many insects to overcome unfavorable environments such as weather changes and seasonal food shortages (Tauber et al., 1986; Hopper, 1999; Saunders, 2000; Soula and Menu, 2003; Fordyce et al. 2006). Our study shows that both *O. primorskyensis* and *O. agrili* produce disproportions of diapausing and non-diapausing individuals under normal rearing condition (warm and long-day photoperiod), depending upon the diapause status and age of the parental parasitoids. Over their lifetime, adults of both diapaused *O. primorskyensis* and *O. agrili* produced a majority of non-diapaused progeny, which continued development to adults and reproduction under the normal rearing condition. However, diapaused *O. primorskyensis* gradually produced higher proportions of the diapaused progeny as the parental adults become older, with the non-diapaused parasitoids producing nearly 100% of diapaused progeny even under the favorable (long-day photoperiod) condition. In contrast, parental parasitoid age did not have a significant effect on the proportion of diapaused *O. agrili*

progeny after their parents had diapaused, and non-diapaused parasitoids continue to produce a majority of non-diapaused progeny under the normal rearing condition. These findings strongly suggest that *O. primorskyensis* and *O. agrili* may have evolved different diapause mechanisms for aestivation and overwintering. The difference in their diapause mechanisms may also result in different risk-spreading or bet-hedging strategies against the unpredictable, temporal variation in their host egg abundance in a growing season (May to July), and thus has implications for their effectiveness in biological control of *A. planipennis*. However, we point out that our study was conducted in the laboratory under the optimal (warm and long-day photoperiod) rearing condition. Further field studies would be needed to determine if the differences in diapause patterns between *O. primorskyensis* and *O. agrili* hold true in nature, where the seasonal temperature and photoperiod varies throughout the seasons.

To date, *O. primorskyensis* has been collected from parasitized *A. planipennis* eggs only in the Russian Far East region near Vladivostok and Ussuriysk (Yao et al., 2016). In contrast, *O. agrili* has been collected from parasitized *A. planipennis* eggs in north to northeast China including Heilongjiang, Liaoning and Jilin Provinces and Beijing city (Liu et al. 2007; Wang et al. 2015). Currently it is not known what ecological factors have shaped the evolution of different diapause patterns for risk-spreading or bet-hedging strategies in *O. primorskyensis* and *O. agrili*. Considering the small difference (<30 min) in daytime length between the Russian Far East and northeast China during the growing season (USDC NOAA 2016), we suspect that the co-evolutionary history with the host population in their native ranges favors the parasitoid population that best synchronizes with *A. planipennis* oviposition during the growing season. *A. planipennis* may have a mixed semi-voltine and univoltine in both northeast China and the Russian Far East (Table 1; Wei et al. 2007; Duan et al. 2012). It is possible that adult emergence

and oviposition of *A. planipennis* may be more synchronized and the peak period of viable host eggs may thus be shorter in the colder area such as the Russian Far East than in north or northeast China such as Liaoning province and Beijing city. Thus, the more synchronized adult emergence and shorter oviposition period of *A. planipennis* in the Russian Far East would favor the diapause pattern in *O. primorskyensis*, which would allow the overwintered (or diapaused) generation to reproduce one generation of predominately non-diapausing progeny before the host oviposition activity ceased by the end of July. On the other hand, the less synchronized adult emergence and longer oviposition period of *A. planipennis* in north or northeast China would favor the diapause pattern in *O. agrili*, which would allow the overwintered generation to continue reproducing more (possibly two) generations of non-diapausing progeny that can be induced to diapause by short-day photoperiod that signals the change of season to late summer or the fall when host eggs become scarce (Hoban et al. 2016).

Current classical biological control programs against *A. planipennis* need to consider the distinctive patterns of the diapause and associated fitness consequences in these two parasitoid species. This is particularly important when developing mass-rearing protocols and release strategies for both *O. primorskyensis* and *O. agrili* in North America. For example, continuous mass-production of *O. primorskyensis* for biocontrol releases would be challenging because the diapaused parasitoid could only reproduce one generation of non-diapausing progeny under the normal rearing condition (warm and long-day photoperiod). In contrast, *O. agrili* could be continuously produced under the same normal rearing condition. However, optimal chill temperature and times for both species to maintain and terminate the larval diapause are currently unknown and need to be determined for effective mass production of these parasitoids for field releases against *A. planipennis*. In addition, further investigations into the risk-

spreading strategies of these parasitoids in both their native ranges and North America, as well as the impact of climatic change or temperature variability on their reproductive biology, would be worthwhile for development of an effective biological control program against *A. planipennis*.

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Figure Captions:

Fig 1. Survivorship of parental adults of diapaused and non-diapaused *Oobius primorskyensis* and *O. agrili* reared on *Agrilus planipennis* eggs (A) and estimates of median survival time and 95% confidence intervals (B). Bars followed with the same letter in Fig. 1B indicate no significant differences (Log-rank χ^2 tests with type I error rate corrected by Bonferroni methods).

Fig. 2. Weekly realized fecundity or oviposition rate (A) and lifetime realized fecundity (B) of parental adults of diapaused and non-diapaused *Oobius primorskyensis* and *O. agrili* reared on *Agrilus planipennis* eggs. Bars followed with the same letter in Fig. 2B indicate no significant differences (ANOVA and LSD student's t tests).

Fig. 3. Diapause rates of the progeny produced by adults of diapaused and non-diapaused *Oobius primorskyensis* and *O. agrili* on a weekly basis (A) and over lifetime or generation (B). Bars followed with the same letter in Fig. 3B indicate no significant differences (likelihood ratio χ^2 tests with type I error rate corrected by Bonferroni method).

Table 1. Life cycle of the emerald ash borer (EAB) egg parasitoids (*Oobius* spp.) in relation to its host *Agrilus planipennis* life cycle and seasonal time. The synthesis is based on field data collected in Northeast China for *Oobius agrili* (Liu et al. 2007) and its host *A. planipennis* (Wei et al. 2004; Wang et al. 2010).

Month of the Year	Egg parasitoid: <i>Oobius agrili</i> *	Host EAB: <i>Agrilus planipennis</i>
May - July	Adults/Eggs: emerging/laying eggs inside host eggs Larvae: Feeding on tissue inside host eggs - Non-diapausing larvae may complete two or more generations prior to producing diapausing larvae; - Diapausing larvae stop feeding and development for aestivation and overwintering.	Adults/Eggs: emerging/mating/ovipositing on host trees (bark crevices) Larvae: Feeding on cambium tissue under the bark; - Mature larvae stop feeding, excavate a chamber, and form a J-shape for aestivation and overwintering. - Non-mature larvae continue to feed
August - November	Diapaused larvae: Aestivating	Mature J-larvae: diapausing inside the chamber for aestivation and overwintering Non-mature larvae: continue to feed and develop (resulting in semi-voltine generation)
December - February	Diapaused larvae: Overwintering	Mature J-larvae: overwintering Non-mature larvae: overwintering (semi-voltine generation)
March - April	Pupae: Diapaused larvae resume development to pupae	Pupae: Overwintering mature J-larvae resume development to pupae Non-mature larvae: continue to feed and develop through next season to result in semi-voltine life cycle.

*Currently, there has been no published information on the life history of *O. primorskyensis* in the Russian Far East. Based on anecdotal field collection of parasitized eggs and a laboratory study (Yao et al. 2016), the life cycle of *O. primorskyensis* appears to be similar to that of *O. agrili* except that a majority of overwintering *O. primorskyensis* may only be able to complete one generation during May through July because nearly 100% of the progeny produced by the first non-diapaused generation will diapause for aestivation and overwintering.

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