

cold climate zones may be suboptimal due to low temperatures, as may mid-summer releases in hot climate zones due to high temperatures.

1. Introduction

Classical biological control is an ecologically-based tool that can be used for the long-term management of invasive pests. The success of classical biological control programs hinges on the establishment of specialized natural enemies introduced from the native range of the invasive pest. To increase the probability that these natural enemies establish after introduction to new regions, it is important to have an in-depth understanding of how climatic factors influence the development and reproductive biology of the agents (Hoelmer and Kirk, 2005). Parasitic wasps, or parasitoids, are often used as biocontrol agents of invasive insect pests. Because parasitoids like other insects are poikilothermic, ambient temperatures directly affect their developmental rates, reproduction, mortality, body size, and/or behavior (e.g. Howe, 1967; Atkinson, 1994; Wang et al., 1999; Golizadeh et al., 2009; Mawela et al., 2013; Rodrigues et al., 2013; Yu et al., 2013). Knowledge of temperatures that favor the development and rapid population growth of insect natural enemies is important not only for optimizing biocontrol agent release strategies (Pilkington and Hoddle, 2006), but also improving the efficiency of natural enemy mass-rearing programs (e.g., Qiu et al., 2012; Favero et al., 2015). Moreover, the likelihood of natural enemy establishment in the newly introduced region may reasonably be predicted if it is known how temperature affects key parameters of its development, reproduction, biology, and population growth (Pilkington et al., 2014).

Tetrastichus planipennis Yang (Hymenoptera: Eulophidae) is a specialized larval endoparasitoid of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), and plays an important role in regulating the pest population in its native range of northeast Asia (Liu et al., 2003, 2007; Wang et al., 2015). In 2007, five years after EAB was first detected in the United States (USA), this parasitoid, along with the egg parasitoid *Oobius agrili* Zhang & Yang and the larval ectoparasitoid *Spathius agrili* Yang, were first introduced from northeast China to the USA as EAB biocontrol agents (Federal Register, 2007). The initial releases of these three EAB parasitoid species (*T. planipennis*, *O. agrili*, *S. agrili*) were made in Michigan, the epicenter of EAB invasion, and only a few hundred of each species were released due to limitations in insect rearing. After USDA initiated the EAB Biocontrol Program and a mass-rearing facility became operational in 2010, larger numbers of parasitoids became available for release at more sites (Bauer et al., 2014, 2015). To date, 27 of 33 United States and two of the three Canadian provinces with known EAB infestations have released one or more of the EAB biocontrol agents (Duan et al., 2018). Although these three biocontrol agents were recovered one year following their releases at some early Michigan study sites, only *T. planipennis* and *O. agrili* are consistently recovered and expanding their range in EAB populations (Bauer et al., 2015; Duan et al., 2018).

While the role of the egg parasitoid *O. agrili* in suppressing EAB population growth remains to be determined, *T. planipennis* appears to play a significant role in suppressing EAB population growth in young ash trees and saplings in long-term study sites in Michigan (Abell et al., 2014; Duan et al., 2013a, 2015, 2017). However, parasitism rates by *T. planipennis* varied among different geographic regions in the USA. For example, the average parasitism rates of EAB larvae by *T. planipennis* (sampled in the fall and early spring) was approximately 11% in Maryland (Jennings et al., 2016), 10.5% in Kentucky (Davidson & Rieske, 2016), and approximately 30% in Michigan (Duan et al., 2013a, 2015, 2017). In northeast China, where *T. planipennis* was originally collected, the parasitism rate of EAB larvae sampled in late summer and fall averaged ~40% (Liu et al., 2007). It is not known if key climatic

factors such as temperature may contribute to geographic, seasonal, and year-to-year variations in parasitism rates of EAB larvae by *T. planipennis*.

A previous study determined some critical life-history parameters relating to immature larval development and adult reproduction of *T. planipennis* at constant 25 °C (Duan et al., 2011). More recently, a laboratory flight-mill study showed that ambient temperatures strongly affected the flight distance and speed, and post-flight mortality of adult *T. planipennis* (Fahrner et al., 2015). To date, however, no studies have reported on temperature ranges that optimize parasitism rates, development, reproduction, and population growth of *T. planipennis*.

In the present study, we determined the effects of different temperature ranges (15 °C–35 °C), which might occur during the growing season (May to September) in the USA, on *T. planipennis*'s host parasitism rates, development and survivorship of its immature stages, as well as adult longevity and fecundity. Based on these laboratory results and location-specific climatic data, we then estimated the potential for *T. planipennis* population growth in representative areas from climate zones 3 to 7 in the USA (USDOE, 2013), where EAB has invaded or has the potential to invade, and biocontrol has been or could be implemented (MapBiocontrol, 2017; USDA APHIS, 2018). This information may improve the efficiency of mass-rearing and field release strategies for this important EAB biocontrol agent.

2. Materials and methods

2.1. Parasitoids

Tetrastichus planipennis used in this study were F23 - F25 progeny of a laboratory colony that originated from parasitized EAB larvae collected in 2008 in Fengcheng, Liaoning province, China (Wang et al., 2015). This colony was maintained on EAB larvae infesting tropical ash [*Fraxinus uhdei* (Wenzig) Lingels] stems at the USDA ARS Beneficial Insect Introduction Research Unit, Newark, DE. Before being used in various experiments, newly emerging (< 3 d old) adult parasitoids were housed in ventilated acrylic cylinders (20 cm height × 12 cm diameter) using a 3:1 female:male (F:M) sex ratio and maintained in environmental chambers (AR-66L2, Percival Scientific, Perry, IA) at 25 ± 1.5 °C, 65 ± 10% RH, and 16:8 h (L:D) photoperiod. From field collections and laboratory rearing, 2.5:1 F:M is the typical sex ratio for *T. planipennis*, and each male mates with multiple females (Yang et al., 2006; Duan et al., 2011; Duan and Oppel, 2012). A water source was provided inside each acrylic cylinder via a 37-ml clear plastic vial (US Plastics, Lima, OH) fitted with a 10-cm braided cotton dental wick (Richmond Dental, Charlotte, NC), and clover honey was streaked on the ventilation screens of rearing containers as a food source.

2.2. Host larvae

Host EAB larvae used in this study were 3rd–4th instars reared in tropical ash stems (14 cm length × 1–3 cm diameter) (Duan et al., 2013b). Briefly, EAB eggs laid on unbleached coffee filter paper (HOMELIFE, Eden Prairie, MN) were placed onto ash stems in single or pair groups against the surface of the wood and held on with strips of Parafilm® (Bemis Co., Inc., Neenah, WI). After infestation with EAB eggs, the ash stems were placed in water-soaked floral foam bricks (OASIS®, Smithers-Oasis Co., Kent, OH) inside plastic containers (58.4 × 41.3 × 31.4 cm³, Sterilite Corp., Townsend, MA) and incubated in an environmental chamber at 27 ± 1.5 °C, 65 ± 10% RH, and 16:8h (L:D) photoperiod for 4.5–5 weeks to produce the suitable

(3–4th) instars of EAB larvae used in the experiments. To prevent the growth of mold in the plastic containers, approximately 100 ml of 1% methyl paraben solution was applied to the surface of water-soaked floral foam in each container after placement of the infested ash stems.

2.3. Effect of temperature on completion of immature stage development

The development time of *T. planipennisi* at the different temperature treatments was estimated from egg to adult eclosion because it is an endoparasitoid, and its immature stages cannot be observed without dissection of the host body. Each tropical ash stem containing a single host larva was exposed to ten gravid female parasitoid adults (~2-weeks old) under the normal rearing condition ($25 \pm 1.5^\circ\text{C}$, $65 \pm 10\%$ RH, and 16:8 h L:D photoperiod) above for 24 h. These conditions favor the induction of nearly 100% EAB larval parasitism by *T. planipennisi* (Duan et al., 2011, Duan and Oppel, 2012). A polystyrene crisper box ($18.5 \times 13 \times 10\text{ cm}^3$, Tri-State Plastics, Dixon, KY) was used as the exposure arena. After a 24-h exposure to gravid female *T. planipennisi*, ash stems were placed in emergence enclosures (clear plastic 1-L Solo cups with mesh-screened lids; Solo Cup Co., Lake Forest, IL) and randomly assigned to an environmental chamber set at one of five temperature treatments (15, 20, 25, 30, 35°C), 45–75% RH, and 16:8 h L:D photoperiod. Temperature inside each environmental chamber was automatically controlled within the $\pm 1.0^\circ\text{C}$ range of the targeted temperature. Treatments in the five environmental chambers were initiated at the same time. A total of 10 EAB larvae were exposed at each temperature treatment. Emerging *T. planipennisi* progeny of each sex were monitored daily and tallied for a maximum 150 d. However, we should point out that even with use of a high parasitoid-to-host (10:1) ratio that favored nearly 100% parasitism, not all of the parasitized host larva produced viable adult parasitoid progeny. This resulted in the unequal number of sample (host larval) units (or blocks) for each temperature treatment. In addition, the parasitoid brood size emerging from the parasitized host larvae across different temperature treatment varied from 5 to 112 individual adults per parasitized host larva. For data analysis, we first calculated the mean adult eclosion time (day) for both males and females for each replicate (i.e., a parasitized host larva or a brood of parasitoid adults), and then analyzed the effect of treatment temperature on the mean adult eclosion times using the analysis of variance (ANOVA).

To determine the theoretical lower development threshold (T_{lo}), we first calculated the development rates for both sexes by taking the reciprocal of mean development day (1/D) for each replicate (or parasitized larva) that produced adult parasitoids. We then used the non-linear second-order regression model described by Damos and Savopoulou-Soultani (2012) to model the relationship between temperature and development rate for the completion of parasitoid immature stages. Preliminary data analysis indicated that there were no significant differences in mean development time between males and females (t -test, $P = 0.4780$); thus sex was not included as a predictor for the development rate in the final, nonlinear second-order model. By using the nonlinear second-order model, T_{lo} was then calculated by solving the second-order equation when development rate was equal to 0. Degree days required to complete development from egg to adult eclosion were calculated for each temperature treatment by multiplication of the reciprocal of the development rate (1/D) and the temperature above T_{lo} . All analyses were conducted using JMP Pro 12.1.0 (SAS Institute, 2015).

2.4. Effect of temperature on adult longevity, pre-oviposition period, parasitism rate, and fecundity

One pair of newly emerging (< 3 days old) female and male adult parasitoids (from our rearing colony) were placed inside each rearing enclosure (16-cm long \times 6-cm diameter, Polycarbonate tubing fitted with plastic caps, US Plastics Corp., Lima, OH), and randomly assigned

Table 1

Mean development time (days) of *T. planipennisi* immature stages (from egg to adult eclosion) in relation to temperature treatments.

Temperature ($^\circ\text{C}$) ^a	N ^b	Mean development time (d) \pm SE ^c		
		Female (n)	Male (n)	Both sexes
15	2	124.3 \pm 4.17a	138.9 \pm 4.57a	139.8 \pm 3.86a
20	9	39.3 \pm 1.39b	38.9 \pm 2.15b	39.5 \pm 1.82b
25	10	28.6 \pm 1.32c	28.4 \pm 2.05c	28.8 \pm 1.72c
30	4	26.1 \pm 2.08c	25.8 \pm 3.23c	26.3 \pm 2.73c
35	–	–	–	–
ANOVA		F _{3, 20} = 168.6, P < 0.0001	F _{3, 21} = 172.7, P < 0.0001	F _{3, 22} = 245.1, P < 0.0001

^a No data available at 35°C due to 100% egg mortality.

^b Number of replicates or blocks (i.e., parasitized host larvae).

^c Mean (\pm SE) values in each column with different letters are significantly different according to ANOVA and Least Squared Means Difference Student t -tests.

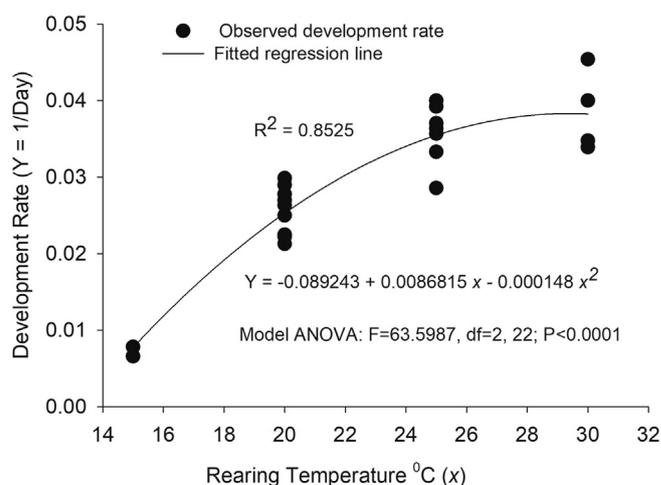


Fig. 1. The relationship of development rate (1/day) of *T. planipennisi* (from egg to adult eclosion) and rearing temperature (X) as described by the non-linear (quadratic) regression model.

to an environmental chamber set at one of five temperature treatments (15, 20, 25, 30, and 35°C). Each test pair of *T. planipennisi* was provided with two to three ash stems infested with two to three EAB larvae (3rd–4th instar), and clover honey was streaked onto the enclosure screening as a food source. Larval host stems were removed twice a week and replaced with new larval host stems, at which time adult parasitoid mortality was recorded. This twice-a-week supply of EAB larvae, provided to each pair of *T. planipennisi*, was expected to result in low parasitism and thus not limit parasitoid host utilization (Duan et al., 2011). A total of 15–18 pairs (or replicates) of adult parasitoids was tested at each temperature treatment. Parasitoid-exposed ash stems were placed in emergence containers (same design as the exposure arena) at $25 \pm 1^\circ\text{C}$ for ~6 weeks to monitor adult wasp emergence, and thereafter, the stems were dissected to determine EAB larval parasitism. Dead EAB larvae were reconstituted in water for 24 h and then dissected to look for unsuccessful parasitism (based on presence of parasitoid eggs, larval and adult remains).

The survival functions of adult parasitoid longevity in weeks at different temperatures were fitted with the Kaplan-Meier (or product-limit survival fit) procedure and compared with log-rank Chi-square tests among different treatments. The means (\pm SE) of parasitoid longevity in weeks estimated from the fitted survival functions were presented. The adult parasitoid pre-oviposition period (in weeks) and realized life-time fecundity (number of adult progeny produced) at each temperature was analyzed with ANOVA, while the life-time parasitism

Table 2

Average female adult longevity (weeks), pre-oviposition period (days), lifetime parasitism rates, and lifetime fecundity (progeny produced per female) of *T. planipennis* adults exposed to emerald ash borer larvae at different temperature treatments.

Temperature (°C)	No. of Replicates	Female adult longevity (± SE) ^a	Pre-oviposition time (± SE) ^b	% Parasitism (± SE) ^c	Lifetime fecundity (± SE) ^b
15	15	23.8 ± 1.6 ^a	81.2 ± 5.7 ^a	1.4 ± 0.3 ^d	26.8 ± 8.8 ^b
20	15	7.8 ± 0.6 ^b	13.4 ± 5.0 ^b	13.0 ± 2.2 ^c	69.8 ± 7.7 ^a
25	15	6.6 ± 0.6 ^b	7.9 ± 4.7 ^b	18.9 ± 2.4 ^b	81.6 ± 7.2 ^a
30	15	4.1 ± 0.4 ^c	7.9 ± 4.7 ^b	28.2 ± 3.2 ^a	81.1 ± 7.2 ^a
35	15	1.8 ± 0.2 ^d	–	0.5 ± 0.5 ^d	0.0 ± 0.0 ^b

^a Mean (± SE) value in columns with different letters are significantly different according to comparisons of the product-limit survival fit functions by Log-Rank χ^2 tests ($\alpha = 0.05$).

^b Mean (± SE) value in columns with different letters are significantly different according to ANOVA and multiple mean comparison procedure (Student-t tests, $\alpha = 0.05$).

^c Mean (± SE) value in columns with different letters are significantly different according to Log-likelihood χ^2 tests ($\alpha = 0.05$).

rate by test parasitoids in the different temperature treatments were analyzed with the likelihood Chi-square tests based on the binomial logistic regression model (SAS Institute, 2015). The binomial logistic regression procedure modeled the relationship of the probability of EAB larval parasitism (dichotomy response) by the parasitoid with the temperature treatment (independent variable).

2.5. Calculation of demographic parameters

Parasitoid fertility tables were constructed for each temperature by using the weekly fecundity and longevity data from each of the females according to methods described in Watt et al. (2016). The fertility table consists of m_x as age-specific female fecundity (number of daughters born to females at age x) and l_x as the probability of surviving to age x . Net reproductive rate ($R_0 = \sum l_x m_x$) expresses the per generation growth rate of a population ($R_0 > 1$, population increasing; $R_0 < 1$, population declining; $R_0 = 1$, population at equilibrium). Intrinsic rate of increase (r_m) is the exponential growth potential for a population with a stable age distribution living under defined environmental conditions (Birch, 1948) and was calculated by iterating the value of r_m in the equation $1 = \sum l_x m_x e^{-r_m x}$ until the equation was solved. Finite rate of increase ($\lambda = e^{r_m}$) is the constant factor by which a population multiplies per generation. Population doubling time ($T_d = \ln(2)/r_m$) is the amount of time required for a population growing at a given r_m to double in size. The generation time (T_c), i.e., the average time separating births of one generation to births of the next, was estimated directly for each temperature by adding the immature development time (egg to adult eclosion) to the female pre-oviposition period. All demographic parameters (R_0 , r_m , λ , T_c , T_d) were calculated at each temperature treatment from the fertility table with the formula developed by Southwood and Henderson (2000).

2.6. Prediction of *T. planipennis* generations in the USA

Temperature data (average daily temperature) for 11 locations in the USA from 2014 to 2016 (Table 4) were obtained from the National Centers for Environmental Information, National Oceanic and Atmospheric Administration (NOAA NCEI, 2017). These stations are located in climate zones ranging from 3 (southern limit latitude approximately 29° N in Texas) to 7 (northern limit latitude approximately 49° N in Minnesota) (USDOE, 2013), and comprise regions with and without known EAB infestations and associated releases of *T. planipennis* (mapbiocontrol, 2017; USDA APHIS, 2018). Degree-days (DD) were calculated for each location using the basic equation (McMaster and Wilhelm, 1997): $\left(\frac{T_{max} + T_{min}}{2}\right) - T_{lo}$, where T_{max} = maximum daily temperature (°C), T_{min} = minimum daily temperature (°C), and T_{lo} = the theoretical lower development threshold for complete development as estimated by the nonlinear second-order regression model. When the daily mean temperature $\left(\frac{T_{max} + T_{min}}{2}\right)$ is less than the base (T_{lo}), it is set

equal to the base temperature (T_{lo}). To predict the number of *T. planipennis* generations at each location, the number of DDs accumulated $> T_{lo}$ was divided by the summation of the estimated DDs required from parasitoid egg to adult eclosion. However, we pointed out that the pre-oviposition period was not included in the DD calculation because the low temperature threshold for oviposition could not be determined in our study. However, under normal rearing temperature range (20–30 °C), *T. planipennis* begins to lay eggs within 7 days after adult emergence (Duan et al., 2011). Nevertheless, this might have resulted in a slight underestimate of DDs required for adult parasitoid development.

3. Results

3.1. Immature parasitoid development

Temperature significantly affected the total development time from parasitoid egg to adult eclosion (ANOVA $P < 0.0001$ for males, females and both sexes combined, Table 1). As temperature increased from 15 °C to 30 °C, the mean (± SE) development time for both sexes combined decreased from 139.8 (± 3.86) to 26.3 (± 2.73) d (Table 1). The relationship of the development rate ($1/D$ or Y) for both sexes with the temperature (X) was significantly described by the second-order regression model $Y = -0.089243 + 0.0086815X - 0.000148X^2$ (Fig. 1. $P < 0.0001$ for all parameter estimates). The lower threshold temperature (T_{lo}) was calculated to be approximately 13.29 °C for immature parasitoid development by solving the quadratic equation $0 = -0.089243 + 0.0086815X - 0.000148X^2$ from on the above regression model. No adult parasitoids emerged from the parasitoid-exposed hosts in ash stems at 35 °C, and post-trial dissections confirmed that although parasitoid eggs were present, none hatched in the parasitized larvae at this highest temperature treatment.

3.2. Adult parasitoid longevity, parasitism rate, pre-oviposition period, and fecundity

Temperature significantly affected adult parasitoid longevity (Log-Rank test, $\chi^2 = 113.7$, $df = 4$, $P < 0.0001$), parasitism rate (Log-likelihood Ratio $\chi^2 = 317.85$, $df = 4$, $P < 0.0001$), pre-oviposition period ($F = 31.57$, $df = 4, 52$, $P < 0.0001$), and lifetime fecundity ($F = 9.57$, $df = 3, 52$, $P < 0.0001$). Mean (± SE) longevity of adult female parasitoids decreased from 24 (± 1.6) to 2 (± 0.2) weeks as temperature increased from 15 to 35 °C, respectively (Table 2). While the parasitism rate increased from 1.4% at 15 °C to 28% at 30 °C, few hosts (< 0.5%) were parasitized at 35 °C (Table 2). Pre-oviposition time decreased from 81.2 d at 15 °C to 7.9 d at 25 and 30 °C, while the lifetime fecundity of females increased from 27 at 15 °C to 81–82 progeny/female at 25–30 °C (Table 2). The dynamics of weekly survivor and fecundity of adult parasitoids varied with temperature treatments (Fig. 2A–E).

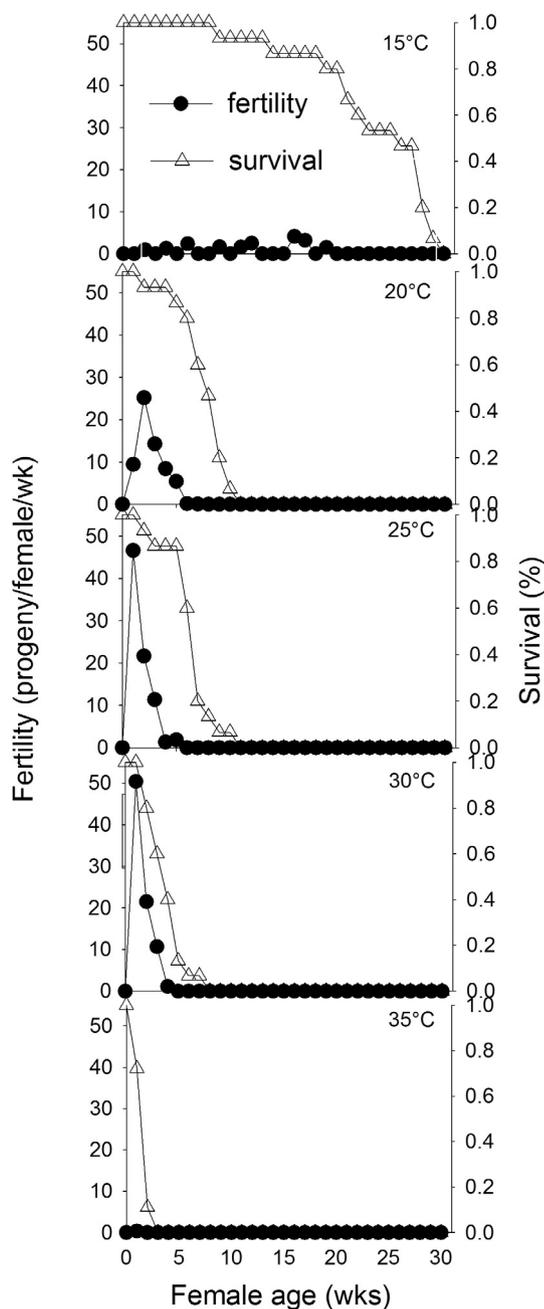


Fig. 2. Weekly longevity and fertility of *T. planipennisi* as influenced by constant ambient temperature treatments. Solid lines with black circles are the mean number of progeny (male and female) produced by a single ovipositing female for each week of life. Solid lines with white triangles are female adult longevity.

Table 3
Demographic parameters for *Tetrastichus planipennisi* reared at constant ambient temperatures in emerald ash borer larvae.

	15 °C	20 °C	25 °C	30 °C
Net reproductive rate (R_0)	13.1800	42.9100	58.0614	52.6233
Intrinsic rate of increase (r_m)	0.0120	0.0716	0.1117	0.1161
Finite rate of increase (λ)	1.0121	1.0742	1.1181	1.1231
Population doubling time (T_d ; wks)	57.6985	9.6797	6.2081	5.9696
Generation time (T_c ; wks)	30.6640	7.5000	5.1970	4.8750

3.3. Demographic parameters

The demographic parameters (R_0 , r_m , λ , T_d or T_c) varied with temperature treatments (Table 3). While the net reproductive rate of the parasitoid ($R_0 = 58.0614$) was the highest at 25 °C, the intrinsic rate of increase ($r_m = 0.1161$) and finite rate of increase ($\lambda = 1.1231$) were highest at 30 °C. Population doubling time (T_d) was longest (58 wk) at 15 °C and shortest (6 wk) at 30 °C, and similarly, generation time (T_c) was longest at 15 °C (31 wk) and shortest at 30 °C (5 wk).

3.4. Prediction of *T. planipennisi* generations in the USA

The number of degree-days required for completion of immature development of a single generation (from egg to adult eclosion) of *T. planipennisi* was calculated as 319.79 DD (averaged across temperature treatments from 15 to 30 °C) above an estimated lower developmental threshold of 13.29 °C (Table 1). The degree-days accumulated above the theoretical lower development threshold from 2014 to 2016 at the 11 locations in climate zones of 3–7 in the USA ranged from 2962.7 DD in Dallas, Texas to 596.24 DD in Duluth, Minnesota (Table 4). These data indicate that *T. planipennisi* can complete more than one generation in climate zones 3 to 7, with fewer generations in colder regions (Table 4).

4. Discussion

The temperature range (15–35 °C) tested in our study is ecologically relevant to climatic conditions that *T. planipennisi* and its host EAB encounter during the growing season (spring to fall) in the USA. Our findings support that temperature strongly influenced several biological parameters of this important EAB biocontrol agent including immature developmental time, adult longevity, pre-oviposition period, and parasitism rates, indicating that *T. planipennisi* establishment and impacts on EAB populations will vary in different climatic regions of North America. For example, at 15 °C, slow immature parasitoid development (124–136 d from egg to adult eclosion) and limited EAB attack rate (1.4% larval parasitism rate) by *T. planipennisi* suggest that its release in early spring (e.g., March–April) or in late fall (e.g., October–November) may be too early or too late, respectively, in most EAB-infested regions of the USA (northeastern, mid-Atlantic, and Midwestern regions), despite the availability of suitable EAB larval stages for parasitism (Duan et al., 2010). On the other hand, the success of *T. planipennisi* released during the summer heat typical of the southern USA may also hinder parasitoid establishment and performance due to drastic reductions in parasitism rates and mortality of parasitoid eggs at 35 °C. However, additional research is needed to estimate the upper developmental threshold temperatures (> 30 °C) for *T. planipennisi* larval and pupal stages.

Our data on key demographic parameters (R_0 , r_m , λ , T_d , and T_c) showed that *T. planipennisi* had the fastest population growth rates from 25 to 30 °C. For the purpose of mass-rearing programs, this larval parasitoid may be most effectively reared at this temperature range. On the other hand, *T. planipennisi* had the lowest values in R_0 , r_m , and λ at 15 °C, largely because of the prolonged immature developmental time and few host attacks. From 15 to 30 °C, the values in R_0 , r_m , λ , increased with the increase in temperature and appeared to peak at ~30 °C. However, the exact peak above 30 °C could not be determined because of few host attacks and 100% mortality of parasitoid eggs at 35 °C. A recent study with a congener *Tetrastichus brontispae* Ferrière, a larval endoparasitoid of *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), revealed that temperatures between 24 and 26 °C were optimal for parasitism, but no adult emergence was observed at 32 °C (Liu et al., 2014). Such interspecific variations in response to temperature (also see Gage and Haynes, 1975) should not be surprising as the genus *Tetrastichus* includes a large number of diverse species that attack a wide variety of hosts in agriculture, horticulture, and forest habitats.

Table 4Number of *T. planipennisi* generations estimated to occur in climatic zones in the USA, where EAB has been detected or could have become established in the future.

Location (USA)	Climate zone ^a	Known EAB infestations	Mean degree-days (mean \pm SE) ^b (> 13.3 °C)	Predicted number of <i>T. planipennisi</i> generations (mean \pm SE)
Dallas, TX	3	no	2962.7 \pm 108.7	9.3 \pm 0.3
Shreveport, LA	3	yes	2717.9 \pm 151.3	8.5 \pm 0.5
Atlanta, GA	3	yes	2279.1 \pm 135.6	7.1 \pm 0.4
Charlotte, NC	3	yes	2082.0 \pm 113.1	6.5 \pm 0.4
Sacramento, CA	3	no	1700.8 \pm 64.77	5.3 \pm 0.2
Washington, D.C.	4	yes	1506.4 \pm 115.0	4.7 \pm 0.4
Seattle, WA	4	no	749.36 \pm 37.92	2.4 \pm 0.1
Detroit, MI	5	yes	1198.5 \pm 91.85	3.7 \pm 0.3
Boston, MA	5	yes	1122.5 \pm 46.6	3.5 \pm 0.2
Minneapolis, MN	6	yes	1127.8 \pm 68.5	3.5 \pm 0.2
Duluth, MN	7	yes	596.24 \pm 41.02	1.9 \pm 0.1

^a Climate zones are from the US Department of Energy (USDOE 2013).^b Means and associated SE values were calculated from three years (2014–2016) of degree-day data (NOAA NCEI, 2017).

Previous climatic matching analysis indicated that all northern and most central parts of the USA had high values of climate-matching indices (> 0.6 at the scale from 0 to 1) to the locations of Northeast China (40–50° N latitude) where both EAB and *T. planipennisi* were known to occur (Federal Register, 2007; Bray et al., 2011; Bauer et al., 2014; Wang et al., 2015). However, EAB has now spread as far south as Webster County, Louisiana (32.7°N latitude), where the average temperature (~33 °C) during the growing season (June to August) is much higher than in other regions of the USA where EAB has invaded (USDA APHIS, 2018; Duan et al., 2018), and the lethal temperature of 35 °C or above for *T. planipennisi* eggs may be frequently reached.

Based on our calculation of the number of degree-days (319.79 DD) required for completion of a single generation of *T. planipennisi*, this introduced biocontrol agent will be able to complete more generations in central and southern parts of USA. Although releases started more recently in southern states, the warmest regions where *T. planipennisi* establishment has been confirmed to date are in climate zone 4 in Wayne County, Missouri (~37°N latitude) and Fayette Co., Kentucky (~38°N latitude) (MapBiocontrol, 2017; Duan et al., 2018). However, the frequent occurrence of high temperatures > 35 °C in climate zone 3 in states such as Louisiana and Texas may hinder the establishment and effectiveness of *T. planipennisi* in suppressing EAB population densities. Therefore, as EAB continues spreading south and west in the USA, expanded foreign exploration in Asia is needed to find species or populations of EAB natural enemies adapted to warmer climate zones.

The populations of *T. planipennisi* currently being reared for release in North America by EAB biocontrol programs were collected in Liaoning and Jilin provinces, China from ~40 to 44° N latitude, where Liu et al. (2007) estimated this parasitoid completed up to four generations in 2005. The results of a recent cold tolerance study suggest that cold-acclimated larval stages of the EAB parasitoids from northeast China, *T. planipennisi*, *S. agrili*, and *O. agrili*, could tolerate moderate level of freezing temperatures during the winter season in North America (Hanson et al., 2013). However, this and other studies have revealed that prolonged exposure to cold temperatures could result in some mortality of cold-acclimated larvae (Crosthwaite et al., 2011; Jones et al., 2017).

The results of laboratory studies (Hanson et al., 2013 and our current study), as well as field data from North America that document where *T. planipennisi* is establishing and spreading (MapBiocontrol, 2017; Duan et al., 2018), support that warm climates in general, particularly from climate zone 4–6 in the USA and southern Canada, favor high population growth and lower winter mortality of *T. planipennisi*. Colder climate zones (7 or higher) may result in higher overwintering mortality and slower population growth for *T. planipennisi*. We recommend further laboratory research to validate the low developmental temperature threshold for *T. planipennisi* larvae (e.g. 10 °C) (see reviews in Damos and Savopoulou-Soultani, 2012), although the best predictor of population growth, establishment, and performance of

natural enemies is field-collected data. To date, the coldest release sites in North America where *T. planipennisi* establishment has been confirmed are climate zones 6 (Hennepin County, Minnesota, ~45°N latitude) and 7 (Houghton County, Michigan, ~47°N latitude) (MapBiocontrol, 2017; Duan et al., 2018; USDOE, 2013). However, as EAB continues spreading north, more cold-adapted EAB natural enemy species or populations may be needed.

Like many laboratory studies, prediction of the population growth, establishment and performance of natural enemies under field conditions needs serious caution and should be validated by field data. Unfortunately, there have been few field studies attempting to document the demography of *T. planipennisi* as well as other introduced EAB parasitoids under field conditions. This is largely due to the logistic difficulty of observing these parasitoids under field conditions because their immature stages are associated with EAB host larvae or eggs and concealed under or in the bark of host ash trees. Furthermore, our study design did not include a low treatment temperature (e.g. 10 °C) near or below the predicted threshold temperature (13.3 °C) mainly because the practical difficulty of observing the development of the larval parasitoid inside its host and concealed under the bark in a prolonged period (> 6 months). As many previous studies have noted (see reviews in Damos and Savopoulou-Soultani, 2012), this could lead to an inaccurate estimate of the predicted low developmental temperature threshold.

In addition to seasonal and regional temperature, the successful establishment and impact of *T. planipennisi* on EAB populations depends on the availability of suitable host larval stages when adult parasitoids are foraging (Ulyshen et al., 2010). In Michigan and other colder regions of the USA, the development of EAB is less synchronized, leading to the presence of different EAB larval stages throughout the year (Cappaert et al., 2005; Duan et al., 2010). This facilitates the establishment of EAB larval parasitoids such as *T. planipennisi* by providing available hosts from early spring through late fall (Duan et al., 2010). However, it is not known if EAB has a similar phenology in the warmer region of the USA that would favor the establishment and impact of *T. planipennisi* on EAB populations. Long-term field studies in geographic regions with distinctive climatic conditions in the USA are needed to predict establishment and impacts of *T. planipennisi*, as well as other introduced EAB biocontrol agents on EAB populations.

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