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Effect of temperature on life history of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an egg parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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Abstract

The effect of temperatures ranging from 5 to 40 °C on *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) oviposition and development were investigated. There were significant differences in the rate of development among the temperatures for all life stages. The egg stage lasted approximately 7 times longer at 15 than at 25 or 30 °C and the larval stage, consisting of 4 instars, took significantly less time at each increasing temperature from 15 to 30 °C. *A. vaquitarum* development from egg to adult took 16.3 days at 30 °C, significantly faster than the other temperatures tested. At 15 °C *A. vaquitarum* reached the pupal stage after a mean of 41.6 days but did not successfully complete pupation. *A. vaquitarum* eggs hatched on 66% of the parasitized egg masses at 35 °C but did not survive past the first instar. *A. vaquitarum* did not develop past the egg stage at 5 or 40 °C. Host *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) eggs also did not survive at temperatures of 35 and 40 °C on live plants. Upper and lower temperature thresholds for *A. vaquitarum* development were calculated to be 33.0 and 16.0 °C, respectively. Maximum development rate occurred at 30.9 °C and a thermal constant of 494.2 DD was calculated. Photoperiods ranging from 10L:14D to 16L:8D did not significantly affect development time from egg to adult at 25 °C. Oviposition was significantly higher at 30 °C than at the other temperatures tested; oviposition was also relatively high at 25 and 35 °C and several eggs were laid at 20 °C. Relatively cool winter temperatures in central Florida may be contributing to the limited range of *A. vaquitarum* in the state.
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Keywords: *Diaprepes abbreviatus*; *Aprostocetus vaquitarum*; Biological control; Citrus IPM; Development; Oviposition

1. Introduction

Diaprepes abbreviatus (L.) is a root weevil native to the Lesser Antilles of the Caribbean (Woodruff, 1985). First reported in Florida in 1964, it was presumably introduced from Puerto Rico and is now established across the citrus-producing regions of the state (Woodruff, 1964). *D. abbreviatus* is polyphagous and has been associated with more than 270 species of plants from 59 families (Simpson et al., 1996). Citrus and various woody ornamentals support the entire life cycle of *D. abbreviatus* (Mannion et al., 2003; Schroeder et al., 1979), which has resulted in a significant pest for both ornamental growers and the citrus industry where it is estimated to cost producers over 70 million dollars annually (Stanley, 1996). Adult weevils feed along the edges of leaves, leaving characteristic semi-circular notches. Eggs are laid in the canopy, glued between two leaves. Upon hatching, larvae fall to the ground to enter the soil to feed on the roots of host plants. Damage from root feeding can be significant, leading to reduced productivity and possibly death of the host plant. Root feeding may also leave citrus plants more susceptible to

root rot organisms such as *Phytophthora* spp. (Timmer et al., 2005).

In pursuit of an integrated control strategy for *D. abbreviatus*, efforts have been made to identify and introduce classical biological control agents. In the late 1990's, programs were initiated to introduce hymenopteran egg parasitoids from the Caribbean islands into Florida (Peña et al., 2001). In 2000, *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) was introduced into Florida from the Dominican Republic where it is a principal parasitoid of *D. abbreviatus* eggs (Jacas et al., 2005). *A. vaquitarum* has been released in several Florida counties and is now considered to be established in parts of southern Florida where parasitism levels of 70–90% have been achieved (Peña et al., 2006). However, attempts to establish *A. vaquitarum* in other areas of Florida have been unsuccessful to date.

Temperature requirements of this tropical parasitoid may constrain its ability to establish in central Florida. Through the winter months the mean and minimum temperatures are considerably higher in the regions of south Florida where *A. vaquitarum* has established than in areas of central Florida where establishment has been unsuccessful (Anonymous, 2005). The aim of this study was to evaluate the effects of a range of temperatures on *A. vaquitarum* oviposition and development. Effects of photoperiod on development and the number of larval instars were also examined.

2. Materials and methods

2.1. Stock colonies

Adult *D. abbreviatus* root weevils were collected from the foliage of ornamental trees at nurseries within a five mile radius of Homestead, FL (25.46 °N, 80.45 °W, 1 m alt.). Weevils were placed in plexiglass cages (30 × 30 × 30 cm) with water and foliage of the host plant *Conocarpus erectus* L. (Myrtales: Combretaceae). Foliage was formed into bouquets containing 20–25 terminal branches (each 15–20 cm long and with approximately 10 leaves) and placed in a 500-ml plastic container of water. Foliage was renewed every 2–3 days. Leaves containing *D. abbreviatus* eggs were removed and placed inside a similar cage in a room maintained at 26.5 ± 1 °C, 12:12 L:D, and approximately 78% RH. Adults of *A. vaquitarum* were introduced into the cage and provided honey on blotting paper and water on a cotton wick. Presumably parasitized egg masses were removed from the cage 4–5 days later and placed in plexiglass emergence cages (same dimensions as above) supplied with both a water and a honey food source until adult emergence (approximately 15 days). The *A. vaquitarum* colony was initiated from specimens collected on *Diaprepes* spp. eggs during the summer of 2000 in the Dominican Republic (Jacas et al., 2005). Voucher specimens of *A. vaquitarum* were retained by the USDA-APHIS and the Florida Department of Agriculture and Consumer Services.

2.2. Development and number of larval instars

Leaves containing 150–200 *D. abbreviatus* egg masses (<3 days old), approximately 37 eggs per mass, obtained as above were removed from rearing cages, placed inside a 30 × 30 × 30 cm plexiglass cage, and exposed to approximately 700 *A. vaquitarum* females (<3 days old) for 6 h. Females were provided honey and water, and oviposition was conducted in a room maintained at 26.5 ± 1 °C, 12:12 L:D, 78% RH. After 6 h of exposure, *A. vaquitarum* females were removed and the cage containing parasitized egg masses was transferred into an incubator at constant conditions of 25 °C, 14:10 L:D, and 85% ± 10 RH. Beginning 15 h after the females were removed, samples of 1–3 egg masses were opened at 3–6 h intervals to determine the number of *A. vaquitarum* at each development stage. A minimum of 34, up to >350, specimens were included in the development time means for all life stages at each temperature. Larvae were preserved in ethanol (70% vol.) and subsequently digested in lactic acid (65% vol.) at 45 °C. They were then mounted on a slide in Hoyer's medium (distilled water, arabic gum, chloral hydrate, and glycerin, 5:5:20:2 weight) for microscope observation. We measured mandible length using of the Eclipse Net® software (Nikon®, Fig. 1). Results were subjected to an ANOVA and mean separation was achieved with least significant difference (LSD) test (Statistix®8 Analytical Software, 2003). According to the significance of the differences obtained, the number of larval instars was established (Dyar, 1890). Pupae were also removed from parasitized egg masses and placed into gelatin capsules in groups of six; pupae were checked at 4–8 h intervals to determine adult emergence. This experiment was repeated at temperatures of 5, 15, 20, and 30 °C. Host plant material desiccated after several days at temperatures of 30 °C and above, so an additional experiment was conducted with live host plants at 30, 35, and 40 °C. Because of the restricted number of egg masses on live plants, egg masses were not sampled to determine the development times for all life stages at the higher temperatures.

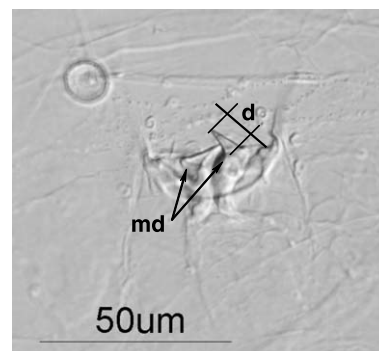The experiment was also repeated as above (25 °C) at photoperiods of 10:14 L:D and 16:8 L:D. Developmental

Fig. 1. Mouthparts of a 65–70 h-old *A. vaquitarum* larvae showing how mandible length (d) was measured. md: mandible.

data were subjected to the GLM ANOVA Procedure and mean separation was achieved with the Student–Newman–Keuls test. Photoperiod data were analyzed with a Kruskal–Wallis ANOVA (SAS Institute, 1999).

2.3. Temperature thresholds and thermal constant

Once development times (*y*) were established for each temperature treatment, developmental rates ($r(T) = y^{-1}$) were calculated. These rates were plotted against temperatures and fitted with modification 2 of the Logan model for non-linear regression (Lactin et al., 1995; Logan et al., 1976): $r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} \pm \lambda$, where $r(T)$ is development rate at temperature T, $T\,max$ is temperature of maximum development rate, and ρ, Δ, and λ are fitted parameters. The regression curve was fitted by iterative non-linear regression (STSC, 1987). Both upper and lower temperature thresholds (UTT and LTT, respectively) were estimated from this regression. Because the model is considered unrealistic for estimating LTT (Logan et al., 1976), we also estimated this parameter using linear regression.

Based on the LTT obtained, we calculated the thermal constant required for egg to adult development using the following equation (Varley et al., 1974): $K = \Sigma[y_i (t_i - x)]/n$, where K is the thermal constant, y_i is the development time, t_i is the temperature, x is lower temperature threshold and n is the number of replicates.

2.4. Oviposition

To examine the influence of temperature on *A. vaquitarum* oviposition, females were held in the presence of host eggs at constant temperatures between 10 and 40 °C. In a preliminary experiment 100 females (<4 days old) were offered 55 *D. abbreviatus* egg masses in a $30 \times 30 \times 30$ cm plexiglass cage and maintained at constant temperatures of 10, 15, 20, 25, 30, and 35 °C. After 24 h of exposure the host egg masses were dissected, *A. vaquitarum* and host eggs were counted. Based on the results of the preliminary work, the primary experiment was conducted at 15, 20, 25, 30, 35, and 40 °C. Twenty females (<4 days old) were offered 10 host egg masses and maintained at a constant temperature for 24 h. Host egg masses were then removed and dissected, the numbers of *A. vaquitarum* eggs and host eggs were recorded. The experiment was conducted in Bell® one-liter wide mouth jars, the lids had a 7-cm-diameter opening covered with fine mesh to allow ventilation. Each jar was supplied with a moist cotton wick and a smear of honey applied with the pointed end of a needle. The experiment was repeated 6 times at each temperature. Data were subjected to an ANOVA and mean separation was achieved with least significant difference (LSD) test (Statistix®8 Analytical Software, 2003).

2.5. Temperature

Mean minimum and mean maximum monthly air temperatures were based on data from the Southeast Regional Climate Center for Miami WSCMO airport (25.5 °N, 80.2 °W, 2.3 m alt.; station #085663—from 1948 to 2004) and Lake Alfred experimental station (28.1 °N, 81.4 °W, 42.1 m alt.; station #084707—from 1924 to 2000). These stations were chosen as representative of the southeast region of Florida where *A. vaquitarum* has established (Miami-Dade County) and central Florida where it has not established (Lake Alfred). Individual months missing more than five days of temperature data were not included in the means.

3. Results

3.1. Development and number of larval instars

There were significant differences in the rate of development among the temperatures tested for all life stages of *A. vaquitarum*. The egg stage lasted approximately 7 times longer at 15 than at 25 or 30 °C, where development was most rapid ($F_{4,1446} = 703.3$, $P < 0.001$) (Table 1). The larval stage took significantly less time at each increasing temperature interval from 15 to 30 °C ($F_{3,609} = 26825$, $P < 0.001$). The prepupal stage also took significantly less time at each increasing temperature interval from 15 to 25 °C ($F_{2,193} = 69263$, $P < 0.001$). Pupation was significantly more rapid at 25 than 20 °C ($F_{1,211} = 8573$, $P < 0.001$). Due to a limited number of egg masses at 30 °C, data for the prepupal and pupal stage were not attained at this temperature. Overall, *A. vaquitarum* development from egg to adult was significantly more rapid at 30 °C than at the other temperatures tested, 10% faster than at 25 °C and 43% faster than at 20 °C ($F_{2,378} = 7785$, $P < 0.001$) (Table 1).

At 15 °C *A. vaquitarum* reached the pupal stage after a mean of 41.6 days but did not successfully complete pupation. *Aprostocetus vaquitarum* eggs hatched on 66% of the parasitized egg masses at 35 °C but did not survive past the first instar. No *A. vaquitarum* development past the egg stage occurred at 5 or 40 °C. *D. abbreviatus* eggs also did not survive at temperatures of 35 and 40 °C. Host eggs at 35 °C contained fully formed head capsules but no lar-

Table 1
Mean developmental time, days (±SE), for each life stage of *A. vaquitarum* at four temperatures when reared on *D. abbreviatus* egg masses

Stage	15 °C	20 °C	25 °C	30 °C
Egg	11.6 (0.20) a	4.9 (0.20) b	1.8 (0.20) c	1.7 (0.20) c
Larva	25.9 (0.30) a	8.8 (0.02) b	5.6 (0.00) c	4.7 (0.03) d
Prepupa	4.1 (0.10) a	1.2 (0.06) b	0.5 (0.03) c	**
Pupa	*	13.7 (0.07) a	10.1 (0.08) b	**
Total	*	28.6 (0.07) a	18.0 (0.08) b	16.3 (0.06) c
% Female	*	99	99	99

Means in each row followed by the same letter are not significantly different ($P < 0.05$).

* *A. vaquitarum* did not develop past the pupal stage at 15 °C.

** No data due to limited egg masses.

vae successfully enclosed, those at 40 °C died earlier in development.

Photoperiods ranging from 10:14 L:D to 16:8 L:D did not significantly affect development time from egg to adult at 25 °C ($F_{2,17} = 1.50$, $P = 0.25$). Development time ranged from 17.4 ± 0.7 days at 10:14 L:D to 18.4 ± 0.4 days at 14:10 L:D; development at 16:8 L:D was intermediate in duration at 17.5 ± 0.6 days. Overall, the data collected at different photoperiods support the previous experiment which showed *A. vaquitarum* development time to be 18.0 days at 25 °C (Table 1).

In total 140 larvae of *A. vaquitarum* were processed for mandible length measurements. At least 6 larvae from each age group were considered. Based on these measurements, four larval instars were established (Fig. 2; $F_{8,131} = 27.82$, $P < 0.001$).

3.2. Temperature thresholds and thermal constant

Development rates (day-1) were fitted with non-linear regression (Lactin et al., 1995; Logan et al., 1976) (Fig. 3) $[r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} \pm \lambda$; $\rho = 0.0050 \pm 0.0001$; $\Delta = 0.3358 \pm 1.9483$ $\lambda = 1.0816 \pm 0.0030$;   estimate $\pm$ asymptotic standard error]. Upper and lower temperature thresholds estimated from this equation were 32.99 and 15.75 °C, respectively, and the maximum development rate occurred at 30.86 ± 1.3 °C. Lower development threshold (LTT) was also estimated by further fitting a linear regression between development rates from 10 to 30 °C and temperature $[r(T) = 0.00564\, T - 0.09036$; $r = 0.9434$ ($F = 7319.66$; $df = 1, 904$; $P < 0.00001$) and was calculated to be 16.0 °C. Using this estimate of the LTT, a thermal constant of 494.2 ± 3.0 DD ($n = 381$) was calculated.
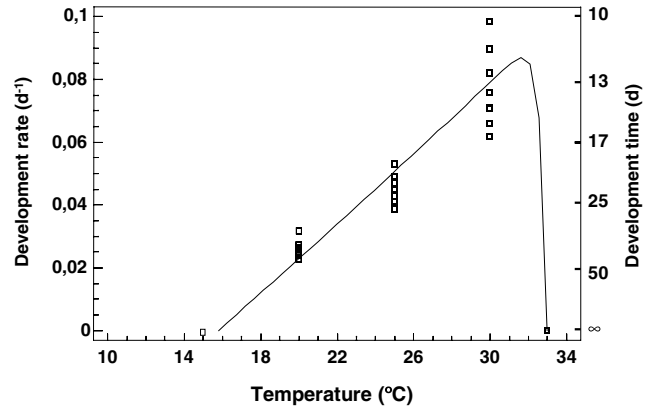

Fig. 3. Development rate (d^{-1}) of *A. vaquitarum* when reared on *D. abbreviatus* egg masses plotted against temperature (°C). Fitted curve: Logan model and modification 2. $r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} \pm \lambda$; $\rho = 0.0050 \pm 0.0001$; $\Delta = 0.3358 \pm 1.9483$; $\lambda = 1.0816 \pm 0.0030$; estimate $\pm$ asymptotic standard error.

3.3. Oviposition

In the preliminary test with 100 *A. vaquitarum* females, maximum oviposition occurred at 25 and 30 °C, no oviposition occurred at 10 or 15 °C; 20 and 35 °C were intermediate. Based on these results a more comprehensive experiment was conducted at temperatures ranging from 15 to 40 °C. Oviposition was significantly higher at 30 °C (14.1 eggs/female) than at the other temperatures tested ($F_{5,30} = 21.4$, $P < 0.001$). Oviposition was also relatively high at 25 and 35 °C and several eggs were laid at 20 °C (Fig. 4). Very few eggs were laid at temperatures of 15 or 40 °C (0.18 and 0.22 eggs/female, respectively). Many of the females were not active during the 24-h exposure period
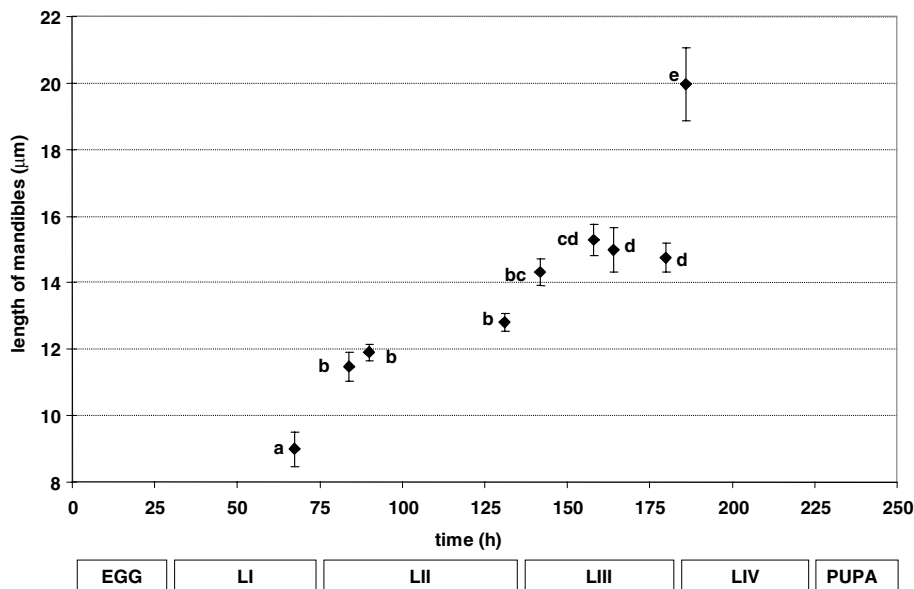

Fig. 2. Mean (±SE) mandible measurements of larvae of *A. vaquitarum* of different ages. Means with different letters are significantly different (LSD, $P < 0.05$). According to these differences, four larval instars are indicated at the bottom of the figure.
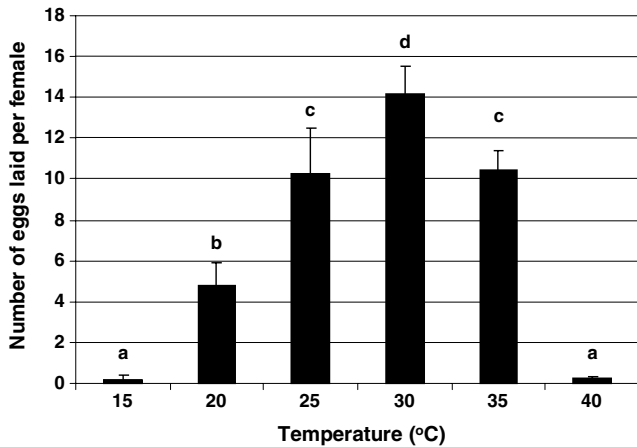
Fig. 4. Mean number (+SE) of eggs laid in a 24-h period by *A. vaquitarum* females on *D. abbreviatus* egg masses at different temperatures. Means with different letters are significantly different (LSD, $P < 0.05$).

at 15 °C and did not appear to move from their initial release point on the bottom of the cage. Though all the females did move at 20 °C, activity was visibly reduced compared to that of higher temperatures. Females were actively searching host plant material at temperatures of 25–40 °C and no difference in behavior was readily apparent. There was no significant difference in the number of *D. abbreviatus* eggs that were offered to *A. vaquitarum* females at the different temperatures ($F_{5,30} = 1.29$, $P = 0.29$).

4. Discussion

Development time of *A. vaquitarum* decreased with increasing temperature up to 30 °C for all life stages examined. Though *A. vaquitarum* was able to successfully develop at temperatures from 20 to 30 °C, development was almost twice as long at 20 compared to 30 °C. Developmental response of *A. vaquitarum* to various temperatures is comparable, though less pronounced, to that of *Quadrastichus haitiensis* Gahan (Hymenoptera: Eulophidae), an endoparasitoid of *D. abbreviatus* eggs (Castillo et al., 2006). A similar relationship, peaking at 30 °C, has also been observed between temperature and development for several other eulophid parasitoids (Acosta and O'Neil, 1999; Bazzocchi et al., 2003; Kfir et al., 1993; Rahim et al., 1991; Urbaneja et al., 2002, 2003). As expected from the estimated LTT (16.0 °C), *A. vaquitarum* did not complete development past the pupal stage at a constant temperature of 15 °C. Similarily, *Q. haitiensis* was shown not to develop past the prepupal stage at 15 °C (Castillo et al., 2006). Given the Caribbean origin of *D. abbreviatus* and its egg parasitoids it is not surprising that sustained temperatures of 15 °C or below are lethal.

At constant temperatures of 35 °C and above *A. vaquitarum* did not survive, this is in agreement with the estimated UTT (32.99 °C). Host *D. abbreviatus* eggs also did not survive at these temperatures. Similarly, Lapointe (2001) found that development rate of *D. abbreviatus* eggs increases with temperature up to 30 °C but larvae do not emerge at temperatures of 32 °C or higher. *A. vaquitarum* is an important primary parasitoid of *D. abbreviatus* in its native range (Jacas et al., 2005) and it is likely that the comparable relationship between temperature and development rates for parasitoid and host are a result of close evolutionary ties. However, it is interesting that females laid a substantial number of eggs at 35 °C and many *A. vaquitarum* eggs hatched at this temperature; the UTT was also calculated to be 33 °C. Our results indicate that *A. vaquitarum* may be able to survive on another host at temperatures of 30–33 °C if the host eggs could tolerate these temperatures.

Eulophid wasps usually have three to five larval instars (Askew, 1968; Gauld and Bolton, 1988) and *A. vaquitarum*, with four larval instars, fits within these limits. Also in agreement with what is usual among eulophid larvae, those of *A. vaquitarum* did not produce any cocoon after completion of their development (Gauld and Bolton, 1988).

Photoperiod did not significantly affect total development time of *A. vaquitarum*; however, mean development time was lowest at the shortest daylength tested. Obrycki et al. (1985) found that development of *Edovum puttleri* Grissell (Hymenoptera: Eulophidae), an egg parasitoid of the Colorado potato beetle (Coleoptera: Chrysomelidae), was significantly affected by photoperiod. Obrycki et al. (1987) later showed that *E. puttleri* developed slightly, but significantly, faster under short compared to long daylength. The effect of photoperiod on the development rate of eulophid egg parasitoids warrants further study.

The areas where *A. vaquitarum* has established in southeast Florida have higher mean temperatures and less volatile temperature fluctuations throughout the year, comparable to its native Caribbean range, than do areas in central Florida where this parasitoid has been released but shows no signs of establishment (Fig. 5). Minkenberg (1989) and Minkenberg and Helderman (1990) demonstrated that for both *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) and its eulophid parasitoid *Diglyphus isaea* (Walker), the effect of an alternating temperature regime
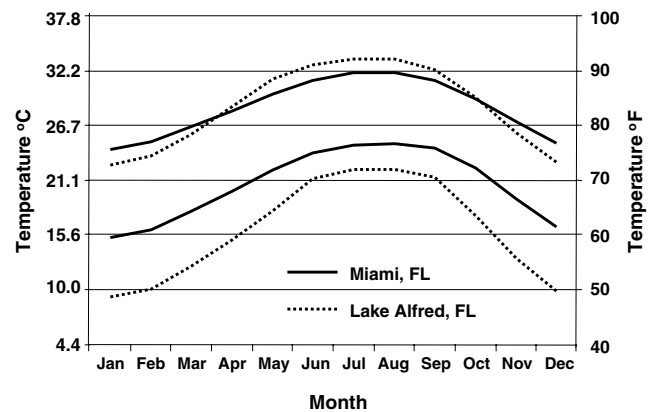

Fig. 5. Mean minimum and mean maximum monthly temperatures at Miami (southeast) and Lake Alfred (central), Florida.

on development parameters could be predicted from regression equations obtained at constant thermoregimes. If this is also true for *A. vaquitarum*, we can infer parasitoid development should not stop in south Florida where January mean temperature (Miami: 20 °C) does not reach the LTT of 16 °C (Anonymous, 2005). However, in central Florida (Lake Alfred) a mean temperature above 20 °C only occurs from April to October. In January the mean temperature of 15.9 °C is below the LTT and the mean minimum temperature is below 10 °C (Anonymous, 2005). Temperatures below 20 °C are less than ideal for *A. vaquitarum* oviposition or development and sustained temperatures below 15 °C are lethal. It is likely that the relatively cool winters and the more extreme temperatures experienced throughout the year in central Florida are a constraint to the establishment of *A. vaquitarum* across some regions of *Diaprepes* infestation. However, an effort to locate populations of *A. vaquitarum* adapted to lower temperatures may provide an opportunity to expand the range of this parasitoid in Florida. Obrycki et al. (1987) demonstrated geographical variation in the ability of *E. puttleri* to tolerate low temperature. It is possible that higher altitudes in the Caribbean may harbor biotypes of *A. vaquitarum* that are adapted to lower temperatures.

Other factors such as host availability and the seasonal changes in relative humidity experienced in central Florida may also be unfavorable for the establishment of *A. vaquitarum*. Given the relatively short lifespan of *A. vaquitarum*, extended periods without the presence of host eggs could be catastrophic. Host-free periods and low temperatures during the winter months have hindered the establishment of *E. puttleri* in the northeastern United States (Obrycki et al., 1985) and *Tetrastichus gallerucae* (Fonscolmbe) (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle (Coleoptera: Chrysomelidae), in northern California (Dreistadt and Dahlsten, 1991); neither species is known to diapause. Similarly, *A. vaquitarum* has not been shown to enter diapause under various light and temperature regimes in >4 years of insectary production and may not be able to overcome extended winter periods of cool temperatures or host absence. Pesticides use may also be contributing to the lack of establishment of *A. vaquitarum* in the citrus producing areas of central Florida; various products used in citrus production are harmful to this parasitoid (Ulmer et al., 2006).

Aprostocetus vaquitarum has established and is an important part of an integrated control program for *D. abbreviatus* in parts of southeast Florida (Peña et al., 2006). Though *A. vaquitarum* is successful in the regions of the state where climatic conditions are similar to its native Caribbean range, recovery of this parasitoid from release sites in the citrus producing regions of central Florida has been sporadic. The present study has shown that the relatively cool temperatures in the winter are not favorable for development of *A. vaquitarum* and may be hindering its establishment in some regions. Further research is required to establish the role of other factors, such as host availability, in limiting the range of *A. vaquitarum* in the United States.

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