El Colegio de la Frontera Sur

Atributos biológicos de *Doryctobracon areolatus*, parasitoide de moscas de la fruta del género *Anastrepha* emergidos de diapausa.

Tesis

presentada como requisito parcial para optar al grado de Maestra en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en Entomología Tropical

Por

Jassmin Cruz Bustos

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examinador de: Jassmín Cruz Bustos

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Atributos biológicos de *Doryctobracon areolatus*, parasitoide de moscas de la fruta del género *Anastrepha* emergidos de diapausa

para obtener el grado de **Maestra en Ciencias en Recursos Naturales y Desarrollo Rural**

	Nombre	Firma
Director	Dr. José Pablo Liedo Fernández	
Co-director	Dr. Pablo Jesús Montoya Gerardo	
Asesora	Dra. Irma Gabriela Pérez Lachaud	Gabriela Pérez
Sinodal adicional	Dra. Ariane Liliane Jeanne Dor	
Sinodal adicional	Dr. Jaime Gómez Ruiz	
Sinodal adicional	M. en C. Javier Francisco Valle Mora	
Sinodal suplente	Dr. Alfredo Castillo Vera	

A mi madre,

mi amada leona,

mi guerrera de luz y amor:

Ludis Bustos Gonzales… inspiración, bendición y fuerza.

A mi padre:

Guerrero y consejero huave,

ejemplo de constancia y superación:

Juan Cruz Nieto.

A mi hija:

Cielo mío

Esperanza de mi Amor

Quetzalli Pérez Cruz

A mi compañero: José Ángel Pérez Lara

Fuego que arde en mi pecho, sin consumirse, ni apagarse.

A todxs los que me han heredado una sonrisa y un aprendizaje en mi Vida Bohemia.

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• Resumen

Doryctobracon areolatus (Szépligeti) es un endoparasitoide solitario nativo de la región Neotropical que se caracteriza por presentar diapausa y por atacar estadios tempranos de moscas de la fruta del género *Anastrepha.* El objetivo de este trabajo fue comparar atributos biológicos como el tamaño, habilidad de vuelo, longevidad y fecundidad entre adultos emergidos de desarrollo directo (sin diapausa) y emergidos de diapausa en condiciones de laboratorio. Los parasitoides se obtuvieron a partir de una colonia de laboratorio criada en larvas de 2° instar de *Anastrepha ludens*, las muestras de puparios parasitados se clasificaron en dos cohortes de acuerdo con su tipo de desarrollo. El tiempo de desarrollo desde el huevo hasta el adulto varió de 18 a 32 días en los parasitoides de desarrollo directo, y de 70 a 278 días para los individuos con diapausa. Los puparios con parasitoides de desarrollo directo tuvieron mayor peso y los adultos son significativamente más grandes que aquellos que pasaron por diapausa. Los parasitoides hembra fueron de mayor tamaño que los machos, independientemente de su tipo de desarrollo. No hubo diferencias en la longevidad de los adultos, la resistencia a la inanición y la emergencia entre parasitoides de desarrollo directo o diapáusicos. La habilidad de vuelo y la fecundidad fueron mayores en la cohorte de desarrollo directo. Las hembras que pasaron por diapausa y las de desarrollo directo iniciaron la oviposición desde el primer día de edad, sin embargo, la proporción de progenie hembra (58%) fue mayor en parasitoides de desarrollo directo, mientras que en parasitoides de diapausa la proporción de progenie macho fue mayor (68.7%). Ambas cohortes produjeron progenie que a su vez entró en diapausa, pero la cohorte de desarrollo directo produjo más (26.55%) que la cohorte de diapausa (9.12%). La edad materna en los parasitoides de desarrollo directo tuvo un efecto significativo en la proporción de descendientes que entraron en diapausa; en las hembras de desarrollo directo más longevas, de 26 a 34 días de edad, el 78.91% de su progenie entró en diapausa, mientras que las hembras de la cohorte diapáusica del mismo rango de edad no presentaron esta particularidad. La diapausa tiene un efecto sobre los atributos biológicos de *D. areolatus*. Las diferencias observadas contribuyen a comprender mejor las causas y las consecuencias de la diapausa en esta especie.

Palabras clave: Aptitud, tamaño, fecundidad, longevidad, sobrevivencia, Tephritidae, desarrollo, Braconidae.

• Introducción.

La familia Tephritidae, incluye diversas especies que ocasionan grandes pérdidas económicas para la fruticultura a nivel mundial (Hernández-Ortiz y Guillén-Aguilar 2010). En México, se encuentran los géneros *Anastrepha* (Schiner), *Rhagoletis* (Loew) y *Toxotrypana* (Gerstaecker) (Hernández-Ortiz 1992).

Los daños directos e indirectos que ocasionan las moscas de la fruta del género *Anastrepha* son muy cuantiosos (Salcedo 2010, Gutiérrez 2010). Están consideradas entre las plagas de mayor importancia comercial y cuarentenaria a nivel internacional, por lo que para su control se establecen programas fitosanitarios a gran escala (Montoya y Cancino 2004), en donde se destaca el uso de enemigos naturales, principalmente avispas parasitoides.

Los parasitoides son los agentes de control biológico más utilizados, y se caracterizan porque el desarrollo de sus estados inmaduros se realiza en el interior o sobre un hospedero, del cual se alimentan y le ocasionan la muerte. Solamente un hospedero es necesario para completar el desarrollo de un parasitoide, mientras que los adultos son de vida libre (Godfray 1994, Quicke 1997). Actúan como un parásito del hospedero, pero su comportamiento ecológico poblacional corresponde al de un depredador porque terminan matando a su presa o huésped (Vinson 1984, Quicke 1997).

Los parasitoides de moscas de la fruta han sido estudiados en cuanto a diversidad de especies e impacto (Aluja et al. 1998, Avendaño 2006, Boscan y Godoy 1996, Cabrera et al. 2006, Hernández-Ortiz et al. 1994). Han desempeñado un papel importante en la reducción de poblaciones de algunas especies de moscas de la fruta (Jiménez-Jiménez 1956, Wong et al. 1991, Sivinski 1996, Montoya et al. 2000).

Los parasitoides de moscas de la fruta se agrupan en diversas familias del Orden Hymenoptera: Braconidae, Aphelinidae, Ichneumonidae, Trichogrammatidae, Encyrtidae, Eulophidae, Diapriidae y Figitidae (Greathead y Waage 1986, Ovruski et al. 2000). Son un grupo muy amplio y diverso que se desarrolla en diferentes estados inmaduros de sus hospederos (huevos, larvas o pupas), por lo que su biología y comportamiento puede presentar grandes variaciones (Cancino y Ruiz 2010). Estos son holometábolos y el tiempo de desarrollo es muy variable entre las diferentes especies. Por ejemplo, los bracónidos parasitoides de larva-pupa como *Diachasmimorpha longicaudata* (Ashmead) y *Diachasmimorpha tryoni* (Cameron) tienen un tiempo de desarrollo de 15 días (Cancino y Ruiz 2010). En *Doryctobracon areolatus* (Szepligeti) es de 27 días (Murillo et al. 2015), mientras que en *Utetes anastrephae* (Viereck) es de 24 días (García-Medel et al. 2007). Algunas especies de parasitoides de moscas de la fruta presentan diapausa, lo cual implica una marcada interrupción en alguna etapa del desarrollo del insecto (Aluja et al. 1998, Carvalho 2005).

Mansingh (1971) clasifica a la diapausa como un tipo de dormancia; señala que es una condición de detención metabólica y del desarrollo. La diapausa desempeña un papel fundamental en los ciclos de vida de invertebrados, lo que les permite pasar a través de períodos de adversidad ambiental, explotar los recursos estacionales fluctuantes y sincronizar la reproducción (Koštál y Denlinger 2011). La diapausa es considerada una estrategia de adaptación en insectos que habitan en regiones de clima templado para sobrevivir el invierno (Koštál y Denlinger 2011). Sin embargo, Denlinger (1986, 2002) señala que la diapausa también puede ocurrir en los insectos que habitan ambientes tropicales, aunque los factores que regulan este fenómeno no han sido completamente comprendidos. Según Carvalho (2005), en regiones tropicales la diapausa permite a los insectos sobrevivir durante la estación seca, que se caracteriza por la falta de alimento. Según Hahn y Denlinger (2007) es una respuesta de desarrollo genéticamente programada y controlada por hormonas que ocurre en una etapa específica para cada especie. En algunas especies se ha observado que tiene efectos ("trade-off") sobre la sobrevivencia y reproducción (Ishihara y Shimada 1995), es decir puede haber una compensación entre la cantidad de energía gastada en la diapausa y el fitness del adulto (Ellers y van Alphen 2002).

La diapausa es también esencial para comprender la historia de vida de los insectos a nivel de población, así como para modelar y predecir el tamaño y la actividad estacional de sus poblaciones. El desarrollo de estrategias de manejo de plagas requiere un buen conocimiento de la diapausa en especies de importancia económica (Koštál y Denlinger 2011).

Para la región Neotropical, en estudios de campo se reportan algunas especies nativas de parasitoides de moscas de la fruta que presentan diapausa, como *D. areolatus*, *Doryctobracon brasiliensis* (Szépligeti), *U. anastrephae*, *Opius sp. Opius bellus* (Gahan) (Hymenoptera: Braconidae), *Aganaspis pelleranoi* (Brèthes) y *Odontosema anastrephae* (Borgmeier) (Hymenoptera: Figitidae). También se ha observado en la especie introducida *D. longicaudata* (Aluja et al. 1998, Carvalho 2005, Ovruski et al. 2016).

La presente investigación se realizó con la especie *D. aerolatus*, un endoparasitoide solitario koinobionte (permite que los hospederos continúen su alimentación y crecimiento después de ser atacados) que se reporta ampliamente distribuido desde Argentina a México y hasta Florida (Wharton y Marsh 1978, Murillo et al. 2015). Esta especie tiene la capacidad de ovipositar en huevos y larvas de 1er, 2do y 3er instar de *A. obliqua* (Murillo et al. 2015), además ha sido reportada parasitando *Anastrepha ludens* (Loew), *Anastrepha striata* (Schiner), *Anastrepha fraterculus* (Wiedemann*), Anastrepha serpentina* (Wiedemann), *Anastrepha bahiensis* (Costa Lima), *Anastrepha crebra* (Stone), *Anastrepha spatulata* (Stone) y *Rhagoletis pomonella* (Walsh) en el centro y sur de México (Aluja et al. 2013).

Aluja y colaboradores (1998) reportaron que *D. areolatus* es la especie que presenta diapausa con mayor frecuencia y con la mayor duración en México, hasta 11 meses cuando los hospederos provenían de frutos de *Spondias mombin* L.; mientras que Carvalho (2005) reportó una diapausa máxima para *D. areolatus* de 414 días cuando provenían de hospederos en frutos de *Eugenia uniflora* L. en Brasil. En Argentina, Ovruski y colaboradores (2016) reportaron diapausa prolongada de entre 369 y 373 días en 10% de los individuos.

La factibilidad del control biológico de moscas de la fruta está íntimamente ligada a la posibilidad de producir masivamente al enemigo natural seleccionado (Montoya 1999). Actualmente, en el laboratorio de Control Biológico del Programa Moscafrut se realizan bioensayos para el establecimiento de una cría de *D. areolatus*. De lo anterior se deriva la necesidad de conocer la frecuencia de individuos de esta especie que presentan diapausa, así como determinar si algunos parámetros de aptitud de los organismos adultos emergidos de condiciones diapáusicas son diferentes de aquellos no diapáusicos (de desarrollo directo).

La relevancia del estudio de la diapausa en parasitoides de moscas de la fruta radica en que no sólo ofrece un potencial para la investigación fundamental sobre la diapausa, sino que además, los individuos que entran en diapausa pueden poseer habilidades diferentes de aquellos no diapáusicos que pudieran ser de utilidad en el control biológico o limitar su acción como agente biológico. Por ejemplo, efectos sobre la sobrevivencia, reproducción, tolerancia al estrés ambiental, habilidad de vuelo, etc., (Ishihara y Shimada 1995, Denlinger 2002, Hahn y Denlinger 2007, Denlinger 2008, Koštál y Denlinger 2011).

El objetivo de este trabajo fue determinar sí existe diferencia entre los atributos biológicos (tamaño del adulto, emergencia, habilidad de vuelo, longevidad, resistencia a la inanición, fecundidad) de individuos que pasaron por diapausa e individuos de desarrollo directo en *D. areolatus*.

•Artículo científico, enviado para su publicación.

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Biological attributes of diapausing and non-diapausing *Doryctobracon areolatus* (Hymenoptera: Braconidae), a parasitoid of *Anastrepha* spp. (Diptera: Tephritidae) fruit flies.

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Corresponding author:
Pablo Liedo
El Colegio de la Frontera Sur (ECOSUR)
Carretera Antiguo Aeropuerto Km 2.5 Tapachula, Chiapas, CP 30700 México
Tel. +52-962-628-9800 ext. 5471
E-mail: pliedo@ecosur.mx

Running head: Diapause in *Doryctobracon areolatus*

Biological attributes of diapausing and non-diapausing *Doryctobracon areolatus* (Hymenoptera: Braconidae), a parasitoid of *Anastrepha* spp. (Diptera: Tephritidae) fruit flies

Jassmin CRUZ-BUSTOS[a], Pablo MONTOYA[b], Gabriela PÉREZ-LACHAUD[c], Javier VALLE-MORA[a], and Pablo LIEDO[a]

[a] El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, Tapachula, Chiapas CP 30700, México (jacruz@ecosur.edu.mx, pliedo@ecosur.mx, jvalle@ecosur.mx)
[b] Programa Moscafrut SAGARPA-SENASICA, Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas CP 30860, México (pablo.montoya@iica-moscafrut.org.mx)
[c] El Colegio de la Frontera Sur, Ave. Centenario km 5.5, Chetumal CP 77014, México (igperez@ecosur.mx)

Abstract

Doryctobracon areolatus (Szépligeti) is a solitary endoparasitoid native to the Neotropics that attacks eggs and young larvae of *Anastrepha* fruit flies, and can enter diapause under tropical and subtropical conditions. Our aim was to compare biological attributes, such as size, flight ability, starvation resistance, longevity and fecundity of diapausing and non-diapausing pupae and adult parasitoids under laboratory conditions. Parasitoids were obtained from a laboratory colony reared on *Anastrepha ludens* larvae. Parasitized host pupae were sorted in two cohorts according to their diapause condition. Developmental time from egg to adult ranged from18 to 32 days in non-diapausing parasitoids, and from 70 to 278 days for diapausing individuals. Pupal weight and adult measurements were greater in non-diapausing than in diapausing parasitoids. Female parasitoids were greater in size than males, regardless their diapause condition. There were no differences in adult longevity, starvation resistance, and emergence between diapausing and non-diapausing wasps. In the diapause cohort, females showed greater longevity than males, but in those from direct development, there were no differences between sexes. Flight ability and fecundity rates were greater in the non-diapausing than in the diapause cohort. The proportion of female offspring was greater in the non-diapausing cohort (58%), whereas in the diapausing cohort the male offspring proportion was greater (68.7%). Both cohorts produced diapause offspring, but the non-diapause cohort produced more (26.55%) than the diapausing cohort (9.12%). Maternal age had a significant effect on the proportion of diapause offspring, in 26 to 34 days old non-diapausing females, 78.91% of their offspring entered into diapause. We confirmed that diapause has an effect on the biological attributes of *D. areolatus.* The observed differences contribute to better understand the causes and consequences of this diapause that is not dependent of environmental conditions. The effect of maternal age on the proportion of offspring entering into diapause might represent a cue for this understanding.

Keywords: Diapause, size, survival, starvation resistance, fecundity, flight ability

1. Introduction

Fruit fly parasitoids are grouped in five families of Hymenoptera: Braconidae, Diapriidae, Eulophidae, Figitidae, and Ichneumonidae (Ovruski et al., 2000). This represents a wide group that exploit different immature stages of their hosts (eggs, larvae and pupae) with important variations in their biology and behavior (Godfray, 1994). All identified species are holometabolous and their developmental type and time are highly variable, depending on the species and the parasitized host stage. Some species may present diapause (Aluja et al., 1998; Carvalho, 2005; Ovruski et al., 2016).

Diapause is defined as a type of dormancy in which metabolic and developmental arrest occur in the life cycles of many invertebrates (Mansingh, 1971). This allows them to survive in periods of environmental adversity, exploit fluctuating seasonal resources, and synchronize their reproduction (Koštál and Denlinger, 2011). Diapause is considered an adaptive strategy in insects that live in temperate regions for winter survival (Denlinger, 2002; Koštál and Denlinger, 2011). However, it has also been observed in organisms that live in tropical regions (Denlinger, 2002). Several species of tephritid fruit fly parasitoids have been reported to enter into diapause: *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti), *Utetes anastrephae* (Viereck), *Opius bellus* (Gahan) (Hymenoptera: Braconidae), *Aganaspis pelleranoi* (Brèthes) and *Odontosema anastrephae* (Borgmeier) (Hymenoptera: Figitidae), that are native to the Neotropics, as well the introduced species *Diachasmimorpha longicaudata* (Ashmead) (Aluja et al., 1998; Carvalho, 2005; Ovruski et al., 2016). Aluja et al. (1998) reported that *D. areolatus* is the species that presents diapause with greater frequency and with the longest duration in Mexico, up to 11 months in fruits of *Spondias mombin* L. Carvalho (2005) reported a maximum diapause for *D. areolatus* of 414 days in fruits of *Eugenia uniflora* L. in Brazil.

The genus *Doryctobracon* Enderlein, 1920, is endemic to the Americas (Ovruski, 2003). *Doryctobracon areolatus* is a solitary endoparasitic koinobiont that is reported widely distributed from Mexico to Argentina and is present in Florida (Wharton and Marsh, 1978; Murillo et al., 2015). This species has the ability to oviposit in *A. obliqua* (Macquart) eggs and larvae of 1st, 2nd and 3rd instars (Murillo et al., 2015). In addition, it has been reported parasitizing *A. ludens* Loew, *A. striata* Schiner, *A. fraterculus* Wiedemann, *A. serpentina* Wiedemann, *A. bahiensis* Costa Lima, *A. crebra* Stone, *A. spatulata* Stone and *Rhagoletis pomonella* Walsh, in Mexico (Aluja et al., 2013).

D. areoloatus developmental time, when it does not enter into diapause, is 27 days (Murillo et al., 2015); adults usually emerge in synchrony with their host. Under laboratory conditions and optimal diet, adult females live around 20 days (Stuhl et al., 2011; Aluja et al., 2013). This neotropical species shares evolutionary history with *Anastrepha* fruit flies (Aluja, et al., 1998; Ovruski et al., 2000; Carvalho 2005).

The study of diapause in fruit fly parasitoids is relevant not only because of its potential to infer evolutionary relationships, but also to provide insights about the use of these species as biocontrol agents. Biological characteristics of diapausing individuals such as reproductive capacity, tolerance to environmental stress or flight ability can influence their use in biological control projects (Denlinger, 2002, 2008; Koštal and Denlinger, 2011).

Our aim in this research was to determine if there are differences between the biological attributes of individuals who enter into diapause and individuals of direct development of *Doryctobracon areolatus*. The biological attributes evaluated were: size, flying ability, starvation resistance, longevity, fecundity, offspring sex ratio and diapause frequency.

2. Materials and methods

2.1 Biological material

Parasitized pupae and adult parasitoids came from the *D. areolatus* colony that is maintained in the Biological Control laboratory of the Moscafrut Program (SAGARPA-IICA), located in Metapa de Domínguez, Chiapas, Mexico. Second instar larvae of *A. ludens* were used as hosts. Pupae were placed in 30 ml plastic containers, covered with organza fabric to allow ventilation. They were kept on a coconut fiber substrate that was kept slightly humid with water applied by spraying until adult emergence. Laboratory conditions were 24 ° ± 1 ° C temperature, 80-90% relative humidity and a 12:12 L:D cycle.

Two cohorts were obtained based on the type of development of the parasitoids: host pupae with parasitoids without diapause (direct development), and host pupae with evidence of containing parasitoids in diapause (hereafter non-diapausing and diapausing, respectively). The pupae containing larvae of the parasitoid in diapause were distinguished by observing under the stereoscopic microscope the 3rd instar parasitoid larva inside the host pupa.

2.2 Size

Fly pupae were individually weighed using an analytical scale (Ohaus, Pine Brook, NJ) and then placed in plastic containers with 24 independent cells. Each cell was conditioned with lightly moistened coconut fiber substrate, where they remained under laboratory conditions until adult emergence.

Pupal and adult measurements were made with a stereoscopic microscope (Carl Zeiss®, Stemi 2000C) fitted with a scale in the right eyepiece. Thirty pupae of each physiological condition were randomly selected and the width and length, from the end of the buccal carinae to the end of the anal pore, were measured. For adult measurements, the cells were checked daily, recording for each emerged parasitoid the date and sex. Each individual was placed in a 1.5 ml vial with an 80% alcohol solution. We measured: 1) length of the left posterior tibia, 2) length of the left wing, 3) thorax length, 4) abdomen length, 5) antenna length, and 6) ovipositor length (Sagarra et al., 2001).

2.3 Emergence and Flight ability

Samples of 100 diapausing and 100 non-diapausing pupae were placed inside a 10 cm diameter X 10 cm height PVC tube, painted black, with the inner wall of the tube covered with neutral powder to prevent the outflow of non-flying parasitoids. These devices were placed inside a 65 x 65 x 45 cm metal frame cage covered with organza fabric (SENASICA, 2012). From the beginning of adult emergence, observations were made every 12 hours. Adult parasitoids that were able to fly out of the tube were collected and their number and sex were recorded. The number of non-emerged parasitoids, parasitoids that opened the puparium but could not get out of it, and non-flying parasitoids (those that despite having emerged completely from the puparium did not manage to leave the tube) (SENASICA, 2012) were recorded. The number of parasitoids that entered or remained in diapause was also recorded. The number of parasitoids in diapause was subtracted from the initial number to determine the percent of emergence and flyers. Two replicates were done for direct development individuals and 3 replicates for diapausing individuals.

2.4 Longevity and Starvation Resistance

Adults that emerged from both, diapausing and non-diapausing pupae, were individually placed in 10x12x16 cm plastic cages. Honey embedded in towel paper placed on a plastic lid (1.5 x .07 cm) was provided as food (Montoya et al., 2012). Water was supplied in 20 ml plastic containers with a cone of absorbent paper. Thirty males and 30 females of each condition were observed. Daily, the number of dead individuals, type of development and their sex was recorded.

For starvation resistance, at emergence, 30 males and 30 females for each type of development (direct development and diapause) were placed in plastic cages (10x12x16cm) without food and water. Daily, the cages were checked and the dead parasitoids were collected and recorded, noting their type of development, sex and age.

2.5 Fecundity

Forty pairs of adults emerged from each, diapausing and non-diapausing pupae, were used. Each pair was placed in a 10x12x16 cm plastic cage. They were provided with food (honey) and water as described above. The food was changed twice a week. Oviposition devices were made of a guava fruit (*Psidium guajava* L.) where the mesocarp and seeds were removed and a layer of approximately 5 mm of epicarp was left. The space inside the fruit was filled with a 2.5 cm in diameter plastic sphere and 30 *A. ludens* larvae of second instar mixed with larval diet. The spheres had a hole in the center through which a piece of galvanized wire 7 cm long was passed, to hang the device inside the cage. One oviposition device was placed in each cage and they were replaced every 24 hours until the death of the female.

A. ludens larvae were removed from the oviposition devices and placed in containers with larval diet for 7 days more. Then, the mature larvae were separated from the diet with a sieve and water and placed in plastic bottles with moist coconut fiber to promote pupation. At emergence, the number and sex of the emerged adult parasitoids was recorded. In the case of non-emerged pupae, they were examined under a stereoscopic microscope to determine if they were in diapause, dead or were non parasitized *A. ludens* pupae. For each female we recorded the number of male, female and diapausing offspring produced per day.

2.6 Statistical Analysis

Differences in development time (mean ± SE) were analyzed by a t test. Morphometric data (mean ± SE) were analyzed using a canonical multivariate analysis of variance (MANOVA) (Fay and Shaw, 2010), and multiple comparisons were made using the first two canonical variables (Friendly and Fox, 2017). Flight ability (mean ± SE) was analyzed by t test. Longevity data were subjected to a survival analysis (Log-rank) (Therneau and Grambsch, 2000) with censorship data by interval and multiple comparisons by orthogonal contrasts with Bonferroni correction, with a level of significance of 5%. Life tables were constructed with data from the survival and fecundity test. The survival of the females was analyzed by an asymptotic Log-rank test for data with interval censorship (Therneau, 2015). All analyzes were performed using Software R version 3.4.2. (Fox, 2005; Fox and Bouchet-Valat, 2017; Fox, 2017; Venables and Ripley, 2002; R Core Team, 2017).

3. Results

3.1 General characteristics

The diapausing organisms used in the various bioassays were obtained from 5,832 pupae with evidence of diapause, while the direct development parasitoids were obtained from 934 pupae. Developmental time from egg to adult, which was from the exposure of the host (*A. ludens* second instar larvae) to adult emergence, ranged from 70 to 281 days for diapausing parasitoids and from 18 to 31 days in non-diapausing ones (Figure 1). Table 1 shows the mean (± SE) development time and mean pupal weight (± SE) of parasitized host pupae that yielded female and male parasitoids in each development condition.

3.2 Size

The multivariate canonical analysis, considering as a whole the length, width and weight of the pupae, indicated a significant interaction of sex and type of development (Manova, $F = 3.86$, $df = 3,115$, $p = 0.01$), which indicates that both factors significantly affect the measured variables. Pupal weight of non-diapausing parasitoids was greater than in diapausing ones. However, pupae of diapausing females tended to be longer and wider, but the only significant difference was in pupal length when compared with non-diapausing females. Likewise, pupae from which female parasitoids emerged were heavier, longer and wider than the pupae containing males (Table 1, Figure 2).

Regarding adult size, statistical differences were found in type of development ($F = 7.06$, $df = 5, 74$, $p < 0.0001$) and sex ($F = 17.78$, $df = 5, 74$, $p < 0.0001$), but there was not a significant interaction between these two factors ($F = 1.07$, $df = 5, 74$, $p > 0.05$). Parasitoids from direct development had longer tibia, wing, abdomen and antennae, compared with parasitoids from diapause (Table 1, Figure 3A). Among females, non-diapausing individuals were greater than diapausing ones in wing, abdomen, antennae and ovipositor length (Table 1, Figure 3B). Among male parasitoids, those from direct development had longer tibia, wing and antenna, than diapause males. Regardless the type of development, females were greater than males in tibia, wing and thorax length (Table 1, Figure 3C). The canonic analysis, considering all six measurements, showed that non-diapausing parasitoids were significantly greater in size than diapausing ones.

3.3 Emergence and flight ability

Adult emergence of non-diapausing parasitoids (75.69% ± 2.96) was greater than in diapausing parasitoids (39.86% ± 9.69). However, the difference was not significant (t test, t = 2.82 df = 3, p> 0.05). The percentage of flying parasitoids from direct development (55.56% ± 3.54) was significantly higher than in parasitoids that passed through diapause (23.36% ± 5.36) (t test, t = 4.03, df = 3, p <0.05) (Table 2).

3.4 Longevity and starvation resistance

There were significant differences in survival (Log-rank test, χ^2 = 98.46, df = 7, p <0.001). When food was provided, diapausing females showed the greatest mean longevity (24 days), but it was not significantly different from non-diapausing males and females. Longevity of diapausing females was significantly different from longevity of diapausing males (Z = 3.46, p = 0.0005).

No significant differences were found in starvation resistance between diapausing and non-diapausing cohorts, nor between females and males (Table 3). In the fecundity bioassays when females were provided with hosts, the difference in the survival of the females of both conditions was not significant (Z = 1.24, p> 0.05, Figure 4).

3.5 Fecundity

Differences in reproduction of parasitoids emerged from direct development and from diapause were observed, both in the fecundity rates and in the distribution of the offspring. Fecundity rates were greater for the non-diapausing cohort than for the diapausing one. Non-diapausing females also produced more daughters and more individuals entering into diapause (Table 4). Females of both cohorts started reproduction from the first day of adult life. In the direct development cohort, daily net fecundity had its maximum value on day 1 (Figure 5). In this case, female offspring represented 42.58% of the total. In diapausing females, the peak fecundity was observed on day 17 and males represented 62.54% of the total offspring. Both cohorts produced offspring that entered into diapause, 26.55% in non-diapausing females and 9.12% in diapausing females (Table 4).

About 67% of the observed pairs produced offspring in both cohorts. Of those pairs with offspring, 86.2% of the non-diapausing cohort and 100% of the diapausing cohort produced males, whereas 62% and 48%, produced females, respectively. The fraction of pairs that produced offspring that entered into diapause was 58% and 48% for the non-diapausing, and diapausing cohorts, respectively. Maternal age in the non-diapausing cohort had an important effect on the production of offspring that enter into diapause. Over 78% of the offspring of 26 to 34 days-old females from the direct development condition entered into diapause, and the rest were only males (Figure 5). Diapausing females that reached this age range did not produced offspring entering into diapause.

4. Discussion

We found that diapausing and non-diapausing *D. areolatus* individuals were different in pupal and adult size, flight ability and reproductive dynamics, whereas there were no differences in their emergence, starvation resistance and adults' survival. Females from both cohorts produced diapausing offspring. However, we found that non-diapausing females produced a much larger proportion of diapausic offspring at old ages (25 to 34 days-old). The physiological, behavioral and evolutionary reasons of this are new research questions.

In insects, diapause can occur at any stage of development and the stage is species-specific (Hanh and Denlinger, 2007; Denlinger and Armbruster, 2014). Here, we found that *D. areolatus* enter into diapause at the third instar larval stage, according to instars description by Murillo et al. (2015). This is in agreement with Ovruski et al. (2016) who found diapausing third instar larvae of *D. areolatus* parasitizing *A. fraterculus* in the Yungas tropical forest in southern Argentina.

The developmental time of diapausing individuals ranged from 70 to 281 days. This time is shorter than the time reported for the same species collected in the field from different fruit species in Mexico, Brazil and Argentina (Aluja, et al., 1998; Carvalho, 2015; Ovruski, 2016). This difference can be attributed to intrinsic traits, such as the faster development of laboratory adapted strains, or to environmental conditions. Those reported in the literature were from wild individuals under a wide range of environmental conditions, such as fruit fly species hosts (*A. obliqua, A. ludens, A. fraterculus, A. serpentina* and *A. striata*), fruit species (*S. mombin*, *P. guajava*, *E. uniflora* and *Tapirira mexicana* March.) and climatic conditions. Our insects were maintained under laboratory-controlled conditions and were reared on mass-reared *A. ludens*.

Another factor affecting diapause duration are the metabolic reserves (Ishihara and Shimada, 1995). Nutrient storage and metabolism rate could determine diapause duration (Hahn and Denlinger, 2007). According to Ellers and van Alphen (2002), the longer the duration of diapause, the higher the mortality rate. This is because only those larvae with enough nutritional resources will survive this period.

Nevertheless, we found no difference in adult emergence between the two cohorts, despite diapausing individuals had lower pupal weight, which could mean a lower amount of fat reserves. In addition, we found that adults emerging from diapause were smaller. Aluja, et al. (1998) and Ovruski et al. (2016) previously noted the smaller larval and pupal size (weight) of diapausing individuals, but no comparisons had been made on adult dimensions. The changes in body size and reserves accumulation are among the most notorious characteristics of diapausing individuals, and this has been related to the energetic demands of the diapause state (Ellers and van Alphen, 2002; Hahn and Denlinger, 2007). The differences in size we observed here are in agreement with this generalization.

The greater wing length of non-diapausing compared with diapausing ones could be associated to their greater flight ability observed in the former cohort. Kölliker-Ott et al. (2003) proposed that wing size, shape and asymmetry influenced the field fitness in *Trichogramma* egg parasitoids.

Diapause did not exert any effect on starvation resistance; furthermore, females emerging from diapause and provided with food and water showed longer longevity than non-diapausing females (Table 3, Fig. 4). This suggest that adults emerging from diapause could be more efficient using the nutritional reserves or had similar reserves than those emerging from direct development.

Non-diapausing females produced a higher proportion of female offspring, whereas diapausing females produce more males. This difference could be explained by a lower mating frequency in the diapause cohort, which would increase the production of parthenogenetic male individuals. However, no difference was observed in the percentage of couples that produced female offspring.

Although there were differences in the fecundity rates of non-diapausing and diapausing parasitoids, the differences in survival were not significant (Figure 4). The greater fecundity rates of the non-diapausing cohort and the greater percent of females indicate that these parasitoids will show greater population growth rates than diapausing individuals. This decrease in reproduction might represent a trade-off for diapause and attributed to the depletion of reserves (Ishihara and Shimada, 1995, Hand and Delinger, 2007, Sadakiyo and Ishihara, 2012).

Our more important findings were the difference between the two cohorts in the proportion of offspring entering into diapause and the effect of maternal age on the production of diapausing offspring. Non-diapausing females produced more diapausing offspring than diapausing females. The proportion of offspring in diapause varied with age and the highest proportion was observed in old females, between 26 and 34 days old. This result suggests that diapause in *D. areolatus* has a physiological trigger that is more important than environmental conditions. Although the frequency of diapause in *D. areolatus* has been associated with the fruit species used by the fruit fly host (Ovruski et al., 2016), in this study, we used the same fruit species (*P. guajava*) and we still found a difference between diapause condition and maternal age.

Some studies proposed that parents might determine whether their offspring enters into diapause (Denlinger, 2002; Hahn and Denlinger, 2007). Environmental factors affecting the parental generation determine the production of diapausing offspring (Saunders, 1965). Here, our study population has been under constant laboratory conditions for 9 to 23 generations. During this time, diapausing individuals were sorted out since only non-diapausing individuals are used to maintain the laboratory colony. Despite the constant environmental conditions and this selection against diapause, the proportion of diapausing individuals did not decreased through generations. How can this be explained?

According to Rahimi-Kaldeh et al. (2018) maternal age together with other abiotic factors (i.e. temperature, humidity, light) can affect the percentage of offspring entering into diapause in some Hymenoptera species, such as *Trichogramma brassicae* Bezdenko, where the percentage of diapause decreases as maternal age increases. An inverse effect has been reported for several Pteromalidae parasitoids, such as *Spalangia* sp, and *Nasonia vitripennis,* where the probability of larvae entering diapause increases if they were born from eggs laid by aging females (Simmonds, 1948; Saunders, 1965). The same occurred in our study with *D. areolatus*, where the oldest females, emerged from non-diapausing pupae, tended to increase the percentage of offspring in diapause.

In conclusion, we found that diapause in *D. areolatus* has effects on some biological attributes that can be trade-offs in parasitoids fitness, such as pupal and adult size, flight ability and reproduction. Other traits, as adult emergence and survival were not affected. We also found that maternal age has an important effect on the proportion of offspring entering into diapause. This represents base line knowledge to understand how diapause affects the population dynamics of the species and what their possible implications for biocontrol applications are. It demonstrates the need to consider diapause in the development of mass-rearing methods. Under field conditions, the use of diapausing individuals could contribute to improve the effectiveness of biocontrol strategies, as part of the offspring of the released population could remain in the area of release during unfavorable environmental conditions and emerge at the optimal time.

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References

Aluja, M., Ovruski, S.M., Sivinski, J., Córdova-García, G., Schliserman, P., Núñez-Campero, S.R., Ordano, M., 2013. Inter-specific competition and competition-free space in the tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae). Ecological Entomology, 38, 485 – 496. doi: 10.1111/een.12039.

Aluja, M, López M, Sivinski J., 1998. Ecological evidence for diapause in four native and one exotic species of larval-pupal fruit fly (Diptera: Tephritidae) parasitoids in tropical environments. Annals of the Entomological Society of America, 91,821 – 833.

Carvalho, R.D.S., 2005. Diapause in fruit fly parasitoids in the Recôncavo Baiano, Brazil. Neotropical Entomology, 34, 613 – 618.

Denlinger, D.L., 2002. Regulation of diapause. Annual Review of Entomology, 47, 93 – 122.

Denlinger, D. L., Armbruster, P. A., 2014. Mosquito Diapause. Annual Review of Entomology, 59, 73 – 94.

Ellers, J., van Alphen, J. J. M., 2002. A trade off between diapause duration and fitness in female parasitoids. Ecological Entomology, 27, 279 - 284.

Fay, M. P., Shaw, P. A., 2010. Exact and Asymptotic Weighted Logrank Tests for Interval Censored Data: The interval R Package. Journal of Statistical Software, 36 (2), 1 - 34. http://www.jstatsoft.org/v36/i02/.

Friendly, M., Fox, F., 2017. candisc: Visualizing Generalized Canonical Discriminant and Canonical Correlation Analysis. R package version 0.8-0. https://CRAN.R-project.org/package=candisc

Fox, J., Bouchet-Valat, M., 2017. Rcmdr: R Commander. R package version 2.4-1.

Fox, J., 2017. Using the R Commander: A Point-and-Click Interface or R. Boca Raton FL: Chapman and Hall/CRC Press.

Fox, J., 2005. The R Commander: A Basic Statistics Graphical User Interface to R. Journal of Statistical Software, 14 (9), 1 - 42.

Godfray, H. J. C., 1994. Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press. Princeton, New Jersey:

Hahn, D. A., Denlinger, D. L., 2007. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. Journal of Insect Physiology, 53, 760 – 773.

Ishihara, M., Shimada, M., 1995. Trade-Off in Allocation of Metabolic Reserves: Effects of Diapause on Egg Production and Adult Longevity in a Multivotine Bruchid, *Kytorhinus sharpianus*. Functional Ecology, 9, 618 - 624.

Kölliker-Ott, U. M., Blows, M. W., Hoffmann, A. A., 2003. Are wing size, wing shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids? Oikos, 100 (3), 563 - 573.

Koštál, V., Denlinger, D.L., 2011. Dormancy and developmental arrest in invertebrates. Journal of Insect Physiology, 57,537.

Mansingh, A., 1971. Physiological classification of dormancies in insects. Canadian Entomology, 103, 983 - 1009.

Montoya, P., Cancino, J., Ruiz, L., 2012. Packing of fruit fly parasitoids for augmentative releases. Insects, 3, 889 - 899. doi:10.3390/insects3030889.

Murillo, F. D., Cabrera, M. H., Barrera, J. F., Liedo, P., Montoya, P., 2015. *Doryctobracon areolatus* (Hymenoptera, Braconidae) a parasitoid of early developmental stages of *Anastrepha obliqua* (Diptera, Tephritidae). Journal of Hymenoptera Research, 46, 91–105. doi: 10.3897/JHR.46.5586

Ovruski, S. M., Aluja, M., Sivinski, J., Wharton, R., 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. Integrated Pest Management Reviews, 5, 81 - 107.

Ovruski, S .M., 2003. Nuevos aportes a la taxonomía de las especies de Opiinae (Hymenoptera: Braconidae), parasitoides de *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) en la provincia de Tucumán, Argentina. Acta Zoológica Lilloana, 47, 15 – 44.

Ovruski S. M., Schliserman, P., Aluja, M., 2016. Occurrence of diapause in neotropical parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in a subtropical rainforest from Argentina. Austral Entomology, 55, 274 – 283.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rahimi-Kaldeh, S., Ahmad, A., Bandani, A., Modarres, S. A. H., 2018. Maternal age influence on diapause induction in sexual and asexual *Trichogramma brassicae*. International Journal of Pest Management, DOI:10.1080/09670874.2017.1422569

Sadakiyo, S., Ishihara, M., 2012. Cost of Diapause on Life-History Traits Under Restricted Resources in Multivoltine Bruchid *Acanthoscelides pallidipennis* (Coleoptera: Bruchidae). Annals of the Entomological Society of America, 105 (3), 422-426. DOI: 10.1603/AN11109

Sagarra, L. A., Vincent, C., Stewart, R. K., 2001. Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera: Encyrtidae). Bulletin of Entomological Research, 91, 363 – 367.

Saunders, D. S., 1965. Larval diapause of maternal origin: induction of Diapause in

Nasonia vitripennis (Walk.) (Hymenoptera: Pteromalidae). Journal of Experimental Biology, 42, 495 – 50.

SENASICA, 2012. Manual de control de calidad de moscas de la fruta estériles. Procedimientos para evaluar en los centros de empaque, 11 - 12. https://www.gob.mx/cms/uploads/attachment/file/109285/Manual_T_cnico_de_Control_de_Calidad_en_Centros_de_Empaque.pdf

Simmonds, F. J., 1948. The Influence of Maternal Physiology on the Incidence of Diapause. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 233, 385 – 414.

Stuhl, Ch., Cicero, L., Sivinski, J., Teal, P., Lapointe, S., Paranhos, B. J., Aluja, M., 2011. Longevity of multiple species of tephritid (Diptera) fruit fly parasitoids (Hymenoptera: Braconidae: Opiinae) provided exotic and sympatric-fruit based diets. Journal of Insect Physiology, 57, 1463 – 1470.

Therneau, T. M., Grambsch, P. M., 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York. ISBN 0-387-98784-3.

Therneau, T. M., 2015. A Package for Survival Analysis in S. version 2.38, https://CRAN.R-project.org/package=survival

Venables, W. N., Ripley, B. D., 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Wharton, R. S., Marsh, P. M., 1978. New world Opiinae (Hymenoptera: Braconidae) parasitic on Tephritidae (Diptera). Journal. Washington Academy of Sciences, 68, 147 – 167.

Figure captions

Figure 1. Duration of development of non-diapausing and diapausing *Doryctobracon areolatus* females and males, parasitizing *Anastrepha ludens* larvae.

Figure 2. Canonical analysis of morphological data from puparia containing non-diapausing and diapausing male and female *Doryctobracon areolatus* parasitoids. The asterisks (*) indicates significant difference.

Figure 3. Canonical analysis of adult parasitoids' morphological data from non-diapausing and diapausing *Doryctobracon areolatus.* A) Comparison by type of development. B) Comparison among females. C) Comparison between sexes. The asterisks (*) indicates significant difference.

Figure 4. Female survival of *Doryctobracon areolatus* parasitoids in the reproduction bioassays.

Figure 5. Net fecundity of *Doryctobracon areolatus* females from (A) non-diapausing and (B) diapausing cohorts.

Figure 1

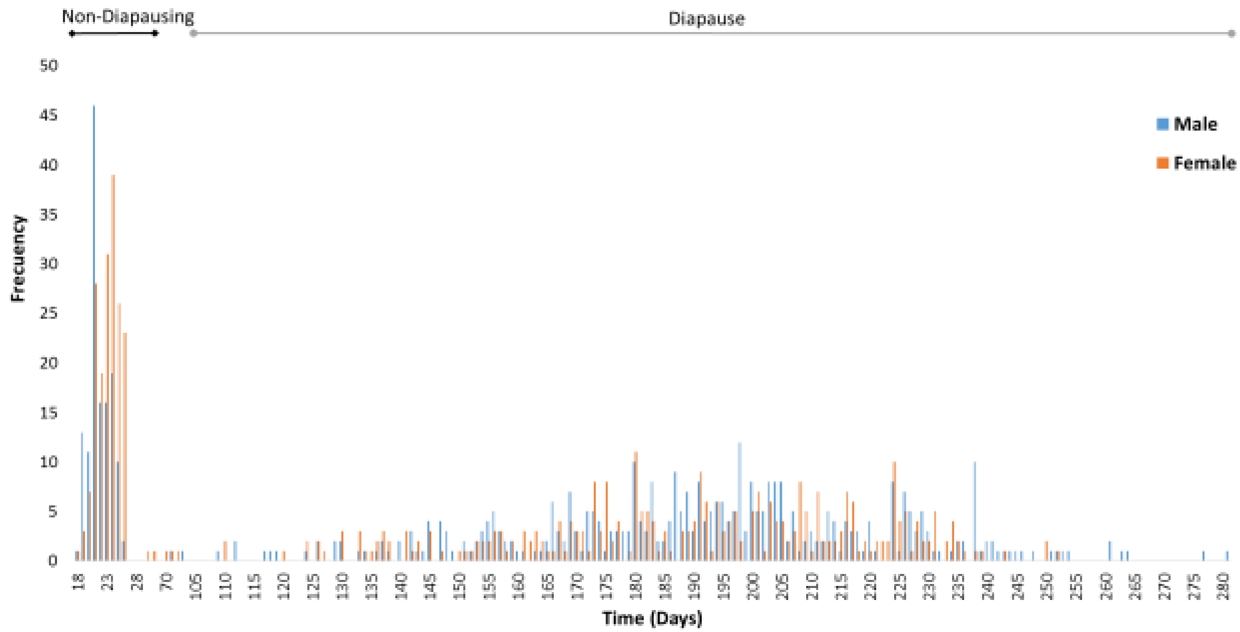

Figure 1. Duration of development of females and males of *D. areolatus*.

Figure 2

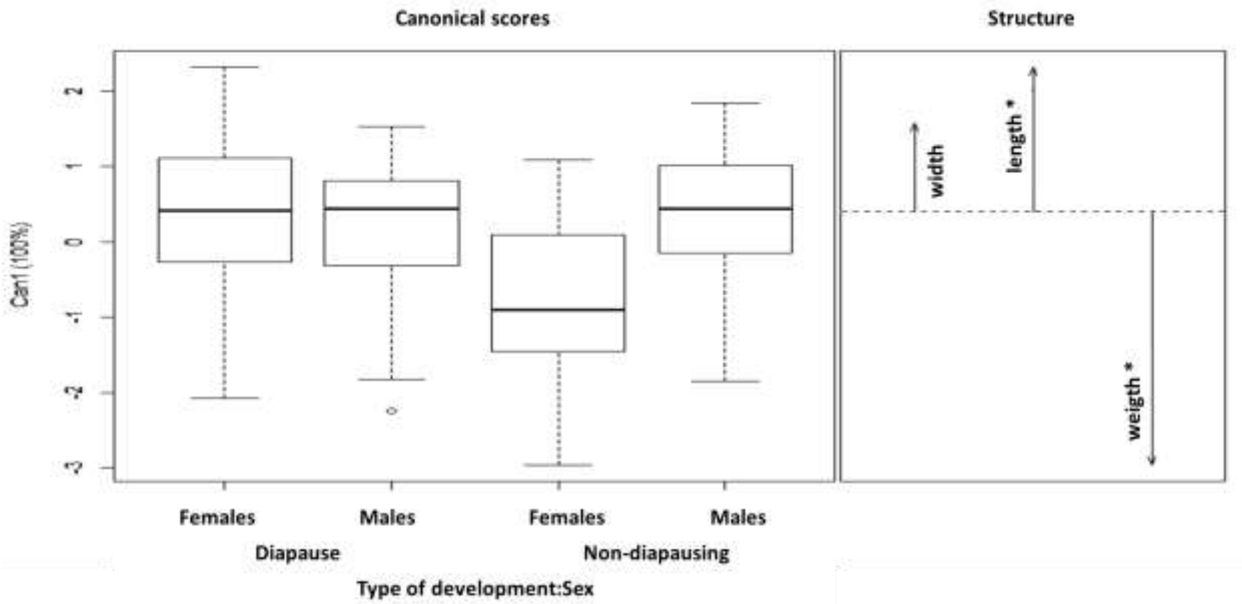

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Figure 3

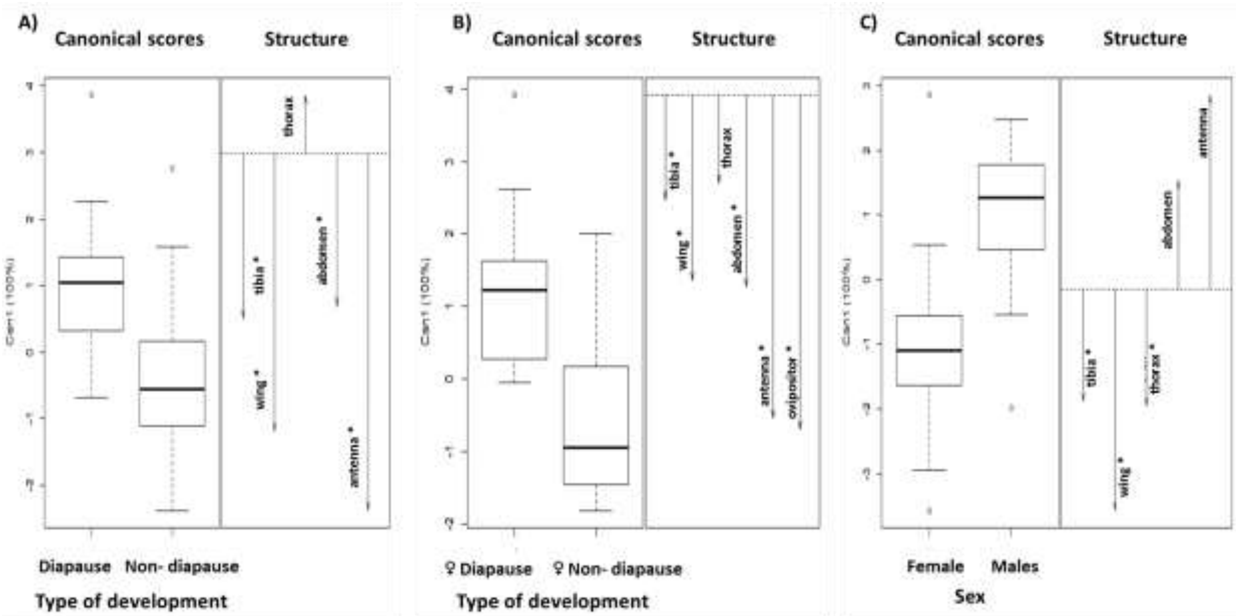

Figure 3. Canonical analysis of adult parasitoids' morphological data from non-diapausing and diapausing *Doryctobracon areolatus.* A) Comparison by type of development. B) Comparison among females. C) Comparison between sexes. The asterisks (*) indicates significant difference.

Figure 4

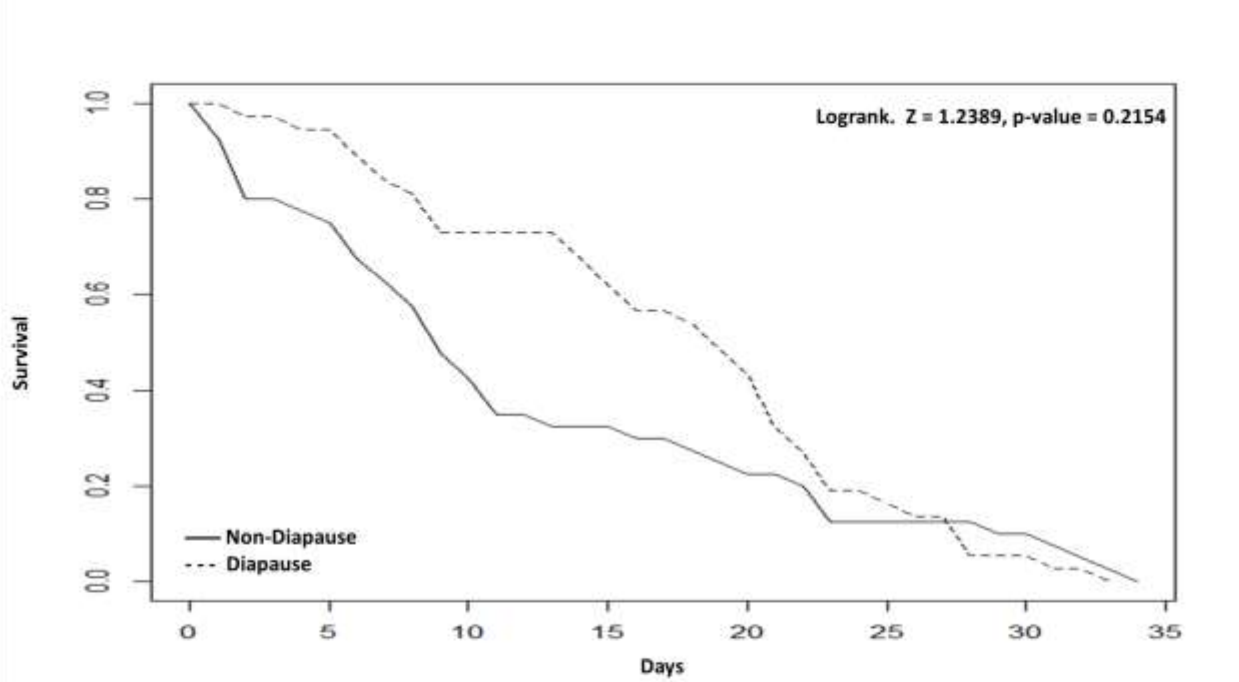

Figure 4. Female survival of *Doryctobracon areolatus* parasitoids in the reproduction bioassays.

Figure 5

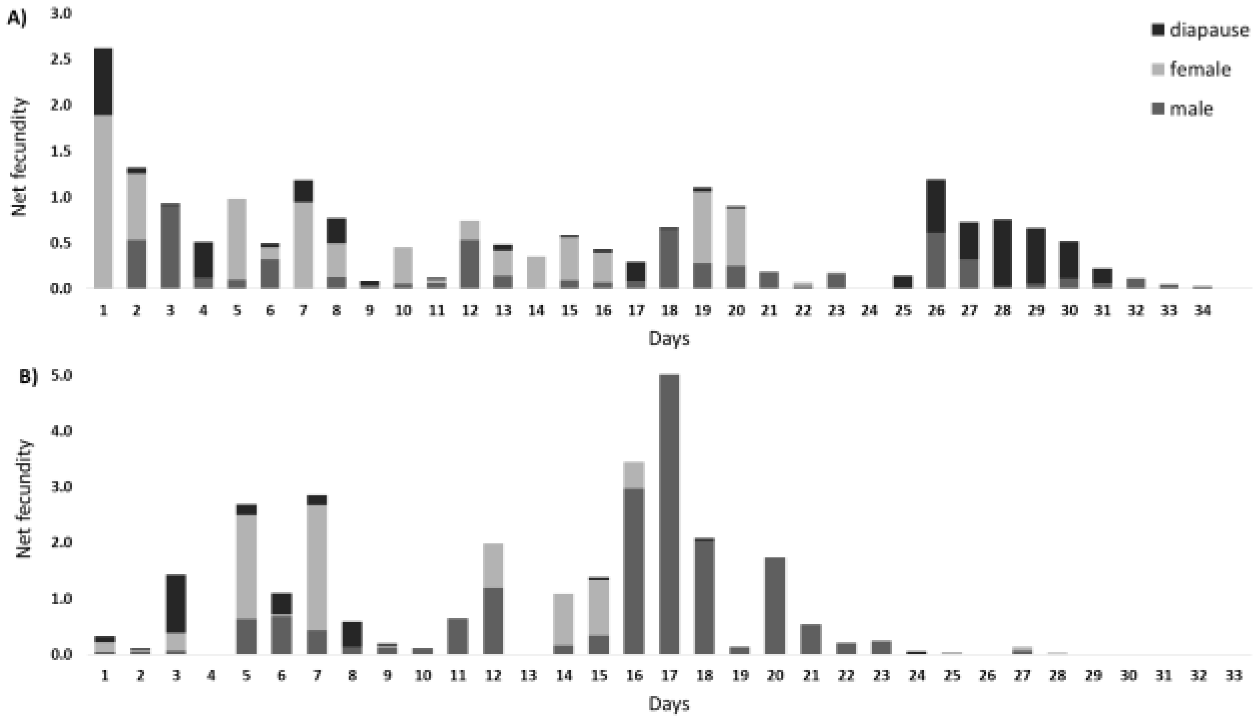

Figure 5. Net fecundity of *Doryctobracon areolatus* females from (A) non-diapausing and (B) diapausing cohorts.

Table 1. Development time and morphological measurements of non-diapausing and diapausing *Doryctobracon areolatus* parasitoids.

Type of development	Non-diapausing				Diapause			
Sex	♂		♀		♂		♀	
Parameter	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n
Development time (days)	21.86 ± 0.16 c	134	23.35 ± 0.15 b	179	191.60 ± 1.62 a	384	188.85 ± 1.76 a	317
Puparia weight (mg)	13.1 ± 0.5 ab	29	15.0 ± 0.6 a	37	12.0 ± 0.4 c	32	14.0 ± 0.5 ab	26
Puparia length (mm)	6.25 ± 0.15 ab	29	6.39 ± 0.09 c	37	6.24 ± 0.06 ab	32	6.52 ± 0.08 a	26
Puparia width (mm)	2.84 ± 0.03 a	29	2.88 ± 0.04 a	37	2.81 ± 0.03 a	31	2.92 ± 0.04 a	24
Tibia length (mm)	1.53 ± 0.02 a	27	1.56 ± 0.02 a	28	1.46 ± 0.03 b	13	1.52 ± 0.02 a	14
Wing length (mm)	4.88 ± 0.04 b	27	5.13 ± 0.08 a	28	4.48 ± 0.07 c	13	4.89 ± 0.04 b	14
Thorax length (mm)	2.03 ± 0.03 a	27	2.13 ± 0.04 a	28	1.99 ± 0.03 a	13	2.29 ± 0.24 a	14
Abdomen length (mm)	3.37 ± 0.07 a	27	3.30 ± 0.06 a	28	3.31 ± 0.07 a	13	3.09 ± 0.09 b	14
Antenna length (mm)	7.64 ± 0.10 a	27	7.27 ± 0.10 b	28	7.08 ± 0.10 b	13	6.64 ± 0.11 c	14
Ovipositor length (mm)	-		4.92 ± 0.07 a	28	-		4.69 ± 0.24 b	14

SE: Standard error, n = sample size. Values followed by the same letter in each row are not significantly different (P> 0.05). Significant difference by canonical discriminant analysis and SE.

Table 2. Flight ability of non-diapausing and diapausing *Doryctobracon areolatus* parasitoids.

Cohort	n	Emergence rate (%) ± SE		Fliers (%) ± SE	
Non-Diapausing	155	75.69 a	2.96	55.56 a	3.54
Diapausing	229	39.86 a	9.69	23.36 b	5.36

Emergence rate is the proportion of adults emerged from 100 pupae. Percentage of flyers is the proportion of parasitoids capable of flying from 100 pupae. n = number of pupae exposed. SE = Standard Error. Values followed by the same letter in each column are not significantly different ($P > 0.05$).

Table 3. Mean longevity (±SE) and starvation resistance in days in non-diapausing and diapausing *Doryctobracon areolatus* parasitoids.

Sex	Type of development	Longevity (days)			
		With food	n	Without food	n
♀	Direct development	14.77 ± 2.27 ab	43	4.18 ± 0.33 a	55
	Diapause	24.00 ± 3.04 a	30	5.40 ± 0.27 a	45
♂	Direct development	12.09 ± 1.48 ab	47	4.40 ± 0.40 a	43
	Diapause	10.67 ± 1.35 b	52	4.33 ± 0.24 a	52

Table 4. Fecundity rates (female offspring per female) of nondiapausing and diapausing *Doryctobracon areolatus* parasitoidd and fraction of females, males and diapausing offspring.

	Non-diapausing	Diapausing
Gross fecundity (daughters / female)	19.99	10.92
Net fecundity (daughters / female)	8.47	8.00
Male offspring (%)	30.87	62.54
Female offspring (%)	42.58	28.49
Diapausing offspring (%)	26.55	9.12

•CONCLUSIONES

A continuación se presenta un listado de las principales conclusiones obtenidas de este trabajo de investigación.

- Los resultados de este estudio demuestran que los adultos de *Doryctobracon areolatus* emergidos de diapausa presentan diferencia en sus atributos biológicos respecto a los de desarrollo directo.
  - Existieron diferencias en cuanto al tamaño, habilidad de vuelo y fecundidad entre parasitoides que pasaron por diapausa y parasitoides que no pasaron por diapausa.
  - Se observó que los puparios que contenían parasitoides en diapausa fueron más anchos y largos, mientras que los puparios que contienen parasitoides de desarrollo directo tuvieron mayor peso.
  - Los parasitoides de desarrollo directo fueron más grandes, comparados con los parasitoides que pasaron por diapausa.
  - En longevidad no existió diferencia en relación al tipo de desarrollo, sin embargo las hembras diapáusicas vivieron más que los machos de su misma condición de desarrollo.
  - En cuanto a efectos de la inanición en la longevidad y emergencia de adultos de los puparios, no existió diferencia significativa entre parasitoides de desarrollo directo y los que pasaron por diapausa.
  - Los parasitoides de desarrollo directo tuvieron mayor porcentaje de voladores.
  - La fecundidad fue mayor en parejas de desarrollo directo. Ambos tipos de cohortes distribuyeron su progenie de manera diferente; en parasitoides de desarrollo directo la mayor proporción de descendencia fue hembra (57.97%), mientras que en los que pasaron por diapausa la mayoría fue macho (68.69%).

- Las hembras de ambas cohortes produjeron descendencia que a su vez entró en diapausa lo que sugiere que la diapausa en *D. areolatus* puede ser una estrategia que no depende de las condiciones ambientales.
- Uno de los principales hallazgos en este trabajo fue el efecto de la edad materna sobre la proporción de descendencia diapáusica: las hembras de desarrollo directo en edad madura (25-34 días) generaron una progenie mayoritariamente diapáusica.

Basándome en mis resultados, para futuras investigaciones encaminadas a conocer las causas y consecuencias de la diapausa en *D. areolatus*, propongo las siguientes consideraciones para futuras investigaciones:

- La competencia intraespecífica e interespecífica entre hembras como un factor que induce a la diapausa (P.E. realizar experimentos en jaulas con 1 hembra, 5 y 10 hembras).
- El efecto de la edad del hospedero en la inducción a la diapausa (P.E. exponer a parasitismo huevos y larvas de diferentes estadios).
- Indagar si la disminución de hospederos induce a la diapausa (P.E. realizar bioensayos ofreciendo diferente cantidad de hospederos 10, 20, 30 larvas.).
- La distribución de la progenie en hembras vírgenes, para averiguar el efecto de la condición reproductiva en la inducción de la diapausa.
- Si la especie de hospedero tiene efecto en la progenie que entra en diapausa. (P.E. *A. ludens* vs *A. obliqua*).
- El efecto de la alimentación en la inducción a la diapausa (P.E. en parasitoides expuestos a inanición, parasitoides alimentados con miel, o parasitoides alimentados únicamente con los exudados de la fruta que se utiliza como sustrato de oviposición).
- El frutal empleado como sustrato de oviposición puede tener influencia en la diapausa.
- La dinámica anual de los parasitoides en diapausa ¿Existe un periodo del año en el cual entren o emerjan más parasitoides en diapausa? ¿Tiene alguna relación con algún fenómeno, como la época de lluvias?

- El efecto de la duración de la diapausa en la fecundidad, para determinar si a mayor duración de diapausa existe un efecto en la cantidad y distribución de la progenie, así como en la aptitud de esta.
- Efecto y resistencia a baja humedad.
- El efecto de aplicar la técnica del adulto frio en parasitoides que son implementados en control biológico de moscas de la fruta.

• Literatura citada.

Aluja M, López M, Sivinski J. 1998. Ecological evidence for diapause in four native and one exotic species of larval-pupal fruit fly (Diptera: Tephritidae) parasitoids in tropical environments. Ann Entomol Soc Am. 91: 821-833.

Aluja M, Ovruski SM, Sivinski J, Córdova-García G, Schliserman P, Núñez-Campero SR, Ordano M. 2013. Inter-specific competition and competition-free space in the tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae). Ecol Entomol. 38 485-496. doi: 10.1111/een.12039

Avendaño FS. 2006. Búsqueda de parasitoides asociados a *Anastrepha spp*. (Díptera: Tephritidae) en frutales de la región de Tapanatepec, Oaxaca. [Tesis de Maestría] Instituto Politécnico Nacional, 73 p.

Boscan MN, Godoy F. 1996. New parasitoids of fruit flies of the genera *Anastrepha* and *Ceratitis* in Venezuela. Agro Trop Maracay. 46, 4: 465-471.

Cabrera MH, Murillo FD, Ortega DA. 2006. Dinámica del parasitismo larva-pupa en *A. obliqua* en frutales hospederos en la región central costera de Veracruz. XXIX Congreso Nacional de Control Biológico, Manzanillo, Colima. 171-175.

Cancino J, Ruiz L. 2010. Biología y comportamiento de parasitoides. pp. 113. En: Montoya P, Toledo J, Hernández E. (Eds.). Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo, 2010. S y G editores, México, D.F.

Carvalho RDS. 2005. Diapause in fruit fly parasitoids in the Recôncavo Baiano, Brazil. Neotrop Entomol. 34:613–618.

Denlinger DL. 1986. Dormancy in tropical insects. Annu Rev Entomol. 31:239–264.

Denlinger DL. 2002. Regulation of diapause. Annu Rev Entomol. 47:93–122.

Denlinger DL. 2008. Why study diapause? Entomol Res. 38:1–9.

Ellers J, van Alphen JJM. 2002. A trade off between diapause duration and fitness in female parasitoids. Ecol Entomol. 27: 279-284.

García-Medel D, Sivinski J, Díaz-Fleischer F, Ramírez RR, 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.

Godfray HCJ. 1994. Parasitoids: Behavioral and Evolutionary Ecology. New Jersey. Princenton University Press. 484pp.

Greathead D, Waage JK. 1986. Parasitoids in classical biological control. In Insect parasitoids. 13th Symposium of the Royal Entomological Society of London, 18-19 September 1985 at the Department of Physics Lecture Theatre, Imperial College, London. pp. 289-318.

Gutiérrez JM. 2010. El programa moscas de la fruta en México. Pp. 3-10. En: Montoya P, Toledo J, Hernández E (Eds.). Moscas de la fruta: Fundamentos y procedimientos para su manejo. S y G Editores, México, D.F.

Hahn DA, Denlinger DL. 2007. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. J Insect Physiol. 53: 760–773

Hernández-Ortiz V, Pérez-Alonso R, Wharton RA. 1994. Native parasitoids associated with the genus *Anastrepha* (Dipt: Tephritidae) in Los Tuxtlas, Veracruz, Mexico. Entomophaga. 39 (2), 171-178.

Hernández-Ortiz, V. 1992. El género *Anastrepha* Schiner en México (Diptera: Tephritidae). Taxonomía, distribución y sus plantas huéspedes. Instituto de Ecología. Xalapa, Ver. Soc Mex Entomol. 162p.

Hernández-Ortiz, V, Guillen-Aguilar J. 2010. Taxonomía e identificación de moscas de la fruta de importancia económica en América. pp. 49-80. En: Montoya P, Toledo J, Hernández E (Eds.). Moscas de la fruta: Fundamentos y procedimientos para su manejo. S y G Editores, México, D.F.

Ishihara M, Shimada M. 1995. Trade-off in allocation of metabolic reserves: Effects of diapause on egg production and adult longevity in a multivotine bruchid, *Kytorhinus sharpianus*. Funct Ecol. 9: 618-624.

Jiménez-Jiménez E. 1956. Las moscas de la fruta y sus enemigos naturales. México. Rev Fitófilo. 16: 4-11.

Koštál V, Denlinger DL. 2011. Dormancy and developmental arrest in invertebrates. J. Insect Physiol. 57:537.

Mansingh, A. 1971. Physiological classification of dormancies in insects. Can Ent. 103: 983-1009.

Montoya P. 1999. Evaluación de *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) como agente de control biológico aumentativo de *Anastrepha* spp*.* (Diptera: Tephritidae). [Tesis de Doctorado] Universidad Nacional Autónoma de México. 87p

Montoya P, Cancino J. 2004. Control biológico por aumento en moscas de la fruta (Diptera: Tephritidae). Folia Entomol Mex. 43:257–270.

Montoya P, Liedo P, Benrey B, Cancino J, Barrera J, Sivinski, Aluja M. 2000. Biological control of *Anastrepha* spp*.* (Diptera: Tephritidae) in mango orchards through augmentative release of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol Control. 18: 216-224.

Murillo FD, Cabrera MH, Barrera JF, Liedo P, Montoya. 2015. *Doryctobracon areolatus* (Hymenoptera, Braconidae) a parasitoid of early developmental stages of *Anastrepha obliqua* (Diptera, Tephritidae). J Hymenopt Res. 46: 91–105. doi: 10.3897/JHR.46.5586

Ovruski SM, Aluja M, Sivinski J, Wharton R. 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. Integrated Pest Manag Rev. 5:81-107.

Ovruski SM, Schliserman P, Aluja M. 2016. Occurrence of diapause in neotropical parasitoids attacking *Anastrepha fraterculus* (Diptera: Tephritidae) in a subtropical rainforest from Argentina. Austral Entomol. 55:274–283.

Quicke D. 1997. Parasitic wasps. London. Chapman and Hall. 470 p.

Salcedo Baca, D. 2010. Evaluación económica de la Campaña Nacional contra moscas de la fruta en los estados de Baja California, Guerrero, Nuevo León, Sinaloa, Sonora y Tamaulipas (1994-2008) (No. IICA H10-26). IICA, México DF (México).

Sivinski JM. 1996. The past and potential of biological control of fruit flies. En: McPheron BA, Steck GJ. (Eds.) Fruit fly pests a world assessment of their biology and management. Delray Beach, Florida. St. Lucie Press. pp 365-375.

Vinson SB. 1984. How parasitoids locate their hosts: a case of insect espionage. En: Lewis T. (Eds.). Insect communication. London, UK. R. Entomol Soc. 325-348.

Wharton RS. Marsh PM. 1978. New world Opiinae (Hymenoptera: Braconidae) parasitic on Tephritidae (Diptera). J Wash Acad Sci. 68: 147–167.

Wong TYY, Ramadan M, McInnis D, Mochizuki N, Nishimoto J, Herr J. 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Maui, Hawaii. Biol Control. 1: 2-7.

• Anexos

Anexo I. Registro de artículo

Dear Miss Cruz-Bustos,

Submission no: IP_2018_190
Submission title: Biological attributes of diapausing and non-diapausing Doryctobracon areolatus (Hymenoptera: Braconidae), a parasitoid of Anastrepha spp. (Diptera: Tephritidae) fruit flies
Corresponding author: Dr Pablo Liedo
Listed co-author(s): Dr. Pablo Montoya, Dr Gabriela Pérez-Lachaud, Miss Jassmín Cruz-Bustos, Mr Javier Valle-Mora

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