OFFSPRING PRODUCTION IN RESPONSE TO HOST EXPOSURE TIMES IN *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE), REARED ON THE GENETIC SEXING STRAIN VIENNA 8 OF *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

Lorena Suarez[1], Guido A. Van Nieuwenhove[2] , Fernando Murua[1], Laura P. Bezdjian[2], Pablo Schliserman[2], Natalia Lara[1], Jorge Escobar[1] and Sergio M. Ovruski[2]

[1]Programa de Control y Erradicación de Mosca de los Frutos de San Juan (ProCEM). Avda. Benavides 8000 (Oeste) CP 5407 Marquesado, Rivadavia, San Juan, Argentina

[2]Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEMEN), División Control Biológico, Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI) – CCT Tucumán – CONICET. Avda. Belgrano y Pje. Caseros, (T4001MVB) San Miguel de Tucumán, Tucumán, Argentina

Corresponding author; E-mail: ovruskisergio@yahoo.com.ar, sovruski@proimi.org.ar

ABSTRACT

The objective of the present study was to assess the effect of different times of exposure to the host on parasitoid emergence rate, parasitoid progeny sex ratio, and on host mortality, as a step toward the development of an efficient mass-rearing system for the braconid *Diachasmimorpha longicaudata* (Ashmead) on larvae of the VIENNA 8 Temperature Sensitive Lethal *Ceratitis capitata* (Wiedemann) strain. The role of host-rearing substrate cues in stimulating the host-searching behavior of parasitoid females was also evaluated. Three exposure times (40, 60, and 120 min) were tested. One hundred 7 d-old host larvae were exposed to 25 female parasitoids per treatment. Larvae mixed with wheat-based rearing medium and larvae without medium were used in each test. A second set of treatments with the same method described above was conducted using late third-instars of the *C. capitata* wild-type strain. These experiments were carried out to assess the quality of the larvae of VIENNA 8 strain in producing *D. longicaudata* adults by comparing them with the larvae of the wild-type strain. Results indicated that the use of larvae of VIENNA 8 strain on their rearing diet at 40 min exposure time significantly increased overall parasitoid offspring production and decreased the host mortality level. Nevertheless, parasitoid emergence recorded from VIENNA 8 strain was notably lower than that recorded from the wild-type strain. Low parasitoid emergence levels and the prevalence of male-biased progeny recorded in all assays are obstacles to development of a parasitoid mass-rearing system using larvae of VIENNA 8 strain as host. Additional studies focusing on host exposure to parasitoids are needed to verify the effect of host larval quality on the production of *D. longicaudata*.

Key Words: biological control, medfly, parasitoids, offspring production, host-searching behavior

RESUMEN

El presente estudio forma parte de una etapa inicial de investigación para el desarrollo de una eficiente cría masiva del bracónido *Diachasmimorpha longicaudata* (Ashmead) sobre larvas de la cepa de *Ceratitis capitata* (Wiedemann) VIENNA 8 TSL. Los objetivos del trabajo involucraron la evaluación del efecto de diferentes tiempos de exposición del huésped sobre la tasa de emergencia del parasitoide, la tasa sexual de la progenie del parasitoide y el nivel de mortalidad del huésped, y la influencia del substrato de cría sobre el comportamiento de búsqueda del huésped por parte de las hembras del parasitoide. Fueron evaluados tres tiempos de exposición (40, 60 y 120 minutos). Se expusieron 100 larvas huéspedes de 7 días de edad a 25 hembras del parasitoide. Por cada tratamiento se utilizaron larvas mezcladas con un medio de cría a base de germen de trigo y larvas sin el medio de cría. Simultáneamente se realizaron tres tratamientos más siguiendo el mismo método previamente descrito pero usando larvas de una cepa salvaje de *C. capitata*. Estos ensayos permitieron comparar la producción de descendientes entre ambas cepas del huésped. Los resultados señalaron que la exposición durante 40 minutos de larvas de la cepa VIENNA 8 con sustrato de cría incrementó significativamente la tasa de emergencia del parasitoide y redujo el nivel de mortalidad del huésped. No obstante, la emergencia de parasitoides registrada de la cepa VIENNA 8 fue notablemente inferior a la obtenida de la cepa silvestre. Los bajos niveles de emergencia de parasitoides y la prevalencia de una progenie con tendencia a machos registrados en todos los ensayos, pueden obstaculizar el avance hacia un adecuado sistema

de cría masiva usando larvas de la cepa VIENNA 8 como huéspedes para el parasitoide. Es necesario efectuar estudios adicionales dirigidos a evaluar el efecto de la calidad de la larva huésped sobre la producción de descendientes en la cría de *D. longicaudata*.

Palabras Clave: control biológico, mosca del mediterráneo, parasitoides, producción de descendientes, comportamiento de búsqueda del huésped

---

The Mediterranean fruit fly (= medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the major pests of fruit crops in Argentina and its presence in many fruit-growing regions is a phytosanitary barrier to the export of fresh fruits (Guillén & Sánchez 2007). In the Province of San Juan, located in the central-eastern region of Argentina known as Cuyo, control strategies against *C. capitata* in temperate pome and stone fruit-producing areas have been implemented by the National Fruit Fly Control and Eradication Program (ProCEM) jointly with the provincial government and the producers. This program aims to establish *C. capitata*-free and/or low prevalence areas based on the concept of area-wide integrated pest management (Guillén & Sánchez 2007). In 1986 the San Juan provincial government constructed the BioPlanta San Juan mass-rearing facility, in order to produce sterile flies to suppress or eradicate *C. capitata* in both urban and rural areas of San Juan Province, Argentina. Therefore, the strategies applied against *C. capitata* through the ProCEM in San Juan, have been based on an integrated use of SIT (sterile insect technique), cultural and chemical controls, jointly with the implementation of a strict quarantine system (Díaz et al. 2008). Furthermore, in 2008 biological control has been incorporated into control activities of ProCEM San Juan by establishing, in a first phase, a laboratory colony of the exotic parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) at BioPlanta San Juan facility.

Diachasmimorpha longicaudata is a larval-prepupal, koinobiont endoparasitoid of several tephritid species, native to Southeast Asia (Wharton 1989) that has been introduced throughout much of Latin American and in the southern United States (Ovruski et al. 2000). It is considered one of the most important biological control agents for augmentative releases against economically important *Anastrepha* (Diptera: Tephritidae) species and *C. capitata* in tropical and subtropical America (Paranhos et al. 2008; Montoya et al. 2011). In 1999, *D. longicaudata* was introduced into Argentina via Mexico with the intent of renewing fruit fly biological control (Ovruski et al. 2003). Therefore, laboratory colonies of *D. longicaudata* were established in Argentina on both wild-type MI94 and CAST191 genetic sexing strains of *C. capitata* (Viscarret et al. 2006) and on both *C. capitata* and *Anastrepha fraterculus*

(Wiedemann), both fly strains originated from wild guava fruits (Ovruski et al. 2011).

The suitability of the VIENNA 8 temperature sensitive lethal (TSL) *C. capitata* strain (Franz 2005) as a host for rearing *D. longicauda* is currently being studied at the BioPlanta San Juan facility. This research is part of a new program of ProCEM-San Juan that includes the use of environmentally friendly strategies to suppress or eradicate *C. capitata*.

Parasitoid emergence and host mortality after exposure are 2 key parameters in the quality control system of the *D. longicaudata* mass-rearing process that must be continuously evaluated (Montoya et al. 2007). Therefore, the present study examined the effect of parasitoid exposure time durations to the host on the parasitoid emergence rate, the parasitoid progeny sex ratio, on the host mortality level, and on the role of host-rearing substrate cues in stimulating host-searching behavior in parasitoid females. The ultimate goal of this study was to identify the factors needed to maximize yields of *D. longicaudata*. This study serves as a first step toward development of an efficient mass-rearing technique for *D. longicaudata* on larvae of VIENNA 8 strain at BioPlanta San Juan facility.

Material and Methods

Study Site and Insects Rearing

The study was performed in the Parasitoid Rearing Laboratory at BioPlanta San Juan facility located in Marquesado, San Juan Province, Argentina. *Diachasmimorpha longicaudata* adults were kept in rectangular iron-framed, mesh-covered cages (0.5 × 0.5 × 0.6 m) at a capacity of 2,000 pairs per cage at 24 ± 1 °C; 65 ± 5% RH and 12:12 h L:D. Each parasitoid rearing cage was provided with water and honey on a daily basis. The colony of *D. longicaudata* was initiated originally in 2008 with 20,000 individuals from the Biological Control Division of the Pilot Plant of Industrial Microbiology Processes and Biotechnology (PROIMI), where *D. longicaudata* had been reared in the laboratory on *A. fraterculus* larvae (Ovruski et al. 2011). Two strains of *D. longicaudata* were used in the experiments. One of them was reared and maintained on third-instar larvae of VIENNA 8 strain, whereas the other one was reared on *C. capitata* larvae of a wild-type strain that had originated from peach (*Prunus persica* (L.)

Batsch; Rosales: Rosaceae) fruit collected in rural areas. The larvae of both *C. capitata* strains were reared on a wheat-based diet fortified with yeast, poplar (*Populus* sp.; Malpighiales: Saliaceae) shavings, sugar, hydrochloric acid, sodium benzoate, nipagin and water.

Experimental Procedure

Exposure times of 40, 60, and 120 min were tested independently to choose between these 3 alternatives a suitable time duration to expose larvae of VIENNA 8 strain to *D. longicaudata* for achieving the greatest parasitoid yield with the highest proportion of female. Late third-instars of VIENNA 8 strain (7 d-old) were used for exposure to the parasitoid. To keep larval weight constant, only male larvae coming from the first batch that dropped out of the larvae rearing tray were collected for assays. In addition, these larvae were not irradiated. Tests were carried out by first placing a group of host larvae into an oviposition device without artificial rearing medium (referred to as "naked" host larvae in the text). This oviposition unit was positioned on the top screen of an experimental cage (0.5 × 0.5 × 0.6 m). Concurrently, another group of host larvae provided with 50 cm³ wheat-based medium (referred to as "covered" host larvae in the text) was placed into an oviposition device on the top screen of another experimental cage. Each oviposition unit was composed of an organdy screen-covered dish (10 cm diameter and 1 cm deep). One hundred of either naked or covered host larvae per oviposition unit were exposed to 25 female parasitoids in each test. This host/parasitoid ratio was based on previous assays for optimizing *D. longicaudata* mass rearing on *A. ludens* at the Moscafrut facility (Metapa de Dominguez, Chiapas, Mexico) (Montoya et al. 2000). Parasitoid females used in the tests were 5 d-old, mated, and had no prior oviposition experience as recommended by Montoya et al. (2000). Parasitoids were released on the floor in the central part of the test cage. The cages were positioned at random on a table and kept under the above-mentioned laboratory conditions. After exposure to the parasitoids, oviposition units were removed from the cages. Host larvae were then placed in emergence cups (0.7 m diam, 0.8 m deep) with meshscreen covers and containing wheat bran as the pupation medium on the bottom. The cups were kept under the above mentioned laboratory conditions until the emergence of adult parasitoids.

A second set of treatments following the same method above described was conducted using late third-instars of the *C. capitata* wild-type strain. These experiments were carried out to assess the quality of the larvae of VIENNA 8 strain in producing *D. longicaudata* adults by comparing with the larvae of wild-type strain.

The number of emerged male and female parasitoids, and the number of noneclosed puparia was recorded daily. Each treatment was replicated 14 times with fresh parasitoids and host larvae. Before starting assays in each replicate, the quality of batches of both strains of host larvae used in the assays was evaluated. For this, a subsample of 10 larvae of both *C. capitata* strains was taken out from each sowing tray and weighed for host quality evaluation. Also, unexposed oviposition units containing either naked or covered larvae of both medfly strains were kept individually to determine the percentages of host mortality. Moreover, all host larvae batches with adult fly emergence percentages below 80% were discarded and were not considered for this study.

Upon release of parasitoids into each test cage, the number of females that landed on the oviposition unit (= females/visit) and also the number of females that inserted their ovipositor into the oviposition unit (= ovipositor insertions) were recorded (García-Medel et al. 2007). The female parasitoids were observed once every 10 min during the first 30 min, each observation lasting 30 sec. Both behavioural observations were used as suitable variables to measure parasitoid preference between host larvae mixed with rearing diet and naked host larvae.

Data Analysis

Percent host mortality was calculated as the fraction of dead host larvae from the total number of larvae exposed to parasitoids plus the fraction of non-eclosed puparia from the total number of recovered puparia. The percentage of emerged parasitoids was determined as the ratio of total number of parasitoid adults divided by the total number of recovered puparia. Parasitoid progeny sex ratio was calculated as the percentage of female offspring from the total number of emerged parasitoids. Data on host mortality, parasitoid emergence, sex ratio of parasitoid offspring, and number of female visits to and ovipositor insertions on the oviposition devices containing either naked or covered host larvae, were compared statistically between treatments by three-way multivariate analysis of variance (MANOVAs) followed by univariate ANOVAs for each response variable at $P = 0.05$. The use of MANOVA followed by ANOVAs, as described above, reduces the probability of inflating the Type I error rate (Zar 1999).

Moreover, host mortalities of each *C. capitata* strain were statistically compared between controls (host larvae not exposed to parasitoids) and between controls and treatments (host larvae exposed to parasitoids) by means of three-way ANOVAs. The exposure times, medfly strains, and the modes of exposing hosts to parasitoids were treated as categorical factors in MANOVAs, and also in the ANOVA performed to compare controls.

Regarding ANOVAs used to contrast host mortality between controls and treatments, the host exposure times and modes, and the host larval status (host larvae exposed to, or not exposed to parasitoids) were the 3 categorical factors. Means were separated with a Fisher's Least Significant Difference (LSD) multiple comparison test at P = 0.05. To meet parametric analysis assumptions, arcsine square root and $\log_e$ (x+1) transformations were applied to the percentage data and to the number of visits and ovipositor insertions, respectively (Zolman 1993). However, the untransformed data are shown as means (± SE) in tables and figures. Pupal weight differences between the 2 *C. capitata* strains were analyzed by a two-sample *t*-test (P = 0.05).

Results

The three-way MANOVA revealed significant effects of the 3 categorical factors and their interaction on *D. longicaudata* performance (Table 1). Corresponding ANOVAs revealed that both host exposure times and medfly strains significantly influenced both parasitoid emergence and host mortality, whereas only the mode in which hosts were exposed to parasitoids (i.e., naked or covered) notably impacted parasitoid emergence (Table 2). The interactions between host exposure times and medfly strains and also between these 2 categorical factors and the host exposure modes were highly significant only for the parasitoid emergence (Table 2). Significantly greater parasitoid emergence (Fig. 1) and lower host mortality (Fig. 2) percentages were recorded from both naked and covered larvae of the 2 medfly strains exposed to parasitoid females at 40 min than at longer exposure times. Furthermore, the percentages of emerged adult parasitoids (Fig. 1) and host mortality (Fig. 2) recorded from both naked and covered wild-type strain larvae were noticeably greater and significantly lower, respectively, than recorded from both naked and covered VIENNA 8 strain larvae exposed to *D. longicaudata* at 40, 60 or 120 min. In addition, parasitoid emergence was significantly higher in covered VIENNA 8 strain larvae at 40 min and at 120 min exposure times, as well as in covered wild-type strain larvae exposed to parasitoids at 60 min (Fig. 1).

The sex ratio of parasitoid offspring did not differ significantly among exposure times, or between the 2 modes of exposing host larvae to parasitoids, or between the 2 medfly strains (Table 1). The mean proportion of parasitoid female offspring recorded from VIENNA 8 strain and from wild-type strain ranged between 32-68% and 47-52%, respectively.

The percent mortalities recorded from host larvae not exposed to parasitoid females (controls) did not differ significantly between the 2 medfly strains, or among exposure times of host larvae or between the 2 modes of exposing host larvae to parasitoids (Table 3). In addition, the interactions among these categorical factors were not significant (Table 3). In contrast, host mortalities recorded from both exposed and non-exposed larvae of the 2 medfly strains differed significantly between controls and treatments (host larvae exposed to parasitoids) and among host exposure times (Table 3). Host mortalities recorded in the controls of both VIENNA 8 and wild-type strains were respectively 3 and 2.3 times lower than for the treatments.

The host larva exposure mode was the only categorical factor that significantly affected the number of visits as well as the number of ovipositor insertions (Table 4). The mean number of *D. longicaudata* female's visits to and ovipositor insertions on the oviposition units containing covered host larvae were 2-3 and 3-4 times significantly higher, respectively, than those recorded in the devices with naked host larvae (visits, $F_{(1, 156)}$ = 132.088, $P < 0.0001$; ovipositor insertions, $F_{(1, 156)}$ = 202.606, $P < 0.0001$) (Table 5).

Wild-type strain larvae were significantly larger than VIENNA 8 strain larvae (t = 56.501, df = 839, $P < 0.0001$, N = 840). Wild-type strain larvae weighed 12.3 ± 0.1 g, while VIENNA 8 strain larvae weighed 10.8 ± 0.1 g.

TABLE 1. SUMMARY OF THREE-WAY MANOVA ON THE EFFECT OF HOST EXPOSURE TIME AND MODE, MEDFLY STRAINS, AND INTERACTIONS OF THESE CATEGORICAL FACTORS ON *DIACHASMIMORPHA LONGICAUDATA* EMERGENCE (EMERGED ADULT PARASITOIDS), PARASITOID SEX RATIO (PROPORTION OF FEMALE OFFSPRING), AND HOST MORTALITY (DEAD HOST LARVAE PLUS NON-ECLOSED HOST PUPARIA).

Source of variation	Wilks' λ	df	Error df	F	P
Host exposure time (HET)	0.36869	6	308	33.207	< 0.0001[a]
Host exposure mode (REM)	0.81073	3	154	11.984	< 0.0001[a]
Medfly strain	0.30521	3	154	116.858	< 0.0001[a]
HET × HEM	0.96723	6	308	0.862	= 0.5229
HET × medfly strain	0.83596	6	308	4.811	< 0.0001[a]
HEM × medfly strain	0.96939	3	154	1.625	= 0.1868
HET × HEM × medfly strain	0.90635	6	308	2.587	= 0.0185[a]

[a]Significant variables

TABLE 2. SUMMARY OF UNIVARIATE THREE-WAY ANOVA ON THE EFFECT OF HOST EXPOSURE TIME AND MODE, MEDFLY STRAIN, AND INTERACTIONS OF THESE CATEGORICAL FACTORS ON PARASITOID EMERGENCE, PARASITOID PROGENY SEX RATIO, AND HOST MORTALITY.

			Dependent variables							
			Parasitoid emergence		Parasitoid offspring sex ratio		Host mortality	
Source of variation	df	Error df	F	P	F	P	F	P
Host exposure time (RET)	2	156	34.330	< 0.0001[a]	0.744	= 0.4770	104.821	0.0001[a]
Host exposure mode (HEM)	1	156	31.824	< 0.0001[a]	1.280	= 0.2595	2.084	= 0.1508
Medfly strain	1	156	297.838	< 0.0001[a]	2.325	= 0.1293	73.833	< 0.0001[a]
HET × HEM	2	156	0.297	= 0.7436	2.067	= 0.1299	0.266	= 0.7667
HET × medfly strain	2	156	13.754	< 0.0001[a]	0.911	= 0.4042	0.489	= 0.6139
HEM × medfly strain	1	156	0.791	= 0.3751	1.809	= 0.1805	2.468	= 0.1182
HET × HEM × medfly strain	2	156	4.425	< 0.0001[a]	1.887	= 0.1549	1.753	= 0.1765

[a]Significant variables
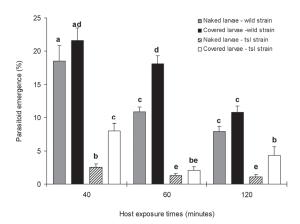

Fig. 1 Mean (±SE) parasitoid emergence percentage recorded from "naked" (without artificial rearing medium) and "covered" (with rearing medium) *Ceratitis capitata* larvae of both VIENNA 8 and wild-type strains exposed to *Diachasmimorpha longicaudata* females. Different letters indicate significant differences among exposure times (Fisher LSD test, P = 0.05).

DISCUSSION

Results from this study indicated that the use of 7 d-old larvae of VIENNA 8 strain with their rearing diet at 40 min exposure time to parasitoid females significantly increased the overall parasitoid offspring production. Nevertheless, the results also revealed that the maximum yield of parasitoids achieved with the 40 min host exposure period was only about 9%. With regard to the latter, it should be noted that the parasitoid emergence rate in the current study were slightly
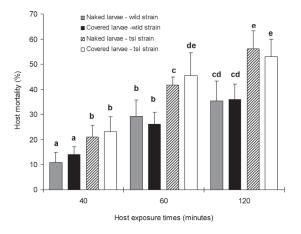

Fig. 2. Mean (±SE) host mortality percentage recorded from "naked" (without artificial rearing medium) and "covered" (with rearing medium) *Ceratitis capitata* larvae of both VIENNA 8 and wild-type strains exposed to *Diachasmimorpha longicaudata* females. Different letters indicate significant differences among exposure times (Fisher LSD test, P = 0.05).

TABLE 3. SUMMARY OF THREE-WAY ANOVAS ON THE EFFECT OF HOST EXPOSURE TIME AND MODE, MEDFLY STRAIN CONTROLS (HOST LARVAE NOT EXPOSED TO PARASITOIDS) OR HOST LARVA STATUS (HOST LARVAE OF THE SAME MEDFLY STRAIN EXPOSED OR NOT EXPOSED TO PARASITOIDS), AND INTERACTIONS OF THESE CATEGORICAL FACTORS ON HOST MORTALITY.

Comparable variables / Source of variation	*df*	Error *df*	*F*	*P*
Comparison between controls (VIENNA 8 strain vs. wild-type strain):				
Host exposure time (HET)	2	156	1.016	= 0.3643
Host exposure mode (HEM)	1	156	0.036	= 0.8501
Medfly strain	1	156	3.588	= 0.0601
HET × HEM	2	156	0.205	= 0.8149
HET × medfly strain	2	156	1.196	= 0.3053
HEM × medfly strain	1	156	0.895	= 0.3696
HET × HEM × medfly strain	2	156	0.181	= 0.8344
Comparison between control and treatment (VIENNA 8 strain):				
Host expposure time (HET)	2	156	41.145	< 0.0001[a]
Host exposure mode (HEM)	1	156	1.360	= 0.2454
Host larva status (HLS)	1	156	439.487	< 0.0001[a]
HET × HEM	2	156	0.336	= 0.7148
HET × HLS	2	156	40.287	< 0.0001[a]
HEM × HLS	1	156	2.621	= 0.1074
HET × HEM × HLS	2	156	0.996	= 0.3718
Comparison between control and treatment (wild-type strain):				
Host exposure time (HET)_	2	156	77.501	< 0.0001[a]
Host exposure mode (HEM)	1	156	0.092	= 0.7618
Host larva status (HLS)	1	156	428.409	< 0.0001[a]
HET × HEM	2	156	1.249	= 0.2895
HET × HLS	2	156	67.435	< 0.0001[a]
HEM × HLS	1	156	0.773	= 0.3807
HET × HEM × HLS	2	156	0.353	= 0.7032

[a]Significant variables

lower than the 13.4% reported for *D. longicaudata* by Lopes & Paranhos (2006), who also worked with third instars of VIENNA 8 strain collected from the first batch at the EMBRAPA Semi-Arid Laboratory, Brazil. Different experimental procedures or differential adaptability to laboratory conditions or distinct host larva quality may be responsible for this difference.

Results from the current study also showed that parasitoid emergence recorded from VIENNA 8 strain was notably lower than that recorded from wild-type strain. This difference in parasitoid emergence rates was previously mentioned by Lopes & Paranhos (2006). Thus, it is possible to consider that the difference between the 2 medfly strains may be the result of distinct internal physiological conditions of host larvae for parasitoid larvae survival or disparities in the quality between larvae of genetic sexing and wild strains. These presumptions may be supported by host mortality data recorded in the present study. Host mortality percentages recorded from both naked and covered larvae of VIENNA 8 strain exposed

to *D. longicaudata* females were significantly 1.4-2.0 times greater than those obtained from wild-type strain larvae exposed to parasitoids. In contrast, host mortality percentages recorded from non-exposure control tests in both medfly strains were substantially similar. Nevertheless, more detailed experimental studies are needed to verify this conclusion.

Data on host mortality showed that the increased exposure time of *D. longicaudata* to the host increased the level of non-eclosed puparia, regardless of the mode in which the host larvae were exposed (i.e., naked or covered). Therefore, the difference between parasitoid emergence and host mortality found in the treatments may be attributed to the action of *D. longicaudata* females during their time of exposure to the host larvae. The high larval mortality levels detected in both *C. capitata* strains at longer exposure times (1- and 2-h) might be caused either by a detrimental effect of the puncture produced when ovipositing parasitoid females insert their ovipositors more than once in an unsuitable host larva, or perhaps

TABLE 4. SUMMARY OF THE THREE-WAY MANOVA ON THE EFFECT OF HOST EXPOSURE TIME AND MODE, MEDFLY STRAINS, AND INTERACTIONS OF THESE CATEGORICAL FACTORS ON THE NUMBER OF PARASITOID FRMALE VISITS TO AND OVIPOSITOR INSERTIONS ON THE OVIPOSITION DEVICES CONTAINING EITHER NAKED OR COVERED LARVAE OF EITHER THE VIENNA 8 OR WILD-TYPE *CERATITIS CAPITATA* STRAIN.

Source of variation	Wilks' λ	df	Error df	F	P
Host exposure time (HET)	0.98322	4	310	0.658	= 0.6213
Host exposure mode (HEM)	0.40556	2	155	113.60	< 0.0001[a]
Medfly strain	0.96394	2	155	2.898	= 0.0581
HET × HEM	0.97881	4	310	0.834	= 0.5040
HET × medfly strain	0.96676	4	310	1.321	= 0.2619
HEM × medfly strain	0.98556	2	155	1.135	= 0.3239
HET × HEM × medfly strain	0.97995	4	310	0.788	= 0.5332

[a]Significant variables

as a consequence of superparasitism, as previously pointed out by González et al. (2007, 2010) and Van Nieuwenhove et al. (2012) to *D. longicaudata* reared on *A. ludens* and *A. fraterculus*, respectively.

The offspring sex ratio, which was preferentially biased toward males in *D. longicaudata* found in almost all tests in the present study involving both *C. capitata* strains, may indicate differential survival of the sexes during parasitoid post-embryonic development (Paranhos et al. 2008) and/or may also reflect low host larva quality that would encourage parasitoid females to lay a reduced amount of fertilized eggs (Montoya et al. 2011). Previous studies conducted by Ovruski et al. (2011) found that *D. longicaudata* reared from *A. fraterculus* larvae, which are twice as large as *C. capitata* larvae, had a considerably higher proportion of female offspring than parasitoids that had developed from larvae of a wild-

type *C. capitata* strain. Thus, physical characteristics, such as size, and/or chemical cues derived from host larvae could influence offspring sex ratio in *D. longicaudata* (Wong 1993; Messing et al. 1993; Eben et al. 2000; López et al. 2009).

Results of behavioral observations provided evidence to explain the high percentages of parasitoid emergence recorded from covered larvae of both medfly strains. *Diachasmimorpha longicaudata* females were more strongly attracted to oviposition units containing host larvae plus rearing medium than to units with only host larvae, albeit the fact that the devices were not offered in a choice situation. These findings are in agreement with earlier observations by Duan & Messing (2000) on ovipositor-probing behaviour in *D. longicaudata* reared on *C. capitata*. It is well known that *D. longicaudata* females exhibit oviposition behaviors in response to chemical cues derived from rotting fruits (Messing & Jang 1992; Eben

TABLE 5. MEAN (±SE) NUMBER OF *DIACHASMIMORPHA LONGICAUDATA* FEMALE VISITS TO, AND OVIPOSITOR INSERTIONS INTO THE OVIPOSITION DEVICES CONTAINING EITHER NAKED OR COVERED LARVAE OF EITHER VIENNA 8 OR WILD-TYPE *CERATITIS CAPITATA* STRAINS.

Host exposure modes	Host exposure times (min)	Medfly strains[a]	Visits[b]	Ovipostior inssertions[b]
Naked larva	40	V8	5.1 ± 0.5 a	2.9 ± 0.5 a
		Wt	7.1 ± 0.8 a	3.8 ± 0.6 a
	60	V8	5.9 ± 0.3 a	5.9 ± 0.3 a
		Wt	8.2 ± 0.9 a	5.1 ± 0.6 a
	120	V8	6.8 ± 0.9 a	4.6 ± 0.4 a
		Wt	7.9 ± 0.7 a	4.1 ± 1.1 a
Covered larva	40	V8	14.8 ± 0.9 bc	9.9 ± 0.9 bc
		Wt	15.2 ± 1.5 bc	11.7 ± 0.9 bc
	60	V8	12.3 ± 1.1 b	10.1 ± 0.8 bc
		Wt	14.1 ± 1.3 bc	8.7 ± 1.0 b
	120	V8	13.4 ± 1.4 b	8.5 ± 0.7 b
		Wt	16.6 ± 1.2 c	11.3 ± 1.1 c

[a]V8, VIENNA 8 strain; Wt, wild-type strain.
[b]Different letters in the same column indicate significant differences (Fisher LSD test, P = 0.05).

et al. 2000; Carrasco et al. 2005) or from fermentation of the artificial rearing medium (Duan & Messing 2000) and also from the host larva themselves (Stuhl et al. 2011).

In conclusion, low parasitoid emergence levels and the prevalence of male-biased progeny found in the present study are potential obstacles to development of a mass-rearing system using larvae of VIENNA 8 strain as host. Certain qualities of the host larva, such as size, age, instar, and species (Montoya et al. 2007; López et al. 2009; Cancino et al. 2009) are among the factors that influence parasitoid emergence, offspring sex ratio, and host mortality, among other quality control parameters in the mass-rearing process of *D. longicaudata*. Therefore, as pointed out by Cancino & Montoya (2004), weight, volume, and age of host larva must be carefully and continuously monitored in the quality control system to achieve more efficient mass rearing of *D. longicaudata*. Following up on the suggestions proposed by Cancino & Montoya (2004) and Montoya et al. (2007), additional studies focusing mostly on the assessment of the VIENNA 8 larvae exposure to parasitoids are needed to verify the effect of host larval quality on production of *D. longicaudata*. Accordingly, a series of future experiments involving different sizes and ages of third instars of VIENNA 8 will be performed.

REFERENCES CITED

CANCINO, J., AND MONTOYA, P. 2004. Desirable attributes of mass reared parasitoids for fruit fly control: a comment. Vedalia 11: 53-58.

CANCINO, J., RUIZ, L., LÓPEZ, P., AND SIVINSKI, J. 2009. The suitability of *Anastrepha* spp. and *Ceratitis capitata* larvae as hosts of *Diachasmimorpha longicaudata* and *Diachasmimorpha tryoni*: Effects PF host age and radiation dose and implications for quality control in mass rearing. Biocontrol Sci. Technol. 19 Suppl. 1: 81-94.

CARRASCO, M., MONTOYA, P., CRUZ-LOPEZ, L., AND ROJAS, J. C. 2005. Response of the fruit fly Parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to mango fruit volatiles. Environ. Entomol. 34: 576-583.

DÍAZ, L. M., MURÚA, F. A., ACOSTA, J. C., AND ESCOBAR, J. M. 2008. Capacidad dispersiva de *Ceratitis capitata* (Diptera: Tephritidae) entre valles agrícolas en San Juan, Argentina. Rev. Soc. Entomol. Argentina 67 (1-2): 155-161.

DUAN, J. J., AND MESSING, R. H. 2000. Effects of host substrate and vibration cues on ovipositor-probing behavior in two larval parasitoids of tephritid fruit flies. J. Insect Behav. 13(2): 175-186.

EBEN, A., BENREY, B., SIVINSKI, J., AND ALUJA, M. 2000. Host species and host plant effects on performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environ. Entomol. 29(1): 87-94.

FRANZ, G. 2005. Genetic sexing strains in Mediterranean Fruit fly, an example for other species amenable to large-scale rearing for the sterile insect technique, pp. 427-451. *In* V. A Dyck, J. Hendrichs and A. S. Robinson [eds.], Sterile Insect Technique Principles and Practice in Area-Wide Integrated Pest Management. IAEA Springer, The Netherlands.

GARCÍA-MEDEL, D., SIVINSKI, J., DÍAZ-FLEISCHER, F., RAMIREZ-ROMERO, R., AND ALUJA, M. 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single- or multiple-species cohorts in field cages: Influence of fruit location and host density. Biol. Control 43: 12–22.

GONZÁLEZ P. I., MONTOYA, P., PÉREZ-LACHAUD, G., CANCINO, J., AND LIEDO, P. 2007. Superparasitism in mass reared *Diachasmimorpha longicaudata* (Asmead) (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Biol. Control 40: 320-326.

GONZÁLEZ P., MONTOYA, P., PÉREZ-LACHAUD, G., CANCINO, J., AND LIEDO, P. 2010. Host discrimination and superparasitism in wild and mass-reared *Diachasmimorpha longicaudata* (Hym.: Braconidae) females. Biocontrol Sci. Technol. 20:137-148.

GUILLÉN, D., AND SÁNCHEZ, R. 2007. Expansion of the national fruit fly control programme in Argentina, pp. 653-660 *In* M. J. B. Vreysen, A. S. Robinson, and J. Hendrichs [eds.], Area-Wide Control of Insect Pests: from Research to Field Implementation. Springer, The Netherlands.

LOPES, F. S. C., AND PARANHOS, B. A. J. 2006. Criação do parasitóide *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) sobre larvas de *Ceratitis capitata* (Diptera: Tephritidae) linhagem *tsl* Vienna 8, pp. 107-114 *In* Jornada De Iniciação Científica Da Embrapa Semi-Árido, 2006, Petrolina. Embrapa Semi-Árido, 2006. (Embrapa Semi-Árido. Documentos, 197).

LÓPEZ, O. P., HÉNAUT, Y., CANCINO, J., LAMBIN, M., CRUZ-LÓPEZ, L., AND ROJAS, J. C. 2009. Is host size an indicator of quality in the mass-reared parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)?. Florida Entomol. 92(3): 441-449.

MESSING, R. H., AND JANG, E. B. 1992. Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to host fruit stimuli. Environ. Entomol. 21: 1189-1195.

MESSING, R. H., KLUNGNESS, L. M., PURCELL, M., AND WONG, T. T. Y. 1993. Quality control parameters of mass-reared opine parasitoids used in augmentative biological control of tephritid fruit flies in Hawaii. Biol. Control 3: 140-147.

MONTOYA, P., LIEDO, P., BENREY, B., CANCINO, J., BARRERA, J. F., SIVINSKI, J., AND ALUJA, M. 2000. Functional response and superparasitism by *Diachasmimorpha*

longicaudata (Hymenoptera: Braconidae), a parasitoid of fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. America 93(1): 47-54.

Montoya, P., Cancio, J., Zenil, M., Santiago, G., and Gutierrez, J. M. 2007. The augmentative biological control component in the Mexican National Campaign against *Anastrepha* spp. fruit flies, pp. 661-670 *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs [eds.], Area-Wide Control of Insect Pests: from Research to Field Implementation. Springer, The Netherlands.

Montoya, P., Cancino, J., Pérez-Lachaud, G., and Liedo, P. 2011. Host size, superparasitism and sex ratio in mass-reared *Diachasmimorpha longicaudata*, a fruit fly parasitoid. BioControl 56: 11-17.

Ovruski, S. M., Aluja, M., Sivinski, J., and Wharton, R. A. 2000. Hymenopteran parasitoids on fruit – infesting tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Intl. Pest Mgt. Rev. 5: 81-107.

Ovruski, S. M., Colin, C., Soria, A., Oroño, L., and Schliserman, P. 2003. Introducción y establecimiento en laboratorio de *Diachasmimorpha tryoni* y *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae, Opiinae) para el control biológico de *Ceratitis capitata* (Diptera: Tephritidae, Dacinae) en la Argentina. Rev. Soc. Entomol. Argentina 62: 49-59.

Ovruski, S. M., Bezdjian, L. P., Van Nieuwenhove, G. A., Albornoz-Medina, P., and Schliserman, P. 2011. Host preference by *Diachasmimorpha longicaudata* (Hymneoptera: Braconidae) reared on larvae of *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomol. 94(2): 195-200.

Paranhos, B. J., Costa, M. L. Z., Ovruski, S. M., Alves, R. M., Lummer, L. B., and Walder, J. M. M. 2008. Offspring in response to parental female densities in the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae: Opiinae). Florida Entomol. 91(4): 628-635.

Stuhl, C., Sivinski, J., Teal, P., Paranhos, B., and Aluja, M. 2011. A compound produced by fruigivorous Tephritidae (Diptera) larvae promotes oviposition behavior by the biological control agent *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environ. Entomol. 40(3): 727-736.

Van Nieuwenhove, G. A., Bezdjian, L. P., and Ovruski, S. M. 2011. Effect of exposure time and ratio of hosts to female parasitoids on offspring production of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) reared on *Anastrepha fraterculus* (Diptera: Tephritidae) larvae. Florida Entomol. 95(1): 99-104.

Viscarret, M. M., La Rossa, R., Segura, D. F., Ovruski, S. M., and Cladera, J. L. 2006. Evaluation of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) reared on a genetic sexing strain of *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). Biol. Control 36: 147-153.

Wharton, R. A. 1989. Classical biological control of fruit infesting Tephritidae, pp. 303-313. *In* A. S. Robinson and G. Hooper, G. [eds.], World Crop Pests: Fruit Flies, Their Biology, Natural Enemies, and Control. Vol. 3b. Elsevier Amsterdam, The Netherlands.

Wong, T. T. Y. 1993. Mass-rearing of larval fruit fly parasitoids in Hawaii, pp. 257-260 *In* M. Aluja and P. Liedo [eds.], Fruit Flies: Biology and Management. Springer-Verlag, New York, Inc. USA.

Zar, J. H. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.

Zolman, J. F. 1993. Biostatistics: Experimental Design and Statistical Inference. Oxford University Press, New York.