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Development cycle of a potential biocontrol agent: the American hoverfly, *Eupeodes americanus*, and comparison with the commercial biocontrol agent *Aphidoletes aphidimyza*

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Abstract

Aphids (Hemiptera: Aphididae) are a major economic problem in numerous crops, including pepper, melon, and potato. Larvae of the American hoverfly, *Eupeodes americanus* (Wiedemann) (Diptera: Syrphidae), are common aphidophagous natural enemies in agrosystems in North America. The objective of the present study was to characterize the development cycle of *E. americanus* in order to evaluate its potential as a biocontrol agent. The development cycle, survival rate, and adult longevity of *E. americanus* were determined and compared with those of the commercial aphid midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) under laboratory conditions, using the green peach aphid, *Myzus persicae* (Sulzer), as prey. The complete preimaginal development time, pupal development time, egg hatching rate, sex ratio, and survival rate were not different between *E. americanus* and the commercial *A. aphidimyza*. The larval developmental time was longer in the syrphid species, increasing the predation window. Finally, the adult longevity of the syrphid was drastically longer than that of *A. aphidimyza*. These results demonstrate a potential for *E. americanus* as a new aphidophagous biocontrol agent.

Abbreviated abstract

Larvae of *Eupeodes americanus* (Diptera: Syrphidae) are common natural enemies of aphids in natural systems and agrosystems in North America. In this study, the development cycle, survival rate, and adult longevity of *E. americanus* were determined and compared with those of the commercial aphid midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) under laboratory conditions, in order to evaluate its potential as a biocontrol agent. The results demonstrated a high potential for *E. americanus*.

Graphic for Table of Contents

graphical abstract.png

Introduction

Aphids (Hemiptera: Aphididae) are a major economic problem in many agricultural crops due to their negative impact on plants by feeding on phloem sap and the consequent transmission of plant viruses (Ng & Perry, 2004; Blackman & Eastop, 2007). Until now, the main tool for controlling aphids is the use of insecticides, leading to increased levels of aphid resistance to certain groups of insecticides, such as neonicotinoids and carbamates, and to negative side effects on non-target species (Herron et al., 2001; Kift et al., 2004; Bass et al., 2015; Cabrera, 2017). The development of new integrated pest management tools for the biological control of aphids, such as their natural enemies, is necessary to constitute an alternative strategy to reduce the level of insecticide use and prioritize environmental preservation and human health (van Lenteren, 2012).

In North America, the predators used to control aphids in greenhouses belong mainly to the families of ladybirds (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), and gall midges (Diptera: Cecidomyiidae) (van Lenteren, 2018). Among the most used species, the cecidomyiid *Aphidoletes aphidimyza* (Rondani) preys upon more than 60 aphid species (Harris, 1973; Warner & Croft, 1982) during their furtive larval stage (van Lenteren, 2012). The predator is sold at the pupal stage, and females demonstrate a high discriminating capacity, laying eggs proportional to aphid population size (Lucas & Brodeur, 1999). However, the very high reproductive capacity of aphids often limits the effectiveness of these predators and most species become even less effective when the temperature is below 20 ºC (Alotaibi, 2008). These shortcomings demonstrate the need to find new species (Barriault et al., 2019; Bellefeuille et al., 2021) or new genetic strains (Dumont et al., 2018) of biocontrol agents.

As new potential biocontrol agents, syrphid species are resistant to low temperatures and have very high fecundity (Honěk & Kocourek, 1988; Hart & Bale, 1997). Furthermore, Hopper et al. (2011) confirmed that aphidophagous hoverflies are the most voracious of all aphid predators. In Europe, three hoverfly species – *Episyrphus balteatus* (De Geer), *Eupeodes corollae* (Fabricius), and *Sphaerophoria rueppellii* (Wiedemann) – are already commercialized against greenhouse aphids (van Lenteren et al., 2018; Biobest, 2020), but no syrphids are available for the North American market.

The American hoverfly *Eupeodes americanus* (Wiedemann) (Diptera: Syrphidae) is a Nearctic species and is widespread in North America (Vockeroth, 1992; Skevington et al., 2019). *Eupeodes americanus* larvae are generalist aphid predators (Rojo et al., 2003), feeding on more than 40 aphid species, including numerous crop pests such as soybean aphid, *Aphis glycines*

Matsumura (Kaiser et al., 2007; Noma et al., 2010), woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Bergh & Short, 2008; Gontijo et al., 2012), green peach aphid, *Myzus persicae* (Sulzer) (Vockeroth, 1992) on numerous crops, lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Smith & Chaney, 2007), foxglove aphid, *Aulacorthum solani* (Kaltenbach) on pepper (Bellefeuille et al., 2019), potato aphid, *Macrosiphum euphorbiae* (Thomas), and melon aphid, *Aphis gossypii* Glover (Rojo et al., 2003). In a previous study, Bellefeuille et al. (2019), *E. americanus* has demonstrated good active flight, oviposition, and larval voracity at low temperatures (12–18 °C). *Eupeodes americanus* has the potential to be an effective biocontrol agent against aphids in a greenhouse. However, little information exists on the biology and ecology of this species such as: its development cycle, reproduction, and voracity. Knowledge of each of these three characteristics is essential for the development of an effective biological control program and for the development of productive mass rearing (Soleyman-Nezhadiyan & Laughlin, 1998; Stiling & Cornelissen, 2005). The objective of the present study was to determine the development time and survival rate of immature stages as well as the adult longevity of *E. americanus*. The American hoverfly was compared to the already commercialized *A. aphidimyza*, as both species are dipterans and they have ecological similarities during their larval furtive predatory stage.

Materials and methods

Insect rearing

All insect rearing was carried out in the biocontrol laboratory (https://www.laboluttebio.uqam.ca) at the Université du Québec à Montréal (UQÀM). Wild adults of *E. americanus* were collected on *Phlox* sp. in 2014, in Sainte-Agathe-de-Lotbinière (46°23'726"N, 71°21'446"W), Québec, Canada. New wild individuals were added to the colony yearly. These individuals were reared using the Frazer (1972) method. Adults were kept in a large rearing cage (81 × 53 × 60 cm) covered with muslin which was kept in a greenhouse. The greenhouse was set to a photo-thermoperiod of L16(22 °C):D8(19 °C) under high-pressure sodium lamps and 60% r.h. Adults were fed with an artificial flower that consisted of a round cotton makeup remover saturated with a honey: water mixture (1:3 vol/vol) and covered with wildflower bee pollen. They were also fed with a sugar: water mixture (1:10 vol/vol) in two cups with a dental cotton roll protruding from the lid. These were replaced twice a week. When rearing cage adults, broad bean plants (*Vicia faba* L., Fabaceae) infested with pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), were

introduced 2× a week to allow females to lay eggs after mating. Once a week, larvae were collected and transferred into two rearing cages (35 × 35 × 35 cm) covered with muslin and put in a growth chamber set to 24 °C, 70% r.h., and L16:D8 photoperiod. These larval cages contained barley plants (*Hordeum vulgare* L., Poaceae), infested with cereal aphids *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae). The adults were collected and introduced to the adult rearing cage kept in the greenhouse as described above.

Aphidoletes aphidimyza specimens were obtained as pupae from a commercial supplier (Plant-Products Quebec, Laval, QC, Canada). They were reared in the same type of cage and in the same growth chamber as *E. americanus* larvae. *Aphidoletes aphidimyza* were reared on potato plants (*Solanum tuberosum* L. var. Norland, Solanaceae) infested with *M. persicae*. A sugar: water mixture (1:10 vol/vol) in a cup was added as a food source for adults.

Experiment on pre-imaginal development, survival, and sex ratio

Tests were done at 24 °C, 70% r.h., and L16:D8 photoperiod. Eggs from both species used for this study were collected on broad bean plants and were <24 h old. These eggs (n = 81 *E. americanus*, n = 75 *A. aphidimyza*) were incubated in Petri dishes (5 cm diameter) lined with a broad bean on agar gel. Eggs were observed daily until larval emergence to measure egg incubation time and egg hatch rate. Young larvae (n = 58 *E. americanus*, n = 62 *A. aphidimyza*) were isolated individually in a Petri dish with a broad bean on agar gel to begin larval development. Larvae were fed ad libitum with *M. persicae* and observed every 24 h until pupation. New aphids were added into the Petri dish when necessary to keep the resource ad libitum and the number of aphids added was evaluated. Larval development time and survival rate were determined. Larval development time is the period between the hatching date and the pupation date. Stages 1 and 2 of *E. americanus* larvae were difficult to distinguish. Stage 3 was easily recognizable by the breathing tubes fusing into one (Rotheray & Gilbert, 2011). We noted the number of days spent in the L1-L2 stage and in L3. It is impossible to differentiate the different larval stages of *A. aphidimyza*. Survival rate is the proportion of larvae that reach the pupation stage. At the beginning of the pupation stage, aphids were discarded from the Petri dishes to remove any possible effects on survival or development. The pupae (n = 35 *E. americanus*, n = 39 *A. aphidimyza*) were observed every 24 h until adult emergence. Pupation time, adult emergence rate, and sex ratio were recorded. Each larva has been identified with a number to distinguish the development time of males and females.

Experiment on adult longevity

Adult longevity is defined as the period between the adult's emergence and its death. Adult longevity of *E. americanus* and *A. aphidimyza* was determined from unmated adults and emerged in <24 h. A single adult (male or female) was placed in a ventilated cylindrical plastic cage (11 cm diameter, 14.5 cm high) and fed with artificial flowers and sugar water as described in the insect rearing section. Adults (n = 40 *E. americanus*, n = 40 *A. aphidimyza*) were observed daily until death, measuring mean longevity.

Statistical analysis

A Shapiro-Wilk test was applied to the data to determine whether they were following a normal distribution before further analysis. The mean duration of each pre-imaginal life stage (egg-larva-pupa) were not normal, a Wilcoxon rank-sum test was used to compare the variables between predator species. A Pearson χ^2 analysis was done to compare the proportion of time spent for each pre-imaginal stage between predator species. When considering the data for the individuals that have emerged (*E. americanus*, n = 11 males, n = 10 females; *A. aphidimyza*, n = 12 males, n = 17 females), mean hatching time, larval development time, pupation time, and adult longevity (dependent factors) were normal (Shapiro-Wilk). A two-way ANOVA was used on these variables to determine the effect of predator species and sex (dependent variables). Sex ratio between predators, egg hatching rates, larval survival rates, and pupal emergence rates between predators were all compared using a Pearson $\chi 2$ analysis. The R statistical software v.3.4.2 (R Core Team, 2017) was used to conduct all statistical analyses.

Results

Immature development and sex ratio

Hatching time for *E. americanus* was shorter than that for *A. aphidimyza* at 2 and 2.7 days, respectively (Wilcoxon: W = 3045, P<0.001; Figure 1). Egg hatching time was not different between sexes in both species (ANOVA: $F_{1,46} = 3.24$, P = 0.078) and there was no interaction between species and sex ($F_{1,46} = 0.66$, P = 0.42; Figure 2A).

Larval development time for *E. americanus* was longer than for *A. aphidimyza* with 6.9 and 6.3 days, respectively (W = 437, P<0.001; Figure 1). Larval development time was not different between sexes in both species ($F_{1,46} = 2.8$, P = 0.10) and there was no interaction between species and sex ($F_{1,46} = 0.3$, P = 0.58; Figure 2B). The total immature development time (egg to imago)

was 16.0 days for *E. americanus* and 15.9 days for *A. aphidimyza* (W = 285.5, P = 0.71). *Eupeodes americanus* has three stages of larval development (L1–L3). L3 development time was almost equal to the sum of L1-L2 development time with 3.2 and 3.7 days, respectively.

Pupation time was not different between *E. americanus* and *A. aphidimyza* with 7.1 and 7.0 days, respectively ($F_{1,46}$ = 2.0, P = 0.17). Pupation time was shorter in males of *E. americanus* and *A. aphidimyza* than in females ($F_{1,46}$ = 20.7, P<0.001) and no interaction between species and sex ($F_{1,46}$ = 0.03, P = 0.87; Figure 2C).

There was no difference in the proportion of time spent in each immature development stage between predators (Pearson: χ^2 = 3.89, d.f. = 2, P = 0.14; Figure 3). The female: male ratio was 10/11 (1:0.91) for *E. americanus* (χ^2 = 0.048, d.f. = 1, P = 0.83) and 17/12 (1:1.42) for *A. aphidimyza* (χ^2 = 0.86, d.f. = 1, P = 0.35). There was no difference in the adult sex ratio between predators (χ^2 = 2.32, d.f. = 3, P = 0.51).

Adult longevity

Adult longevity of *E. americanus* was longer than that of *A. aphidimyza* with 18.7 and 4.6 days, respectively (ANOVA: $F_{1,76}$ = 133.2, P<0.001). Adult longevity was not different between sexes in both species ($F_{1,76}$ = 0.2, P = 0.65) and there was no interaction between species and sex ($F_{1,76}$ = 0.1, P = 0.74; Figure 4). The total period from egg to adult death of *E. americanus* and *A. aphidimyza* was 34.8 and 20.5 days, respectively (Wilcoxon: W = 20.5, P<0.001).

Survival rate

There were no differences between *E. americanus* and *A. aphidimyza* for (Figure 5): egg hatch rate (71.6 vs. 82.7%; Pearson: χ^2 = 2.1, P = 0.15), larval survival rate (60.3 vs. 62.9%; χ^2 = 0.01, P = 0.92), pupal emergence rate (60.0 vs. 74.4%; χ^2 = 0.98, P = 0.32), or total (egg-imago) survival rate (25.9 vs. 38.7%; χ^2 = 2.35, P = 0.13, all d.f. = 1).

Discussion

The main objective of this study was to determine the development cycle of *E. americanus* and to compare it with *A. aphidimyza*, in order to evaluate its potential as a biocontrol agent against aphids. Both species are dipteran and aphidophagous predators during their larval stage. Immature development time (sum of hatching time, larval development time, and pupation time) was similar for both species, but larval development time and the adult longevity of *E. americanus* were

longer than in *A. aphidimyza*. Survival rates were similar as well as sex ratio in both species.

Immature development time obtained in this study was 16.0 days for *E. americanus* and 15.7 days for *A. aphidimyza*. Development time can be influenced by many environmental factors such as the temperature, prey species, abundance of prey, photoperiod, and host plant (Rüzička, 1975; Vanhaelen et al., 2002; Hong & Hung, 2010). A short immature development time (egg-larva-pupa) in a predator such as *E. americanus* and *A. aphidimyza* promotes rapid population development because the generations will be short. This favors a faster control of the prey population in agrosystems (Amano & Chant, 1977). In *E. americanus*, it was shorter than several other predators used for aphid control such as the ladybeetles *Hippodamia variegata* (Goeze) (18.1 days) and *Adalia bipunctata* (L.) (21.3 days) at 23 °C on *M. persicae* (Lanzoni et al., 2004; Jalali et al., 2009), and the hoverfly *E. balteatus* (21.2 days) on *A. gossypii* (at 26.6 °C) (Hong & Hung, 2010), but remains comparable to that of *Sphaerophoria scripta* (L.) (16.3 days) on *Aphis craccivora* Koch (at 22 and 25 °C) and *Allograpta exotica* (Wiedemann) (15.0 days, at 25 °C) (Moetamedinia et al., 2004; Arcaya et al., 2017).

Larval development time was significantly longer in *E. americanus* than in *A. aphidimyza*. It was comparable to that of *E. corollae* (7 days) on *A. pisum* (at 25 °C) (Asyakin, 1973). This is a positive factor for biological control as a long larval development may extend the predation period and generate an overall increased voracity (Karl & Fischer, 2008).

Considering the voracity of the syrphid predator, even if it was not studied in the present paper, it is possible to extrapolate according to previous studies on related species. *Eupeodes americanus* was able to consume between 440 and 472 aphid wheat *Schizaphis graminum* (Rondani) during its larval stage (Wadley, 1931). One *A. aphidimyza* larva can only consume 7–80 aphids during its development depending on aphid size (Uygun, 1971; Nijveldt, 1988; Harizanova & Ekbom, 1997). The voracity of *E. americanus* should therefore be greater than that of *A. aphidimyza*. Moreover, several species of the genus *Eupeodes* have greater voracity than *A. aphidimyza*. The larva of *Eupeodes fumipennis* (Thompson) consumes approximately 500 individuals of stage-3 *N. ribisnigri* during its larval development at 19 ºC (Hopper et al., 2011). The larva of *E. corollae* can consume approximately 390 apple aphids, *Aphis pomi* De Geer, at 25 ºC (Jalilian et al., 2016) and 300 individuals of *M. persicae* when the temperature varies between 8 and 28 ºC (Benestad, 1970) during its larval development. The larva of *Eupeodes confrater* (Wiedemann) can consume up to 886 *A. gossypii* during its larval development (Agarwala & Saha, 1986). According to these results, we can speculate that *E. americanus* would be more voracious

than *A. aphidimyza*.

Our results showed that pupation time was not different between *E. americanus* and *A. aphidimyza*. The males in both species had a quicker pupal developmental than females; such shorter developmental time for male syrphid species has been reported previously for *E. corollae* (Barlow, 1961; Benestad, 1970). *Aphidoletes aphidimyza*, *E. balteatus*, and *S. ruepelli* pupae are exported commercially and applied in biocontrol programs (Alotaibi, 2008; Yukawa et al., 2008; van Lenteren et al., 2018). Consequently, *E. americanus* pupae could be conditioned and commercialized in a similar way. It is therefore advisable to determine the appropriate temperature for pupal conservation before release into greenhouses.

The egg-to-adult survival rates were not significantly different between *E. americanus* (25.9%) and *A. aphidimyza* (38.7%). The survival rate of *E. americanus* was similar to that obtained for other aphidophagous hoverflies such as *P. clavatus* fed on *Aphis spiraecola* Patch (24%) (Belliure & Michaud, 2001) or *E. balteatus* fed on *A. craccivora* and *A. pisum* (30%) (Geusen-Pfister, 1987). Also, the larval survival rates did not differ between *E. americanus* (60.3%) and *A. aphidimyza* (62.9%); however, it was higher in *E. americanus* than in the syrphid *P. clavatus* (36%) fed on *A. spiraecola* (Belliure & Michaud, 2001). The absence of differences between the commercialized *A. aphidimyza* and *E. americanus* is encouraging, but the survival rate remains low compared to that obtained in the commercialized syrphid *E. balteatus* (77%) (Geusen-Pfister, 1987). As the larval survival rate is a key component of mass rearing, it will be important to optimize this aspect, for example, by comparing larval survival on several of the 40 aphid species consumed by the hoverfly (Rojo et al., 2003).

Adult longevity for *E. americanus* was drastically longer than for *A. aphidimyza*. Other syrphid species also have a higher adult longevity than *A. aphidimyza* (Moetamedinia et al., 2004; Hong & Hung, 2010). Adults of *E. americanus* are long-lived compared to other syrphid species such as *S. scripta* (14.9 days) (Moetamedinia et al., 2004), *Syrphus serarius* Wiedemann (15.2 days) (Xuan, 1993), and *A. exotica* (13.0 days) (Arcaya et al., 2017). The longevity of *E. americanus* was similar to that reported for *E. corollae* (18.4 days) (Huifang & Hanzhong, 1988). Furthermore, adult longevity is a factor usually correlated with the egg-laying-period duration: the longer adult longevity, the longer the egg-laying period, and the more eggs laid (Coll, 1996). It is usually the case in syrphids; for example, the fecundity in *E. collorae* is directly related to female longevity (Scott & Barlow, 1984). *Eupeodes corollae* was able to lay 436 eggs on *M. persicae* at 28 °C (Benestad, 1970) with a longevity of 18 days. In a study by Bellefeuille et al. (2019), *E.*

americanus was able to lay 100 eggs in just 7 days. The highest fecundity in this study was 232 eggs in 7 days. These values are clearly much higher than those obtained in *A. aphidimyza* on *R. padi* (19.9 eggs), on *M. persicae* (40.1 eggs) (Higashida et al., 2016), and on *A. gossypii* (39 eggs) (Watanabe et al., 2014) with a longevity not exceeding 4 days at 25 °C. In our study, the average longevity was 18.9 days; if *E. americanus* were as fecund as *E. corollae*, it would be highly superior to *A. aphidimyza*. The longevity of *E. americanus* females could be higher than that obtained in our study. Previous studies on other syrphid species such as *E. balteatus* (Geusen-Pfister, 1987), *I. escutellaris* (Alfiler & Calilung, 1978), and *P. clavatus* (Belliure & Michaud, 2001), showed that females lived longer than males and that mating was linked to a decrease in the longevity of syrphid males and an increase in the longevity of females (Makhmoor & Verma, 1987; Tawfik et al., 1974).

Future studies will establish for *E. americanus* (1) the link between larval developmental time and the overall voracity of the larval stages, (2) the relation between female longevity and egg-laying period duration (and of course total fecundity), and (3) the effect of mating incidence on the longevity of males and females.

In conclusion, the present results provide several points that may be of interest for the potential commercialization of the syrphid *E. americanus*. First, the complete preimaginal development time, the pupal development time, and the survival are similar between the candidate syrphid and the commercialized midge *A. aphidimyza*. Second, the larval developmental time is longer in the syrphid, increasing the predation window. And, third, the adult longevity of the syrphid is considerably longer than that of the commercialized midge.

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Figure captions

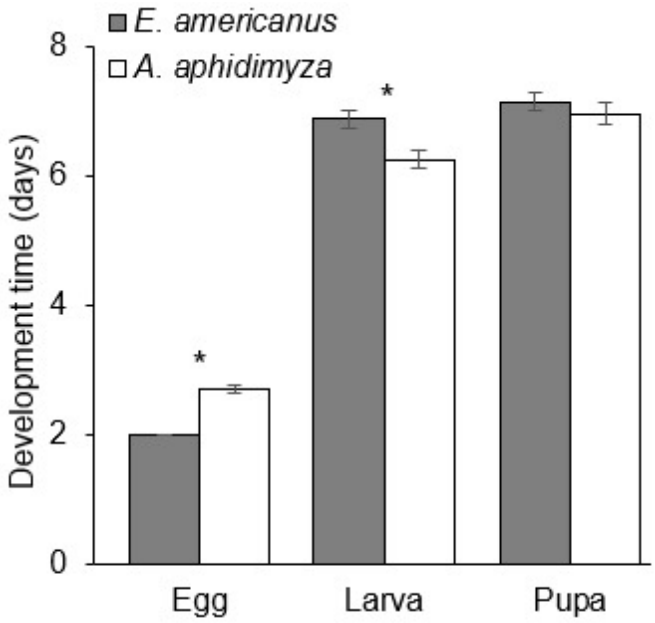
Figure 1 Mean (± SE) development time (days) for *Eupeodes americanus* and *Aphidoletes aphidimyza* eggs (n = 58 and 62, respectively), larvae (n = 35 and 39), and pupae (n = 21 and 29). An asterisk indicates a significant difference between species (Wilcoxon rank-sum test: *P<0.05).

Figure 2 Mean (± SE) development time (days) for (A) eggs, (B) larvae, and (C) pupae of *Eupeodes americanus* and *Aphidoletes aphidimyza* males (n = 11 and 12, respectively) and females (n = 10 and 17). An asterisk indicates a significant difference between species (Wilcoxon rank-sum test: *P<0.05).

Figure 3 Time spent (%) in egg, larva, and pupa stadium in relation to total pre-imaginal development for *Eupeodes americanus* and *Aphidoletes aphidimyza*.

Figure 4 Mean (± SE) adult longevity (days) for *Eupeodes americanus* and *Aphidoletes aphidimyza* males and females (all n = 20). An asterisk indicates a significant difference between species (ANOVA: *P<0.05).

Figure 5 *Eupeodes americanus* and *Aphidoletes aphidimyza* egg hatching rate (n = 81 and 75, respectively), larval survival rate (n = 58 and 62), and pupal emergence rate (n = 35 and 39).



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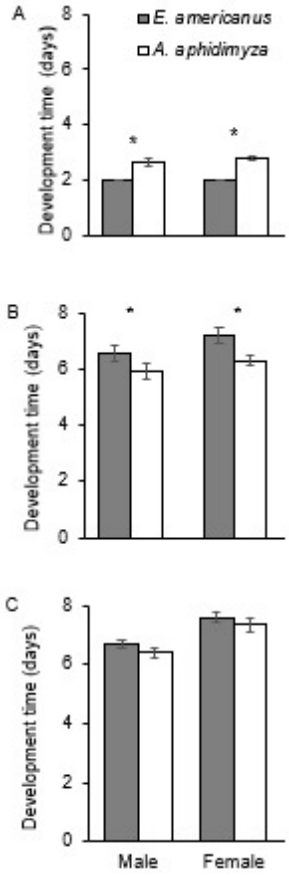

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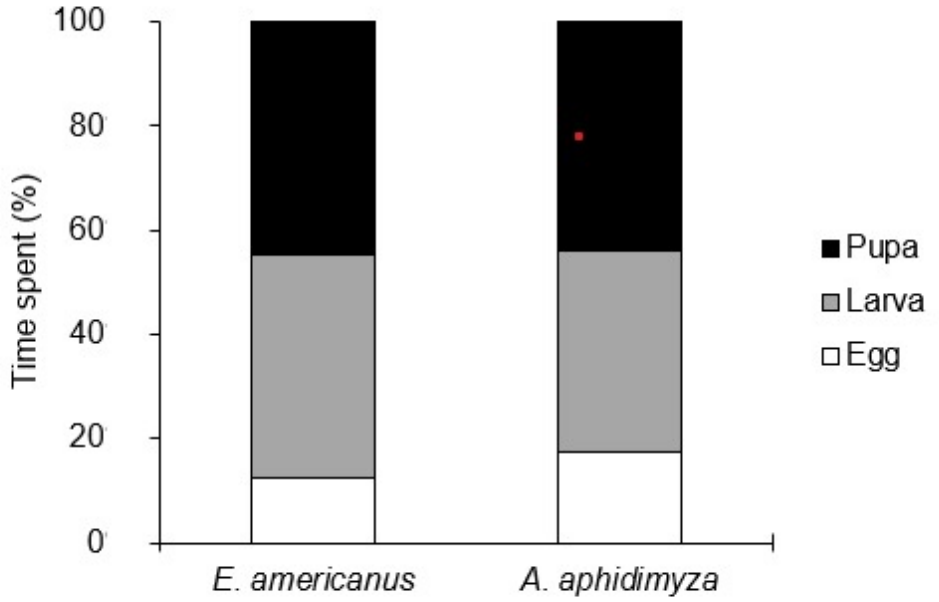

Figure 3

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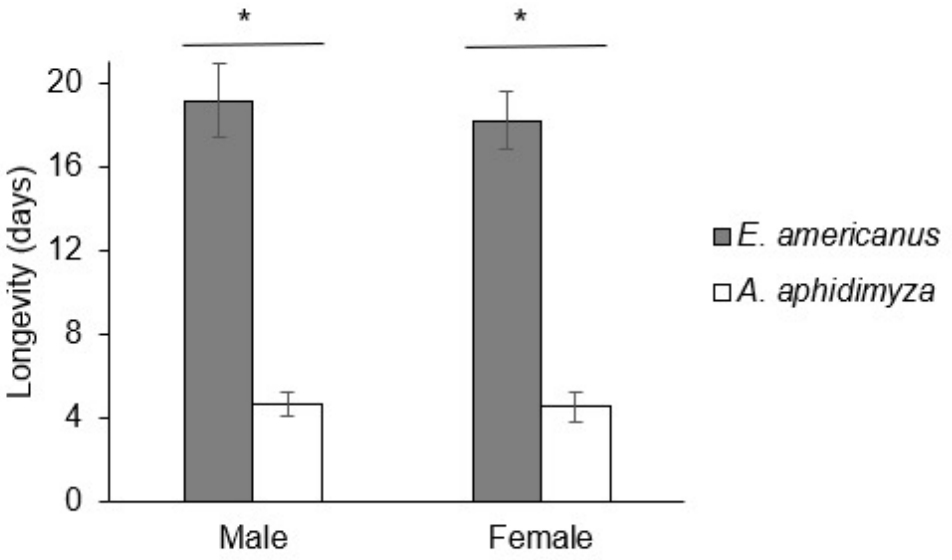

Figure 4

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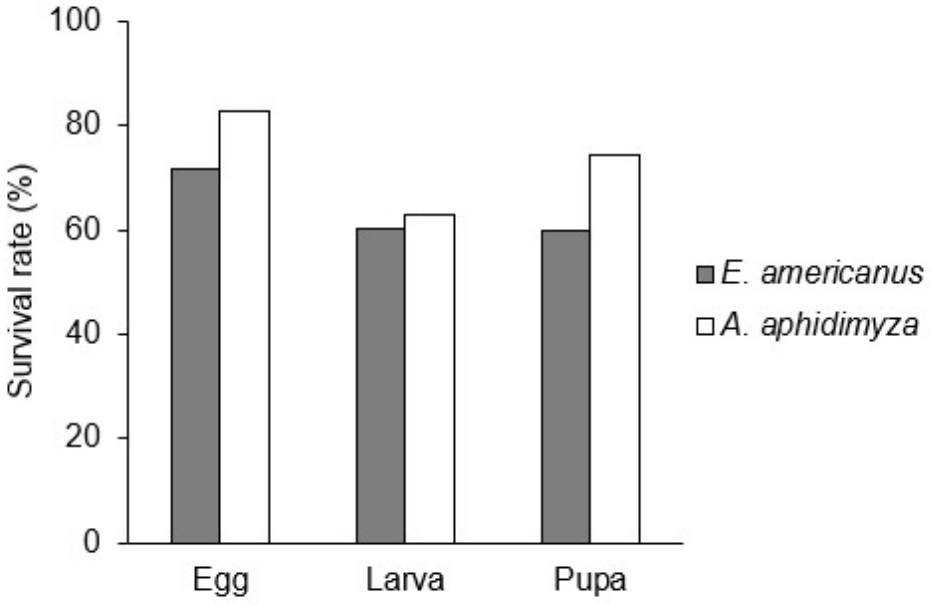

Figure 5

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