

# Successful oviposition and reproductive biology of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae): A predator of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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## Abstract

*Aprostocetus vaquitarum* (Wolcott) causes 78–91 percent mortality to eggs of *Diaprepes abbreviatus* (L.), under field conditions in southern Florida. In the laboratory, *A. vaquitarum* was reared on *D. abbreviatus* eggs at 25 °C, a photoperiod of 12:12 (L:D) and with abundant hosts, *A. vaquitarum* adult females lived around 15 days. Oviposition was significantly affected by the age of the host egg mass. Egg masses aged 0- to 3-day-old were accepted significantly better than those aged 4–6 days. The mean number of eggs deposited per female was around 53, with extreme values of 124 and 19 eggs per female. Using these data in combination with the sex ratio observed in the field (0.16) and the duration of the preimaginal stages,  $r_m$  (0.168–0.142 day<sup>-1</sup>),  $T$  (22.39–22.89 days), and  $R_0$  (43.03–25.81 females per female) were calculated.

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## 1. Introduction

*Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), a weevil first detected in Florida in the 1960s, is a highly polyphagous species (Simpson et al., 1996), and considered a severe pest of a wide variety of agricultural commodities in Florida and the Caribbean Basin (Hall, 1995; McCoy and Simpson, 1994; Wolcott, 1936). Adult *D. abbreviatus* are 1–2 cm in length, slow moving and especially active at night, sunset and dawn. Mated females lay eggs in concealed sites, usually in the space between adjacent leaves, where egg masses are deposited in a gelatinous cement which seals the leaves together and thus provides protection for the eggs. Neonate larvae fall to the soil, where they begin feeding on small

roots. As development advances, larvae move to increasingly bigger roots. Mature larvae can be found feeding on root tissue in the crown area of big trees such as citrus (Browning et al., 1995).

*Diaprepes abbreviatus* has been the target of numerous integrated pest management (IPM) programs (Bullock et al., 1988; McCoy and Simpson, 1994; McCoy et al., 1995; Schroeder and Sieburth, 1997; Shapiro and Gottwald, 1995). Because of a lack of native parasitoids attacking this weevil in citrus orchards in Florida (Hall et al., 2001) and past failures of classical biological control of this weevil (Beavers et al., 1980), renewed efforts were initiated to introduce, release and evaluate candidate egg parasitoids from the Caribbean Region into Florida (Hall et al., 2002; Peña and Amalin, 2000; Peña et al., 1998). In the Caribbean Region, one of the most important natural enemies of *D. abbreviatus* is *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae)

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(Wolcott). The tetrastichinae *A. vaquitarum* (= *A. gala*) was previously known as *Tetrastichus gala* (Walker) and misidentified as *T. marylandensis* Girault (Schauff, 1987). Females of *A. vaquitarum* deposit their eggs in close contact with those of its host by introducing their ovipositor through the sealed leaves that protect the egg masses of *D. abbreviatus*. On eclosion, larvae of *A. vaquitarum* feed externally on several eggs of *D. abbreviatus* to complete their preimaginal development. Then, they pupate within the sealed leaves and eventually emerge from them. Therefore, *A. vaquitarum* behaves as a predator, a common feature observed among other tetrastichinae (Noyes, 2004).

Specimens of *A. vaquitarum* were collected in the Dominican Republic on *Diaprepes* spp. eggs during 2000 (Peña and McCoy, personal observations) and introduced into Florida. Subsequent to importation and screening under quarantine conditions, the University of Florida (UF) commenced mass-rearing as well as release and evaluation.

Release and recovery efforts were aimed primarily at establishing *A. vaquitarum* using open field releases. Adult wasps obtained from a laboratory culture were released from 2000 through 2003 in seven Florida counties that included areas and commodities severely affected by the weevil (Mannion et al., 2003; McCoy, 1985; Simpson et al., 1996). The total number of adult wasps released in Florida was approx. 700,000 and releases in ornamental sites in southern Florida Miami-Dade County, from April 2001 to September 2003 was 230,270 adults. Because *A. vaquitarum* was considered established in Southern Florida, wasp releases were suspended in that area after September 2003 (Peña et al., 2004). Nevertheless, releases continue nowadays in other parts of the State. Recovery of the parasitoid has been erratic between the south western and central areas of Florida (Peña et al., 2004). However, the parasitoid has been continuously recovered since 2001 in Miami-Dade County in the 16 ornamental fields selected as release sites and it is now established in this area resulting in egg mortality levels that range between 70 and 90% in release sites (Peña et al., 2004). Therefore, this is the first and only case of successful establishment of a natural enemy of *D. abbreviatus* in the USA.

In this paper, we report on the investigations undertaken to quantify the effect of *A. vaquitarum* on the host under field conditions in areas where the parasitoid is dispersing. We also studied in the laboratory the conditions of the host necessary for oviposition and successful parasitism, as well as *A. vaquitarum* daily fecundity. These data should help to understand the process of host selection of *A. vaquitarum*, an example of an under represented group of natural enemies, and to explain the fate of this imported wasp in Florida.

## 2. Materials and methods

### 2.1. Stock colony

Adult *D. abbreviatus* root weevils were obtained from ornamental fields at Homestead, FL (80.2°W longitude, 25.3°N latitude, 1 m altitude). 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 renewed every 2–3 days. Leaves containing *D. abbreviatus* eggs were removed and placed inside a similar cage in a room held 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 and water. Parasitized eggs were removed from the cage 4–5 days later and placed in emergence cages (same dimensions as before) for approximately 14 days. Voucher specimens of *A. vaquitarum* were retained by the USDA-APHIS and the Florida Department of Agriculture and Consumer Services.

### 2.2. Effect of *A. vaquitarum* under field conditions

An ornamental field nursery located in Miami-Dade County, about 7 km north from the nearest parasitoid release site, and showing a considerable infestation of *D. abbreviatus*, was selected as sampling site. Sampled plants were *Thrinax radiata* Lodd. ex Schult. and Schult.f. (Arecaceae). With the exception of May 2004, the field was scouted monthly from December 2003 through June 2004 for *D. abbreviatus* adults, and weevil egg masses. Visual inspections of foliage were conducted by 2–3 people per site by walking around the plant canopy and locating eggs on the lower and mid canopy of plants. Scouts searched for a maximum of 1 h per site. Egg masses were collected, placed in individual test tubes (12 × 75 mm) and sealed with Kimwipes tissue covering one end. These were held for 10 days at 25 °C and 75% RH and 12:12 L:D h until adult emergence (Peña et al., 2004). Adult parasitoids were identified, counted and sexed. Both the total number of eggs per egg mass and the number of eggs consumed by *A. vaquitarum* immatures in each egg mass were recorded. *A. vaquitarum* consumed eggs are characterized by a deflated chorion whereas eggs that hatched normally leave a 'blue print of the chorion' where the egg was originally located. Identification of parasitoids was done by M. Schauff (USDA-APHIS) and G. Evans (FDACS) or by the authors.

### 2.3. Laboratory assays

Assays were conducted at the insectary of the UF's Tropical Research and Education Center (TREC) set at 26.5 ± 1 °C, 12:12 L:D, and approximately 78% RH.

### 2.3.1. Host availability

Because an excess number of hosts should be provided to *A. vaquitarum* to estimate its daily oviposition, presumably mated 0- to 2-day-old female wasps were offered either 1, 2 or 3 egg masses of *D. abbreviatus* 0- to 1-day-old. Each treatment, consisting of a female and the corresponding number of leaves containing egg masses of *D. abbreviatus*, was replicated 20 times. Test units consisted of a 800ml wide mouth Ball glass jar (8.5cm diameter × 16.5cm high) whose lid was replaced by a fine mesh. Inside each jar we introduced a 5.5cm diameter × 4cm high water trough holding one, two or three twigs with two *C. erectus* leaves containing one egg mass of *D. abbreviatus* each. Leaves were obtained from the stock colony maintained at TREC, and a drop of honey was deposited on them as food source for the wasps. Once assembled, one female was released into each arena. One day later, leaves were removed and torn open under a binocular microscope, and the number of eggs of both *D. abbreviatus* and *A. vaquitarum* per egg mass recorded.

### 2.3.2. Assay units

To decide on the type of arena most suitable for the laboratory assays, another preliminary trial was set up to determine if there was a difference in parasitism when *A. vaquitarum* was held either in the glass jars described above or in petri dishes. The petri dish unit consisted of a petri dish (10cm diameter; 1.5cm high: standard sterile polystyrene Fisherbrand petri dishes) containing a 2.5 × 2.5 × 1.0cm plug made of water-saturated florists' sponge wrapped in aluminum foil where the leaves of *C. erectus* containing one less than 24-h-old egg mass of *D. abbreviatus* were inserted. In both cases, leaves came from the stock colony maintained at TREC, and a drop of honey was deposited on them as food source for the wasps. Once assembled, one presumably mated female wasp 0- to 2-day-old was released into each arena. Each treatment was replicated 30 times. One day later, the egg masses were removed and checked as previously described.

### 2.3.3. Host age preference

Egg masses of seven different ages (less than 1- to 7-day-old) were offered to 0- to 2-day-old female *A. vaquitarum* in a no-choice test. Thirty replicates, consisting of one female and one egg mass per age group, were considered. Wasps were removed after 24h of exposure. Fifteen replicates were opened immediately after removal of the wasp and evaluated as previously described. The remaining 15 replicates were left undisturbed for 2 weeks. At that time, the leaves were torn open under a binocular microscope, and the number of pupae counted and their length measured.

### 2.3.4. Daily oviposition

0- to 1-day-old females of *A. vaquitarum* were offered one egg mass of *D. abbreviatus* of the same age daily.

Twenty females were used. Egg masses were replaced by new ones daily until the female died. Immediately after removal, weevil egg masses were checked under a binocular microscope to score daily oviposition. Females not ovipositing during their lifetime were excluded from the analyses. These results were further combined with development time (same authors, in preparation) and the sex ratio estimated from field experiments, to construct life tables for *A. vaquitarum* and to calculate its intrinsic rate of increase ( $r_m$ ), generation time ( $T$ ), and net reproduction ( $R_0$ ) (Birch, 1948; Southwood and Henderson, 2000). The following equation was used for  $r_m$  estimation:  $\sum \exp(-r_m x) l_x m_x = 1$ , where  $l_x$  was the survivorship of the original cohort at age  $x$  and  $m_x$  was the number of female offspring produced per surviving female in each age interval.

Because immature survival could not be determined without seriously damaging egg masses, demographic parameters were calculated using different estimates of this figure (100, 80, and 60%).

### 2.4. Data analyses

Results were subjected to one-way analysis of variance and LSD was used for mean separation at  $P < 0.05$  (STSC, 1987). Proportions, such as sex ratio and percent mortality, were arcsin-transformed before the analysis to meet the assumption of normality and homogeneity of variance. Linear regressions were calculated using the same software package mentioned above.

## 3. Results

### 3.1. Field assays

Average number of eggs per egg mass laid by *D. abbreviatus* on *T. radiata* during the sampling period fluctuated between 45.5 and 37.4 (Table 1). Differences between sampling dates were significant ( $F = 2.62$ ;  $df = 5, 105$ ;  $P = 0.0280$ ). However, no temporal trend could be established ( $F = 2.47$ ;  $df = 1, 110$ ;  $P = 0.1188$ ). Numbers of *A. vaquitarum* emerging from these egg masses did not significantly change during the same period ( $F = 1.03$ ;  $df = 5, 106$ ;  $P = 0.4067$ ), and averaged  $17.1 \pm 1.2$  adults per egg mass (mean  $\pm$  SE). These presented a female biased sex ratio of  $0.16 \pm 0.02$  ( $F = 1.06$ ,  $df = 5, 116$ ;  $P = 0.3866$ ), and developing wasps consumed a mean of  $35.1 \pm 2.0$  *D. abbreviatus* eggs per egg mass to complete their development ( $F = 0.88$ ;  $df = 5, 106$ ;  $P = 0.4981$ ). That indicated that a mean of only  $4.7 \pm 1.2$  larvae of *D. abbreviatus* could eclose per egg mass ( $F = 1.33$ ;  $df = 3, 34$ ;  $P = 0.2805$ ), although during February no eclosion was observed because of the combined effect of parasitism (97.4%) and additional natural mortality (2.6%). Percent mortality, both total and that due

Table 1

Parameters (means  $\pm$  SE) observed on *D. abbreviatus* field collected eggs masses in an ornamental field nursery located 7 km north from the nearest site where the wasp *A. vaquitarum* had been released, Homestead, FL

Date, No. of egg masses	<i>A. vaquitarum</i>		<i>D. abbreviatus</i>				
	No. of adults per egg mass	Sex ratio	No. of eggs per egg mass	No. of eggs consumed by <i>A. vaquitarum</i>	No. of eggs enclosed	Percent total mortality	Percent mortality due to parasitism
23/12/2003, $n = 40$	17.0 $\pm$ 1.2 a	0.17 $\pm$ 0.02 a	45.5 $\pm$ 2.0 a	35.1 $\pm$ 2.0 a	4.8 $\pm$ 1.2 a	90.8 $\pm$ 1.8 a	77.5 $\pm$ 2.6 a
28/01/2004, $n = 15$	17.1 $\pm$ 1.9 a	0.18 $\pm$ 0.03 a	38.5 $\pm$ 2.3 b	32.1 $\pm$ 2.5 a	3.4 $\pm$ 1.1 a	91.1 $\pm$ 2.7 a	83.0 $\pm$ 3.8 a
25/02/2004, $n = 8$	23.1 $\pm$ 4.0 a	0.04 $\pm$ 0.03 a	37.4 $\pm$ 5.4 ab	37.1 $\pm$ 5.6 a	0.0 $\pm$ 0.0 a	100.0 $\pm$ 0.0 a	97.4 $\pm$ 2.6 a
18/03/2004, $n = 18$	17.8 $\pm$ 2.2 a	0.20 $\pm$ 0.04 a	44.1 $\pm$ 2.4 a	31.7 $\pm$ 2.5 a	7.2 $\pm$ 2.2 a	86.3 $\pm$ 3.4 a	73.5 $\pm$ 4.1 a
04/04/2004, $n = 38$	19.2 $\pm$ 1.6 a	0.17 $\pm$ 0.03 a	43.5 $\pm$ 2.3 a	33.0 $\pm$ 1.9 a	6.3 $\pm$ 1.9 a	89.1 $\pm$ 2.5 a	79.1 $\pm$ 3.2 a
06/06/2004, $n = 3$	17.1 $\pm$ 1.2 a	0.16 $\pm$ 0.02 a	45.3 $\pm$ 2.0 ab	35.1 $\pm$ 2.0 a	4.7 $\pm$ 1.2 a	91.0 $\pm$ 2.0 a	77.9 $\pm$ 2.7 a

Means within a column followed by the same letter are not significantly different (LSD,  $P = 0.05$ ).

to the activity of *A. vaquitarum*, did not significantly change during the sampling period and averaged  $91.0 \pm 1.8$  ( $F = 2.07$ ;  $df = 5, 106$ ;  $P = 0.0744$ ) and  $77.9 \pm 2.6$  ( $F = 1.50$ ;  $df = 5, 106$ ;  $P = 0.1960$ ), respectively. Besides *A. vaquitarum*, one specimen of the hyperparasitoid *Horismenus bennetti* Schauff (Hymenoptera: Eulophidae) was collected during a single sampling date (December 23, 2003).

### 3.2. Laboratory assays

#### 3.2.1. Host availability

More than half of the females responded and accepted the egg masses offered for oviposition (Table 2). This percentage increased along with the number of egg masses offered. The mean number of egg masses oviposited by females did not significantly change when the number of egg masses offered increased from 1 to 3 ( $F = 2.59$ ;  $df = 2, 29$ ;  $P = 0.0917$ ). Furthermore, oviposition did not change either ( $F = 1.0$ ;  $df = 2, 29$ ;  $P = 0.3791$ ), and no differences could be observed when considering the ratio between the size of the egg clutches ( $F = 0.61$ ;  $df = 2, 29$ ;  $P = 0.5446$ ). Correspondingly, the

percentage of egg masses parasitized decreased as the number offered increased. Therefore, only one egg mass was offered in subsequent assays.

#### 3.2.2. Assay units

The percentage of females ovipositing was the same in both types of containers (Table 3). Furthermore, no significant differences could be observed both on oviposition ( $F = 0.20$ ;  $df = 1, 34$ ;  $P = 0.6542$ ), and on the ratio between the number of eggs in *D. abbreviatus* and *A. vaquitarum* egg clutches ( $F = 0.12$ ;  $df = 1, 34$ ;  $P = 0.7293$ ) in either type of arena. Therefore, because petri dish arenas were easier to handle than jars, these were eventually selected for the rest of the assays.

#### 3.2.3. Host age preference

From the 15 replicates per egg mass age observed 24 h after oviposition, it appeared that around half of the females offered 0- to 3-day-old egg masses responded and accepted these for oviposition (Table 4). This figure dropped for older eggs, and became nil when offered 7-day-old egg masses. At this age, egg masses were ready to hatch and some neonate *D. abbreviatus*

Table 2

Oviposition of 0- to 2-day-old females of *A. vaquitarum* offered either 1, 2 or 3 egg masses of *D. abbreviatus* (means  $\pm$  SE)

Number of egg masses offered	Number of successfully tested females	% females parasitizing ( $n$ )	No. of egg masses parasitized per female	Oviposition (No. of eggs per female)	<i>D. abbreviatus</i> eggs/ <i>A. vaquitarum</i> eggs	% egg masses parasitized
1	19	52.9 ( $n = 9$ )	1.0 $\pm$ 0.0 a	14.2 $\pm$ 2.8 a	11.3 $\pm$ 3.6 a	52.9
2	19	63.2 ( $n = 12$ )	1.1 $\pm$ 0.1 a	9.9 $\pm$ 2.2 a	19.0 $\pm$ 6.8 a	36.8
3	15	66.7 ( $n = 10$ )	1.4 $\pm$ 0.2 a	14.0 $\pm$ 2.9 a	12.3 $\pm$ 2.9 a	33.3

Initial numbers were 20 females per treatment. Non ovipositing females were excluded from analyses. Means within a column followed by the same letter are not significantly different (LSD,  $P = 0.05$ ).

Table 3

Oviposition (means  $\pm$  SE) of 0- to 2-day-old females of *A. vaquitarum* offered 0- to 1-day-old egg masses of *D. abbreviatus* in different types of arenas for 24 h

Cage type	No. of successfully tested females	% females parasitizing ( $n$ )	Oviposition (No. of eggs per female)	<i>D. abbreviatus</i> eggs/ <i>A. vaquitarum</i> eggs
Jar	29	62.1 ( $n = 18$ )	13.3 $\pm$ 1.0 a	6.1 $\pm$ 1.0 a
Petri dish	29	62.1 ( $n = 18$ )	12.6 $\pm$ 1.1 a	6.7 $\pm$ 1.4 a

Initial numbers were 30 females per treatment. Non ovipositing females were excluded from analyses. Means within a column followed by the same letter are not significantly different (LSD,  $P = 0.05$ ).



Table 4

Host age preference of 0- to 2-day-old females of *A. vaquitarum* offered *D. abbreviatus* egg masses of seven different ages during 24 h

Age of the egg mass (days)	Egg masses torn open 24 h after parasitism			Egg masses torn open 15 days after parasitism		
	% females parasitizing (n)	Oviposition (Eggs per female)	<i>D. abbreviatus</i> eggs/ <i>A. vaquitarum</i> eggs	% egg masses with pupae (n)	Successfully developed pupae (No. per female)	% immature mortality (egg to pupae) <sup>a</sup>
<1	53.3 (n=8)	13.5 ± 2.1 a	6.3 ± 1.5 a	42.9 (n=6)	18.5 ± 4.1 a	0
1–2	60.0 (n=9)	11.8 ± 2.4 a	13.5 ± 6.4 a	71.4 (n=10)	14.5 ± 2.2 a	0
2–3	46.2 (n=6)	15.8 ± 2.2 a	4.9 ± 1.5 a	20.0 (n=3)	15.3 ± 2.3 a	58.0
3–4	26.7 (n=4)	10.7 ± 4.5*	13.4 ± 4.7 a	13.3 (n=2)	13.0 ± 0.9*	54.1
4–5	14.3 (n=2)	28.0 ± 4.0*	3.5 ± 0.3 a	14.3 (n=2)	7.0 ± 0.0*	73.2
5–6	13.3 (n=2)	18.0 ± 2.0*	6.3 ± 3.1 a	6.7 (n=1)	1.0 ± 0.1*	97.1
6–7	0 (n=0)	—	—	0 (n=0)	—	—

Initial numbers were 30 females per age group. Half of the replicates were torn open 24 h after exposure and the remaining 15, 15 days later. Non-ovipositing females were excluded from the analyses. Within a column, data followed by the same letter are not significantly different (LSD,  $P = 0.05$ ).

<sup>a</sup> Calculations based on number of successfully developed pupae and oviposition.

\* Because of low number of females parasitizing these results were not included in the analysis of variance.

larvae were actually found during the counting. A statistically significant strong relationship could be established between the percentage of ovipositing female wasps ( $y$ ) and the age of the egg masses ( $x$ ) ( $y = 71.2857 - 10.1857x$ ;  $F = 61.92$ ;  $df = 1, 5$ ;  $P < 0.0005$ ;  $r = -0.961917$ ). Oviposition in egg masses aged 0- to 3-day-old was not significantly affected by the age of the egg mass ( $F = 0.74$ ;  $df = 2, 20$ ;  $P = 0.04904$ ). Similarly, the ratio between the number of eggs in *D. abbreviatus* and *A. vaquitarum* egg clutches did not change for any of the weevil egg ages where parasitism took place ( $F = 1.24$ ;  $df = 5, 25$ ;  $P = 0.3217$ ). Based on these results, 0- to 1-day-old egg masses were further used to assess the daily oviposition of *A. vaquitarum*.

When the number of pupae obtained per egg mass was considered, important differences were detected. The percentage of egg masses with pupae ( $y$ ) significantly depended on the age of the egg mass ( $x$ ) ( $y = 61.7714 - 9.42143x$ ;  $F = 10.20$ ;  $df = 1, 5$ ;  $P = 0.0242$ ;  $r = -0.819167$ ). Although there were no differences on the number of successfully developed pupae on egg masses aged 0–3 days ( $F = 0.5$ ;  $df = 2, 17$ ;  $P = 0.6130$ ), mortality was high from age 2 to 3 days onwards (Table 4), and almost no *A. vaquitarum* eggs could develop on 5- to 6-day-old *D. abbreviatus* eggs. Although 331 pupae were recovered and their length measured, the number of successfully parasitized egg masses was extremely low for ages older than 2 days (Table 4). The ANOVA performed on these data showed no significant differences for length among ages ( $F = 1.44$ ;  $df = 4, 18$ ;  $P = 0.2605$ ), with a mean pupal length of  $1.42 \pm 0.01$  mm.

### 3.2.4. Fecundity and demographic parameters

The mean longevity of adult females ( $n = 20$ ) was  $15.2 \pm 1.0$  days. It took a mean of  $2.6 \pm 0.3$  days for females to lay their first egg ( $n = 18$ ), although two of them already oviposited on the first day of the assay. The mean oviposition period lasted  $6.5 \pm 0.8$  days, almost the same as the mean postoviposition period, which extended for another  $6.8 \pm 0.9$  days. The oviposition rate

depended on the age of the female (Fig. 1). The oviposition curve rapidly increased until the third day. Then it smoothly declined until no more eggs were observed on the sixteenth day after the start of the assay. The mean number of eggs deposited per female was  $53.4 \pm 6.7$ , with extreme values of 124 and 19 eggs per female. Using these data in combination with the sex ratio observed in the field and the duration of the preimaginal stages,  $r_m$ ,  $T$ , and  $R_0$  were calculated (Table 5).

### 3.3. *Diaprepes abbreviatus*/*A. vaquitarum* egg ratio: field and laboratory

The mean size of the egg masses of *D. abbreviatus* observed in the field and in the laboratory were  $35.1 \pm 2.0$  ( $n = 112$ ) and  $73.6 \pm 3.9$  eggs ( $n = 74$ ), respectively. These figures were statistically different ( $F = 40.68$ ;  $df = 1, 184$ ;  $P < 0.00001$ ). However, the mean size of *A. vaquitarum* clutches did not significantly change ( $F = 0.59$ ;  $df = 1, 184$ ;  $P = 0.442$ ) when comparing field and laboratory results:  $17.1 \pm 1.2$  and  $13.7 \pm 0.8$  eggs, respectively. To further check whether there was a relationship between *A. vaquitarum* and *D. abbreviatus* clutch sizes, both field and laboratory data were analyzed. A statistically significant but relatively weak relationship could be established between the number of eggs deposited by *A. vaquitarum* ( $y$ ) and the size of *D. abbreviatus* egg masses parasitized in the field ( $x$ ) ( $y = 1.18001 * x^{0.645187}$ ;  $F = 21.02$ ;  $df = 1, 110$ ;  $P < 0.00001$ ;  $r = 0.4006$ ) (Fig. 2), showing that on average, one egg of *A. vaquitarum* was laid for every  $4.5 \pm 0.5$  eggs of the weevil. When *D. abbreviatus* clutch size was replaced by the number of weevil eggs consumed, the relationship became stronger ( $y = 0.628598 * x^{0.900972}$ ;  $F = 149.81$ ;  $df = 1, 110$ ;  $P < 0.00001$ ;  $r = 0.7594$ ) (Fig. 3), and indicated that a mean of  $2.6 \pm 0.1$  *D. abbreviatus* eggs were necessary for a larva of *A. vaquitarum* to complete its development. However, the relationship between the number of eggs deposited by *A. vaquitarum* and the size of the weevil egg mass parasitized in all comparable

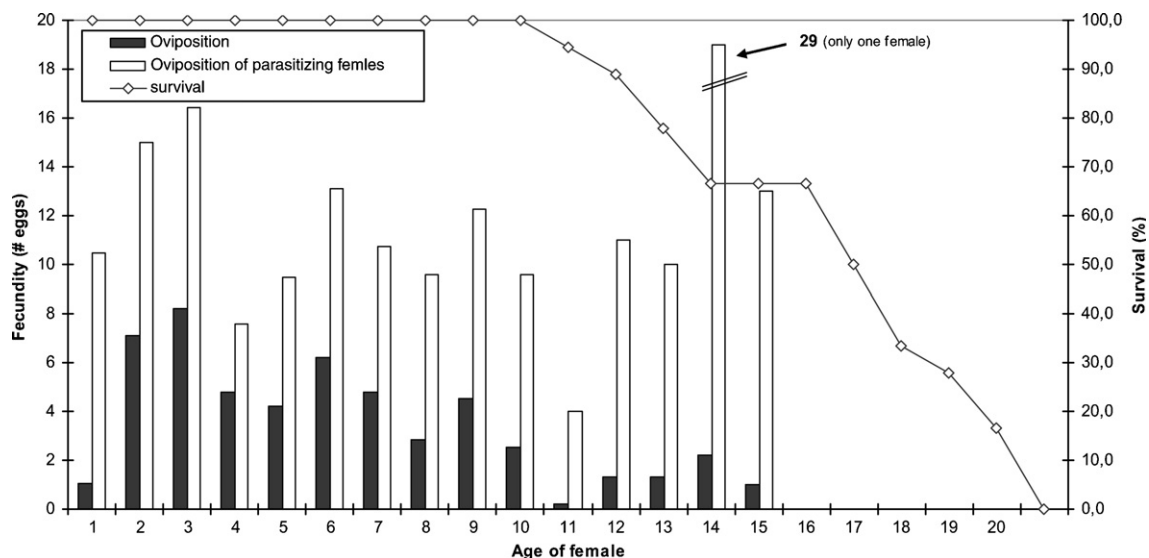


Fig. 1. Daily oviposition (number of eggs per female wasp) of *A. vaquitarum* of different ages (days). Eighteen females were considered.

Table 5

Demographic parameters: net fecundity ( $R_0$ , females per female), generation time ( $T$ , day) and intrinsic rate of increase ( $r_m$ ,  $\text{day}^{-1}$ ) of *A. vaquitarum* at  $26.5 \pm 1$  °C and a photoperiod 12:12 (L:D)

	Immature survival = 1.0	Immature survival = 0.8	Immature survival = 0.6
$r_m$	0.168	0.156	0.142
$T$	22.39	22.63	22.89
$R_0$	43.03	34.42	25.81

A sex ratio of 0.16 was assumed (field results). Duration of immature development was fixed at 17.33 days (unpublished results). Different rates of immature survival were considered.

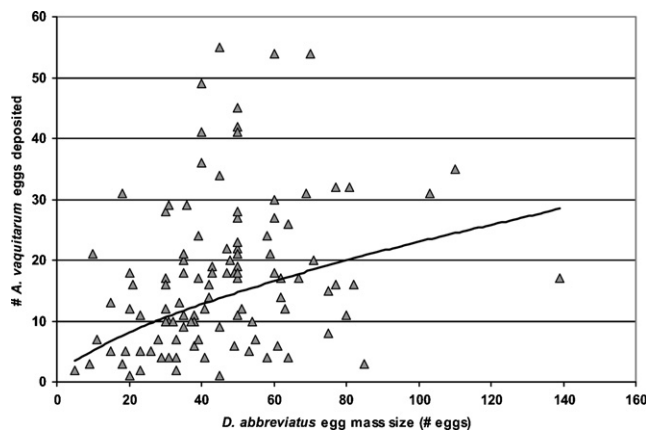


Fig. 2. Relationship between the number of eggs deposited by adult females of *A. vaquitarum* and the size of the oviposited egg mass ( $y = 1.18001 * x^{0.645187}$ ;  $P < 0.00001$ ;  $r = 0.4006$ ). Field results.

laboratory tests (assays using the same female age and number of *D. abbreviatus* egg masses offered) was not significant ( $F = 3.27$ ;  $df = 1, 72$ ;  $P = 0.0747$ ) (Fig. 4), *A. vaquitarum* 0- to 2-day-old females typically laid

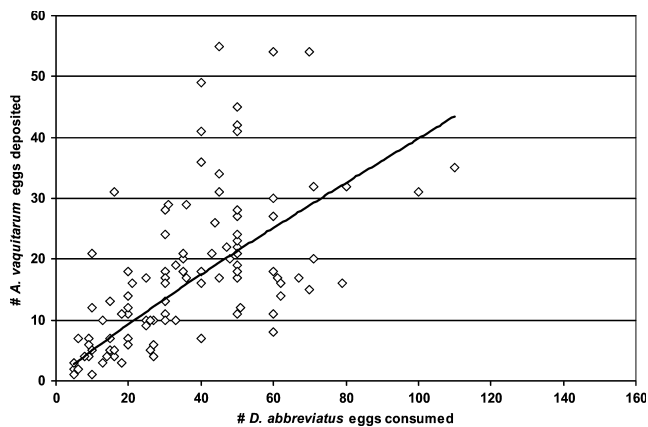


Fig. 3. Relationship between the number of eggs deposited by adult females of *A. vaquitarum* and the number of *D. abbreviatus* eggs consumed by developing immature wasps ( $y = 0.628598 * x^{0.900972}$ ;  $P < 0.00001$ ;  $r = 0.7594$ ). Field results.

about 13–14 eggs irrespective of the size of the host clutch parasitized.

#### 4. Discussion

Mortality rates observed in the present study were similar to rates observed in nursery fields in southern Florida where *A. vaquitarum* was released and has established since 2003 (Peña et al., 2004). This suggests that *A. vaquitarum* is successfully spreading in southern Florida (Miami-Dade County) causing significant mortality to eggs of *D. abbreviatus*. Percent mortality before the release of *A. vaquitarum* was  $7.4 \pm 2.1\%$  ( $2.2 \pm 0.7$  dead eggs/clutch;  $n = 487$  egg masses, 1999; J.E. Peña and R. Duncan, unpublished results). In the

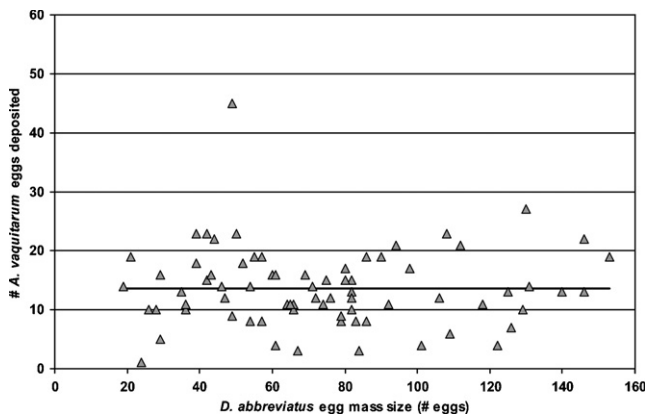


Fig. 4. Relationship between the number of eggs deposited by adult females of *A. vaquitarum* and the size of the oviposited egg mass ( $y = 13.68$ ). Laboratory results.

areas where *A. vaquitarum* is dispersing, percent mortality is higher (Table 1) than in the Caribbean islands where the wasp was originally collected. For instance, during 2002 and 2003 percent predation by *A. vaquitarum* in the Dominican Republic (ex: *D. abbreviatus*) and Dominica [ex: *Diaprepes doublieri* Guerin (Stahl)] was 69 and 43%, respectively (J.E. Peña, unpublished results). One of the possible reasons for this could be low impact of hyperparasitoids (e.g., *H. bennetti*) in *T. radiata* in Florida, which are commonly found in *Diaprepes* sp. parasitized egg masses in citrus in the Caribbean islands (J.E. Peña unpublished results). In our samplings one specimen of *H. bennetti* was collected once.

The mean size of *D. abbreviatus* egg masses observed in the field and in the laboratory were statistically different and this should be attributed not only to the different environment experienced by adult females but, among other possible factors (age, nutrition, competition, wind, etc.), size characteristics of *C. erectus* leaves in comparison to *T. radiata* fronds, which are also more coriaceous than *C. erectus* leaves. However, the mean number of eggs deposited by *A. vaquitarum* per egg mass did not change accordingly. *A. vaquitarum* larvae consumed a mean of 2.57 *D. abbreviatus* eggs per individual to complete their development. This means that an egg mass of 36 eggs of *D. abbreviatus* would be enough to support the development of the mean daily number of eggs deposited by *A. vaquitarum* females in the laboratory (13.68 eggs). If we take into account these figures plus the fact that maximum daily oviposition of *A. vaquitarum* only exceptionally exceeded 20 eggs per female and day (Fig. 2), the average number of eggs in laboratory egg masses of *D. abbreviatus* (73.6 eggs) should not limit oviposition of female *A. vaquitarum*. In contrast, and provided that *A. vaquitarum* females could assess prey availability within egg masses and adjust their egg allocation accordingly, the average of 35.1 eggs

in field egg masses of *D. abbreviatus* could have either limited oviposition or forced young larvae to cannibalism. On the one hand, this could partially explain the weak, but significant relationship found between host and parasitoid clutch sizes recorded in the field. On the other hand this could also account for the high rates of mortality by *A. vaquitarum* found in our study.

Another possible limitation for the oviposition of *A. vaquitarum* is the narrow window for parasitism offered by its host. Successful parasitism by some egg parasitoids occurs only if newly laid eggs are attacked (Strand, 1986). For *A. vaquitarum* this is almost limited to *D. abbreviatus* eggs less than 48-h-old. Oviposition on older egg masses always meant an immature mortality over 50%. The length of the pupae obtained from these egg masses was not different from that obtained on younger eggs. However, occurrence of runts in egg parasitoids feeding on hosts where resources for parasitoid development are just plentiful enough to prevent death have been reported (Jackson, 1958; Salt, 1941). Therefore, further detrimental effects of older eggs on *A. vaquitarum* performance can not be completely excluded, and deserve further research. Based on the results presented, hypothetical immature survival rates used for the estimation of demographic parameters ranged from 100 to 60%. Between these values, generation time almost did not change (2.2% increase), but both net fecundity and the intrinsic rate of increase dropped by 40.0 and 15.5%, respectively. However, even lowest values lie within the range common among other Eulophidae (Urbaneja et al., 2001a). These differences should not hamper the biological control of *D. abbreviatus* by *A. vaquitarum*, but the unavailability of suitable egg masses for oviposition could. Only continuous generations of *D. abbreviatus* or provision of alternative hosts in Florida can guarantee the supply of freshly deposited eggs for *A. vaquitarum* to parasitize, but if discrete generations exist, as it seems to happen in areas of Florida north of Miami-Dade County with winter temperatures below 15 °C, its beneficial role could be seriously impaired. Further research dealing with temperature-development studies of *A. vaquitarum* on *D. abbreviatus* are needed to clarify this situation.

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