El Colegio de la Frontera Sur

Comportamiento de forrajeo y aceptación del huésped de tres especies de parasitoides nativos (Hymenoptera: Braconidae) de la mosca Mexicana de la fruta

Tesis
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Por

Amanda Pricila Ayala Ayala

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Las personas abajo firmantes, miembros del jurado examinador de:

Amanda Pricila Ayala Ayala

Hacemos constar que hemos revisado y aprobado la tesis titulada

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Para obtener el grado de **Doctor en Ciencias en Ecología y Desarrollo Sustentable**

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TABLA DE CONTENIDO

Resumen

El objetivo del presente trabajo fue determinar el comportamiento de búsqueda y aceptación de hospedero, ante la presencia del superparasitismo y su repercusión en la longevidad y fecundidad de tres especies de parasitoides de larva, nativos del género *Anastrepha*. Se observó el comportamiento de oviposición y aceptación de hospedero bajo condiciones de laboratorio, para lo cual ofrecimos simultáneamente larvas de tercer estadio de *Anastrepha ludens* previamente parasitadas por conespecíficos y larvas sin parasitar. Se construyó un etograma para cada especie. Las pruebas se realizaron bajo tres condiciones: a) autosuperparasitismo hembras actuando en solitario; b) superparasitismo conespecífico, grupos de cinco hembras de la misma especie, y c) multiparasitismo, grupos de tres hembras, una de cada especie. Las especies nativas invirtieron mayor tiempo en el proceso de oviposición comparado con el tiempo reportado para las especies introducidas *Diachasmimorpha longicaudata* y *D. tryoni*. La preferencia por ovipositar en hospederos sanos se presentó en las tres especies, lo cual sugiere que la capacidad de discriminación de hospedero es una característica común en las especies. Se observó que el autosuperparasitismo es prácticamente nulo, y que el superparasitismo conespecífico fue más frecuente en *Utetes anastrephae.* En las pruebas de multiparasitismo, no se encontró diferencia significativa en la cantidad de adultos emergidos de las tres especies de parasitoides, sin embargo, cuando se disectaron las pupas de moscas, se observó una mayor cantidad de estados inmaduros de *U. anastrephae.* Las tres especies inmovilizaron a sus hospederos durante el proceso de oviposición, fueron capaces de identificar hospederos previamente parasitados cuando habían sido atacados por conespecíficos y el superparasitismo fue poco frecuente, lo que sugiere que el no superparasitar es una adaptación de las especies nativas.

CAPÍTULO 1

Introducción

1. Introducción

México se encuentra como uno de los principales productores y exportadores de frutas en el mundo, en un mercado donde el consumo de frutas y verduras inocuas es una tendencia que va en aumento y que exige los más altos estándares de calidad. Ante esta situación, los sistemas de producción deben responder garantizando la sanidad e inocuidad de los productos donde las plagas desempeñan un papel importante. En América, las moscas de la fruta del género *Anastrepha* Schiner (Díptera: Tephritidae) son consideradas plagas de interés económico pues ocasionan pérdidas sustanciales al consumir y contaminar la pulpa de los frutos que atacan, lo cual deriva en estrictas regulaciones cuarentenarias (Aluja 1994, Aluja y Mangan 2008).

Para contrarrestar la acción de esta plaga, se han implementado métodos de control que van desde el control biológico hasta el control legal, inmersos en un escenario de manejo integrado, que además de suprimir las poblaciones plaga, evitan la movilización de la fruta infestada.

El control biológico busca reducir las poblaciones de la plaga a un nivel que no cause daño económico, ofreciendo seguridad en materia de protección al medio ambiente ya que no contamina ni genera daños a la salud humana (De Bach 1974, Hokkanen y Pimentel 1989). Sin embargo, existe gran preocupación por el impacto que éste puede tener sobre la biodiversidad (Thomas y Willis 1998), donde el mayor riesgo es el posible efecto sobre especies a las que no va dirigido.

Generalmente se han considerado tres estrategias de control biológico, el clásico, por aumento y por conservación. El control biológico clásico se define como la liberación de enemigos naturales cuyo origen corresponde al de la plaga que se quiere controlar y que fue introducida a una determinada región como “plaga exótica” (De Bach 1974).

El control biológico por aumento fue definido por Greathead y Waage (1983) como una estrategia donde un gran número de enemigos naturales son criados masivamente y liberados en períodos críticos para suprimir las poblaciones plaga a corto plazo. Este tipo de control supone que al manipular las densidades de los

enemigos naturales en las zonas de liberación se pueden solventar deficiencias asociadas al control biológico clásico (Sivinski et al. 1996). La efectividad de éste método se basa en las liberaciones aumentativas (Knipling, 1992, Wong et al. 1991) y puede ser integrado a programas con la Técnica del Insecto Estéril (TIE) (Wong et al. 1992, Sivinski et al. 1996). Un tercer tipo de control biológico, es el control biológico por conservación, que se define como la modificación del ambiente a través de prácticas agrícolas, con el fin de optimizar la eficacia de los enemigos naturales específicos y así reducir el efecto de las plagas (Hajek 2004). En moscas de la fruta los casos más comunes han sido el control biológico clásico y el control biológico por aumento utilizando especies de parasitoides introducidas (Wharton 1989, Sivinski 1996, Montoya et al 2000). Sin embargo el primero no ha sido concluyente, más aún en una plaga como las moscas del género *Anastrepha* (Schiner) (Wharton 1989).

El endoparasitoide introducido *Diachasmimorpha longicaudata* (Ashmead), es empleado operativamente en el programa nacional en México contra moscas de la fruta con el fin de reducir las poblaciones plaga de moscas de la fruta del género *Anastrepha* (Montoya et al. 2000, Montoya et al. 2007), con lo cual se han obtenido resultados satisfactorios. Sin embargo, existe un amplio gremio de parasitoides nativos con potencial para ser usados en programas de control biológico, de los cuales se puede aprovechar su característica coevolutiva con las especies plaga del género *Anastrepha*. Entre estas especies se encuentran *Doryctobracon crawfordi* (Viereck) (Ovruski et al., 2000), *Utetes anastrephae* (Viereck) y *Opius hirtus* (Fischer) (López et al. 1999), las cuales pueden plantear nuevas estrategias para la aplicación del control biológico de estas especies plaga.

En el presente trabajo se planteó caracterizar cualitativa y cuantitativamente el comportamiento de aceptación de hospedero y las estrategias de parasitismo de las tres especies de parasitoides nativos de moscas del género *Anastrepha* enunciadas arriba. En un primer capítulo presentamos el marco teórico que sirve como base y justificación para los objetivos planteados en las diferentes etapas

experimentales. En el capítulo 2 se presentan los resultados del análisis del comportamiento de búsqueda de hospedero y oviposición de las tres especies bajo estudio, así como el efecto que ocasionan las oviposiciones sobre los hospederos atacados. En el capítulo 3 se presentan los resultados del estudio sobre las estrategias usadas por los parasitoides de estudio en tres diferentes condiciones: 1) hembras parasitando en solitario a sus hospederos, 2) hembras conespecíficas atacando simultáneamente a los hospederos disponibles, y 3) hembras de diferentes especies atacando simultáneamente a sus hospederos. Finalmente, en el capítulo 4 se discuten los principales resultados de esta tesis.

Este trabajo aporta información básica que permite identificar aspectos que favorecen o limitan a un enemigo natural en caso de ser considerado como agente de control biológico de moscas del género *Anastrepha*.

2. Objetivos

2.1 Objetivo general

Determinar las estrategias de parasitismo de tres especies de parasitoides nativos de moscas de la fruta del género *Anastrepha*.

2.2. Objetivos específicos

- Caracterizar el comportamiento de forrajeo y aceptación del hospedero de tres especies de parasitoides nativos de la mosca mexicana de la fruta.
- Comparar el efecto de las estrategias de parasitismo sobre la proporción sexual, la longevidad y fecundidad de la progenie.

3. Marco teórico

3.1. Moscas de la fruta

A nivel mundial la producción frutícola representa una de las actividades agrícolas de mayor importancia económica. Sin embargo, los principales productos frutícolas se ven afectados por especies plaga de moscas de la fruta de la familia Tephritidae, que se caracterizan por ovipositar en frutas de importancia comercial.

A este daño directo se le añade un daño indirecto por las restricciones cuarentenarias y de comercialización que representa su presencia (Aluja 1994, Montoya et al. 2007, Aluja y Mangan 2008).

El género *Anastrepha* es endémico de América y se encuentra ampliamente distribuido en las regiones tropicales y subtropicales (Hernández-Ortíz et al. 2010). En México se encuentra distribuido en gran parte del territorio nacional, y se han reportado más de 30 especies de éste género, siendo las de mayor importancia *Anastrepha ludens* (Loew), *Ansatrepha obliqua* (Macquart)*, Anastrepha serpentina* (Wiedemann) y *Anastrepha striata* (Schiner) (Hernández-Ortíz y Aluja 1993).

Las moscas de la fruta son, en su mayoría, especies polífagas, multivoltinas con alta longevidad y fecundidad (Liedo et al. 1993, Aluja et al. 1998), lo que las convierte en un grupo de alto riesgo. Son insectos holometábolos, es decir que presentan una metamorfosis completa, pasando por cuatro estados de desarrollo bien diferenciados: huevo, larva, pupa y adulto.

El daño que ocasionan estos insectos inicia a partir que la hembra deposita su huevo al interior del fruto haciendo un orificio. Sin embargo, el más importante lo hacen las larvas al barrenar los frutos de los que se alimentan. El manejo de estas especies se realiza con base en programas regionales donde se aplican estrategias de acuerdo a un manejo integrado donde confluyen una diversidad de técnicas con los cuales se ha llegado obtener diferentes conceptos de áres libres de moscas para la comercialización de la fruta (Aluja 1994).

3.2. Estrategias de control biológico con enemigos naturales en moscas de la fruta

El control biológico hace referencia al uso y manipulación de enemigos naturales que sirven para reducir las densidades poblacionales de las plagas a niveles que no superen el umbral económico (UE). Además, debe existir una interdependencia entre las poblaciones del enemigo natural y las de la plaga (Van Driesche y Bellows 1996, Huffacker y Dahlstein 1999). Los criterios que prevalecen dentro

del control biológico se basan en el control biológico clásico, por conservación y por aumento (Badii et al. 2000, Montoya et al. 2007).

Se puede considerar que el control biológico de moscas de la fruta en un contexto formal se inició con el desarrollo del programa de control biológico clásico contra la mosca oriental *Bactrocera dorsalis* (Hendel) recién introducida en Hawai en 1945 (Clausen et al. 1965). Sin embargo, al no cumplirse las expectativas se desarrollaron posteriormente crías masivas de diferentes especies de parasitoides que serían liberados periódicamente (Montoya y Cancino 2004), con lo cual se dio inicio a las primera propuestas formales del control biológico por aumento en moscas de la fruta. En México, la cría masiva del parasitoide *Diachasmimorpha longicaudata* (Ashmead) en la planta Moscafrut en Metapa de Domínguez Chiapas, con capacidad de producción semanal de 50 millones de pupas, se considera como un importante referente de los avances actuales logrados en esta disciplina (Cancino et al. 1996).

El control biológico por aumento exige mantener una cría masiva del enemigo natural a liberar (Montoya et al. 2000), cuyos individuos sean eficaces al ser liberados en campo, con la capacidad de suprimir las poblaciones plaga. Éste método de control está soportado por el modelo teórico de Knipling (1992), en donde las liberaciones de parasitoides deben iniciar antes de que las poblaciones de plaga alcancen altas densidades de población preferentemente en las zonas marginales.

En el caso de las moscas de la fruta, la sugerencia más importante para la liberación, es dirigirse hacia las zonas aledañas de los huertos comerciales (Montoya et al. 2007), en donde existan frutos hospederos nativos de poblaciones plaga que posteriormente pueden migrar a las áres comerciales de los huertos. Por ello es importante que al implementar un programa de liberación aumentativa se consideren sus limitantes y los sitios donde puede ser aplicado para que sea exitoso, lo que hace indispensable conocer la fenología de la plaga, así como de las plantas hospederas de la región (Montoya et al. 2007). Un recurso inmediato

son los parasitoides más fáciles para criarlos artificialmente, sin embargo, se requiere de un conocimiento básico previo para su mejor empleo.

3.3. Atributos importantes de insectos parasitoides como enemigos naturales

Como enemigos naturales, los insectos parasitoides deben poseer ciertos atributos para ser considerados candidatos potenciales para los programas de control biológico con liberaciones aumentativas. Algunos de estos atributos son: sobrevivencia, fecundidad, capacidad de búsqueda, de discriminación, de desplazamiento, especificidad, además de un rango de adaptación a las diferentes condiciones climáticas (Montoya y Cancino 2004).

En el proceso de cría masiva, la sobrevivencia es un atributo importante (van Lenteren et al. 2003), ya que los parasitoides emergidos pueden mantenerse por un largo periodo antes de liberarse (Montoya y Cancino 2004). La capacidad de búsqueda también es significativa, considerando que los insectos serán liberados en campo. El proceso de forrajeo mediante el cual los parasitoides localizan y seleccionan a sus hospederos es fundamental para el éxito de la reproducción del parasitoide (Bernal 2007). En general, la búsqueda incluye: (i) localización del hábitat del hospedero; (ii) localización del hospedero; (iii) aceptación del hospedero e (iv) interacción fisiológica con el hospedero (Bell 1990, Tumlinson et al.1993, Speight et al. 2008).

3.4. Aceptación del hospedero

La aceptación de un hospedero es una decisión que depende de las características del mismo (Visser et al. 1992). Una vez localizado el hospedero, las hembras pueden ovipositar en o sobre él, o rechazarlo; sin duda, una característica a considerar es si éste ha sido previamente parasitado o no (van Alphen y Visser 1990).

Los parasitoides en su mayoría poseen la capacidad de distinguir entre hospederos previamente parasitados de aquellos no parasitados y evitan la

oviposición (Pijls et al. 1995). Esta característica se conoce como capacidad de discriminación (van Lenteren 1981) y puede ocurrir en tres niveles: 1) la auto-discriminación (discriminación de hospederos previamente parasitados por la misma hembra), 2) discriminación conespecífica (discriminación de hospederos previamente parasitados por hembra(s) de la misma especie) y 3) discriminación heteroespecífica (especies diferentes), los cuales se han relacionado a tres tipos de superparasitismo: 1) autosuperparasitismo, 2) superparasitismo conespecífico 3) y multiparasitismo o superparasitismo heteroespecífico (Mackauer 1990). En los parasitoides solitarios, sólo un individuo puede desarrollarse por hospedero y las larvas supernumerarias son eliminadas a través de la competencia al interior del hospedero (Hubbard et al. 1987). Generalmente, el primer parasitoide elimina a todos los competidores más jóvenes (Alphen van y Visser 1990). Por lo tanto, un hospedero parasitado es de menor calidad para las hembras parasitoides (Nelson y Roitberg 1995).

Las hembras parasitoides pueden aceptar un hospedero previamente parasitado cuando la probabilidad de que la hembra parasitoide muera es grande o cuando la presencia de dos o más huevos dentro de un hospedero incrementa la probabilidad de sobrevivencia de la especie principalmente porque una acción conjunta puede supera la respuesta inmune generada por el hospedero (Rosenheim y Mangel 1994, Rosenheim y Hongkham 1996). Sin embargo, no todos los parasitoides que se desarrollan a expensas de un solo hospedero superparasitado llegan al estado adulto (Speight et al. 2008); y cuando uno de los estados inmaduros logra completar su desarrollo puede presentar una disminución en su tamaño como consecuencia del superparasitismo (Hajek 2004).

El superparasitismo puede considerarse adaptativo bajo determinadas circunstancias, cuando una hembra se encuentra compitiendo por recursos o bien cuando la respuesta inmune del hospedero es alta que debe contrarrestarla para el desarrollo de la progenie.

3.5. Especies con potencial para el control biológico por aumento de moscas de la fruta

Sin duda alguna el parasitoide *D. longicaudata* originario del sureste de Asia ha sido un caso de éxito en las liberaciones aumentativas contra poblaciones de *Anastrepha*. En México se ha liberado vía terrestre y aérea, en regiones productoras de huertos de mango y naranja, alcanzando niveles de parasitismo de 34 a 70% (Montoya et al. 2000; Montoya et al. 2007). Sin embargo, existen parasitoides nativos que tienen potencial como enemigos naturales para ser candidatos en un programa de control biológico, dentro de un plan de manejo integrado de moscas de la fruta.

Las especies de parasitoides nativos *D. crawfordi*, *U. anastrephae* y *O. hirtus* pueden ser una alternativa importante para la implementación de proyectos de control biológico contra las moscas de la fruta; su colonización y domesticación han sido descritos por Aluja et al. (2009) y Cancino et al. (2009).

Doryctobracon crawfordi es un endoparasitoide solitario, koinobionte que ataca larvas de tercer estadio de diversas especies de *Anastrepha*, su distribución va desde el centro de México hasta el norte de América del Sur (Ovruski et al. 2000). Se localiza en ambientes con temperaturas que fluctúan entre 14 y 29° C, y una humedad relativa superior a 40% (Aluja et al. 1998); es una especie capaz de parasitar larvas de moscas de la fruta presentes en frutos caídos de guayaba (*Psidium guajava* L.) en presencia de conespecíficos (García-Medel et al. 2007).

Respecto a *O. hirtus*, es una especie aparentemente más especializada, se asocia principalmente con especies raras como *Anastrepha cordata* Aldrich, *Anastrepha alveata* Stone sugiriendo que puede ser un buscador superior que no interfiera con otras especies adaptadas para buscar hospederos presentes en altas densidades. (Hernández, Ortiz et al. 1994, Sivinski et al. 2000). Posee una distribución más restringida (México, Costa Rica y República Dominicana) (Ovruski et al. 2000).

Utetes anastrephae es una especie con un ovipositor relativamente pequeño que ataca moscas de *A. obliqua* asociadas a frutos pequeños como

*Spondia*s L. y guayaba (*Psidium guajava* L.), y se distribuye desde el sur de Estados Unidos hasta Argentina (Hernández-Ortiz et al. 1994, López et al. 1999).

Un programa de manejo integrado que considere la liberación de enemigos naturales debe conocer a fondo la biología de los insectos para que la liberación simultánea de dos o más especies de parasitoides pueda resultar en una supresión eficiente de una población de plagas, especialmente si no existe superposición de nicho (Knipling 1992).

CAPITULO 2

Host acceptance by three native parasitoid species (Hymenoptera: Braconidae) attacking larvae of the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae)

Amanda Ayala[1], Gabriela Pérez-Lachaud[2], Jorge Toledo[1], Pablo Liedo[1] and Pablo Montoya[3]

[1] El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, CP 30700, México.

[2] El Colegio de la Frontera Sur, Avenida Centenario km 5.5, Chetumal, Quintana Roo, CP 77014 México.

[3] Programa Moscafrut SAGARPA -IICA. Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, CP 30860 México.

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Host acceptance by three native parasitoid species (Hymenoptera: Braconidae) attacking larvae of the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae)

Abstract

We studied the oviposition and host acceptance behavior of three braconid parasitoid species native to Mexico, *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fischer) and *Utetes anastrephae* (Viereck), with potential to be considered as biocontrol agents against tephritid fruit fly pests in the Neotropics. Third instar larvae of *Anastrepha ludens* (Loew), with and without previous parasitization by conspecifics, were simultaneously offered to females of each species, and the individual behavior was video recorded to construct oviposition flow diagrams. The patterns of foraging and host acceptance were similar in the studied species; all rejected mostly parasitized hosts suggesting that this strategy is common in the guild of larval parasitoids attacking *Anastrepha* spp. The complete searching and host acceptance process took 2.2 ± 0.1 min (mean ± SE) in *D. crawfordi*, 1.7 ± 0.1 s in *U*. *anastrephae* and 1.5 ± 0.1 s in *O*. *hirtus*. Notably, and as a result of toxins injected by parasitoid females during oviposition, the parasitized hosts experienced a transient paralysis of variable duration. Hosts attacked by *U*. *anastrephae* remained immobile for the shortest time (12.5±1 min) (mean±SE), followed by *D. crawfordi* (20.5±3.4 min) and *O. hirtus* (24.1±2 min). Our data revealed a notable discrimination ability in all three species, and that behavioral differences lay mainly in the time of parasitization and in the duration of paralysis experienced by attacked hosts. This suggest that the three species could be valuable as biocontrol agents, but additional studies are necessary to better understand the advantages and limitations of each one as natural enemies of fruit fly pests.

Key words: Host discrimination, transient host paralysis, biocontrol agents, *Doryctobracon crawfordi, Opius hirtus, Utetes anastrephae*

Introduction

Fruit flies (Diptera: Tephritidae) are considered one of the main fruit pests worldwide (Enkerlin 2005). To reduce pest populations, various control tactics have been developed among which the augmentative release of parasitoids has arisen as one sound and well oriented strategy against this type of pests (Sivinski et al. 1996, Montoya et al. 2007).

Parasitoids are insects whose larvae develop by feeding in or on the body of other arthropods, usually insects; larval feeding almost always results in the death of the host (Godfray 1994). Parasitoids are immersed in a multitrophic context (Hassell and Waage 1984, Vet and Dicke 1992), where foraging for nutrients and hosts is performed at different scales (Kramer 2001, Gingras et al. 2002). In general, it is the female parasitoid that locates a suitable host. Because parasitoid development is dependent on limited resources (the host), adult preference and larval performance should be correlated to maximize fitness (Harvey et al. 2015) and the host acceptance procedure is considered the definitive step in host searching behavior (Vinson 1984). The hosts are often hidden in the interior of stems, leaves or fruits (Richerson and Borden 1972), consequently parasitoid females must detect and respond to a number of indirect signals where chemical-sensorial information plays a fundamental role (Doutt 1959, Vinson 1976, 1998, van Alphen and Vet 1986, Vet and Dicke 1992). In addition to chemical stimuli, parasitoids are also capable of identifying vibrations emitted by their hosts through the substrates in which they develop (van Alphen and Janssen 1982, Vet and van Alphen 1985, Mills et al. 1991, Meyhöfer et al. 1997).

Once female parasitoids have located their hosts, they have the capacity to distinguish between parasitized and not parasitized hosts, a strategy known as discrimination ability (van Alphen and Visser 1990). This ability can occur at three levels: (1) self-discrimination, (2) conspecific discrimination and (3) heterospecific discrimination (Mackauer 1990), and has been observed in many species of hymenoptera parasitoids (Vinson 1976), and is particularly important in the case of potential biocontrol agents, since these are expected to be efficient in host

searching and to have the ability to discriminate between parasitized and non-parasitized hosts (van Lenteren et al. 1978). The latter helps females to avoid superparasitism, reducing the time and energy spent in searching behavior (Godfray 1994, Mackauer 1990).

Host location and host acceptance behavior has been widely studied in the generalist fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Greany et al. 1977, Lawrence 1981, Montoya et al. 2003, Carrasco et al. 2005, Gonzalez et al. 2010). This species is exotic in the Americas where it has been successfully reared for augmentative biological control of *Anastrepha* (Schiner) fruit flies in Mexico (Montoya et al. 2000, 2007) and in Florida USA (Sivinski et al. 1996); and for *Ceratitis capitata* (Wiedemann) in Argentina (Suarez et al. 2014, Sanchez et al. 2016). However, there is a guild of native opiine braconid parasitoids (Sivinski et al. 2000, 2001) with potential as biocontrol agents, for which little information exists regarding their foraging and host acceptance behavior. This is the case of *Doryctobracon crawfordi* (Viereck), *Utetes anastrephae* (Viereck) and *Opius hirtus* (Fischer), all of which are solitary, larval-pupal endoparasitoids of *Anastrepha* spp. (López et al. 1999), that coexist in different regions of America (Sivinski et al. 2000). It has been postulated that differences in ovipositor size, as well as specific foraging behaviors, serve to prevent direct competition among these species (Ovruski et al. 2000, Sivinski et al. 2000). Under laboratory conditions, the three species can develop in the third instar larvae of *Anastrepha ludens* (Diptera: Tephritidae) (Aluja et al. 2009).

Doryctobracon crawfordi is native in habitats above 600 masl from the central part of Mexico to Argentina (Ovruski et al. 2005); possess a long ovipositor 5.39 ± 0.08 mm and attacks *Anastrepha* spp. mainly in citrus fruits and is sensitive to both high temperature and low humidity (Sivinski et al. 2000). *Utetes anastrephae* is characterized by a short ovipositor (1.57 ± 0.04 mm, Sivinski et al. 1997, 2001) and can be found associated with small fruits such as those of *Spondias* spp., with 2-5 cm of diam and 4 to 33 g weight (Avitia 2000). *Opius hirtus* is a more specialized parasitoid being recovered from *Anastrepha obliqua*

(Macquart) in *Spondias mombin* L. and from *Anastrepha alveata* (Stone) infesting *Ximenia americana* L. (Olacaceae) (Sivinski et al. 2000). The three species are synovigenic (Sivinski et al. 2001).

The purpose of this study was to compare the foraging and host acceptance behaviors of the parasitoid species *D*. *crawfordi*, *U*. *anastrephae* and *O*. *hirtus* on previously parasitized and non-parasitized larvae of *A*. *ludens,* using video recording equipment under laboratory conditions. This knowledge should allow an improved understanding of the oviposition performance and potential of these parasitoid species as biocontrol agents against fruit fly pest species.

Material and Methods

Study site and biological material

The experiments were conducted in the Biological Control laboratory of the Moscafrut Program SAGARPA-IICA, located in Metapa de Dominguez, Chiapas, Mexico. The parasitoid colonies were initiated from field infested fruits and maintained at 25±1 °C, 70±5% HR with a photoperiod of 12:12 (L:D) h. Eight-day-old larvae of *A*. *ludens* mixed with artificial diet were provided as host by the Moscafrut facility, where this species is mass reared as described by Orozco-Davila et al. (2017). Adult parasitoids of the species *D. crawfordi*, *U. anastrephae* and *O. hirtus* were reared according to Aluja et al. (2009).

Preparation of hosts larvae

Parasitized host larvae were obtained by exposing groups of approximately 100 host larvae for two hours to 100 females and 50 males of each species separately. Larvae with three or more oviposition scars were considered as being successfully parasitized (Montoya et al. 2000, 2003). Host larvae without previous parasitization were allocated to the “not parasitized host group”.

Preparation of the parasitoids

Copulated females, 5-6 days old with previous experience of oviposition were

used. To gain this experience, groups of ~150 recently emerged adults (1female: 1male) were confined in aluminum frame acrylic cages (30x30x30 cm) and provided with water and honey as a source of food. Twenty-four hours before conducting the bioassays, ~200 *A. ludens* larvae mixed with larval diet were offered to these parasitoids in a Petri dish oviposition unit, for 2 h.

Host acceptance test

The host searching and acceptance performance of individual parasitoid females was observed with two different types of *A. ludens* host larvae that were exposed simultaneously: 1) larvae previously parasitized (24 h earlier) by conspecifics, and 2) larvae with no previous parasitization. Bioassays were conducted in oviposition units consisting of Petri dishes (55 mm in diameter x 9 mm in depth) with the edges reduced to five mm in depth and a central division of 5 mm to separate the two type of larvae. Five previously parasitized *A*. *ludens* larvae were placed in one of the two sides, and five non-parasitized larvae, of the same age, were placed in the other side. The oviposition unit was covered with an organza elastic cloth and secured with a rubber band in order to prevent larval escape. This cloth was semi-transparent making possible the observation of the host larvae through it. Guava juice was added on the surface of the cloth in order to attract the females and keep them on the parasitization units until larval detection.

Video recording procedure

The oviposition sequences of thirty females per species were observed and video recordings made with a Samsung KREUZNACH video camera (f=2.3-78.2mm; F:1.6; ø30.5). One female was released onto the surface of the oviposition unit in each observation. The larvae and females were replaced after each observation, as well as the cloth and the oviposition unit. Environmental conditions were 25±1 ^{o}C and 75±5% RH. Bioassays were conducted between 8:30 and 15:00 and the time of observation was ~1 h per female. If the female presented null activity for the first five minutes, it was replaced. Time of latency (defined here as “time that

elapsed between two ovipositions"), the number of ovipositions, oviposition attempts, duration of oviposition and duration of host paralysis following oviposition (from the moment the stung larva remained immobile. to the moment it resumed crawling), were recorded for both host types. Video recordings were independently analyzed using the *Movie Maker* software version 2.6.4037.0, in order to obtain the sequences and transition frequencies of the different behaviors.

Statistical analysis

The number of ovipositions and oviposition attempts on the two larval types were compared using the *t* test for each parasitoid species. In order to compare the time spent on the different activities observed among the three species, a one-way analysis of variance with the Tukey-HSD test was conducted. Prior to analysis, a Box-Cox transformation of the data was conducted. The JMP Starter software version 7.0.1 was used for all of the analyses.

Results

The general behavioral sequences of the three parasitoid species on the two host types were identified. The operational definitions for the observed behaviors are presented in Table 1. The most common sequence for any of the three braconid species included: 1. Walking (W), 2. Searching for a host (S), 3. Detection of a host (D), 4. Oviposition attempt (OP), 5. Oviposition (O), 6. Rejection (RE), and 7. Failure (F) with some variants occurring depending on species (Figs. 1a and 1b; 2a and 2b; 3a and 3b).

Searching and oviposition behavior

In general, the females walked on the surface of the oviposition unit with their antennae in close contact with the surface of the ovipositin unit. Once the females detected a larva, they attempted to establish contact with the host by introducing their ovipositor and began a movement of abdominal vibration (associated with the descent of the egg (Montoya et al. 2009); they then moved the antenna and

extracted the ovipositor. Even though the three species maintained a similar pattern of oviposition behavior, *U. anastrephae* was often observed to perform a wing movement when inserting its ovipositor into a host. *D*. *crawfordi* rotated on its axis once contact was established with the larva. These specific behavioral acts led to successful ovipositions (Figs 1, 2 and 3).

No marked differences in the flow diagrams were observed between non-parasitized hosts and parasitized hosts for any of the braconids studied here. However, females significantly rejected hosts previously parasitized by conspecifics following insertion of the ovipositor compared to those not parasitized ($F= 2.35$; $df = 2$, $P < 0.001$). Overall, 79% of parasitized hosts were rejected by *U*. *anastrephae* females, 74% by *D. crawfordi* and 62% by *O. hirtus*. Furthermore, a more intensive searching was observed when a failure (because the host moved away) occurred when attacking non-parasitized hosts than when attacking parasitized hosts. The complete process of searching and host acceptance (from the beginning of the observation until ovipositor removal) was completed in 2.2 ±0.8 min (mean ± SE) in *D. crawfordi*, 1.7 ± 0.75 min in *U*. *anastrephae* and 1.52 ± 0.75 min in *O*. *hirtus*.

Latency

The time elapsed between ovipositions differed significantly between *U. anastrephae* and the other two species when the hosts had previously been parasitized ($F = 0.5$, $df = 2$, $P < 0.05$; N= 30). Regarding the time of latency with non-parasitized larvae, *U*. *anastrephae* presented the shortest time (3.25 ± 0.3 min) (mean ± SE) (Fig.4) ($F = 10.6$, $df = 2$, $P < 0.001$), compared to that of the other two species (*D*. *crawfordi* = 4.88 ± 0.48 min and *O*. *hirtus* = 5.65 ± 0.75 min) (mean ± SE).

Discrimination ability

The first host choice in the three parasitoid species corresponded mostly to the non-parasitized larvae (*D. crawfordi* 22/30; *U*. *anastrephae* 18/30 and *O*. *hirtus*

19/30). *Utetes anastrephae* parasitized a significantly (F = 3.39, df = 2, P = 0.03) higher quantity of non-parasitized hosts (3.3 ± 0.25) compared to the other two species (*D*. *crawfordi* 2.7 ± 0.23 and *O*. *hirtus* 2.3 ± 0.32). *Doryctobracon crawfordi* performed a greater number of oviposition attempts than *U*. *anastrephae* and *O*. *hirtus* in both types of larvae (Table 2).

The time of ovipositor insertion on previously parasitized larvae differed significantly (F = 4.7, df= 2, P = 0.001) among species, with *D*. *crawfordi* spending more time with the ovipositor inserted and *O. hirtus* the shortest one (Table 3). The time of ovipositor insertion of *O*. *hirtus* was significantly shorter in previously parasitized larvae compared to that in non-parasitized larvae (t = 2.67, df = 67, P = 0.0094, Table 3), while in the other two species no significant difference was found between the two host types. No significant differences were found between the two types of larvae in the duration of abdomen vibration and duration of the host paralysis after the attack by each parasitoid species. However, the duration of paralysis of the host differed among the three species (parasitized hosts, F = 12.8, df = 2, P = 0.00001; non-parasitized hosts, F = 29.5, df = 2, P = 0.000001). All hosts successfully stung showed temporary paralysis: *D. crawfordi* = 21.3 ± 1.2 min, *O. hirtus* = 23.8 ± 1.2 min and *U. anastrephae* = 13.4 ± 0.6 min) (mean ± SE) (Table 3).

Discussion

Knowledge on host acceptance behavior in insect parasitoids is fundamental to improve our understanding on the plant-herbivore-natural enemy tritrophic relations (Vet and Dicke 1992), as well as the population dynamics and their possible implications in pest biological control programs (Minkenberg et al. 1992). Several studies have indicated that responses of natural enemies are mediated mainly by chemical signals detected in the environment (Vinson 1998, Vet and Dicke 1992, van Alphen and Visser 1990), by host-generated vibrations in its microhabitat (Meyhöfer et al. 1997, Vet and van Alphen 1985, Meyhöfer and Casas 1999), and by the individual learning experiences of foraging females. It has been stated that

parasitoids perceive stimuli about host quality once direct contact has been made with the host, influencing the host acceptance process (Brodeur and Boivin 2004, Wajnberg et al. 2008). Here we established the patterns of host acceptance by *D. crawfordi*, *U. anastrephae* and *O. hirtus* in the presence of both non-parasitized and previously parasitized hosts. We further characterized the time spent in different behaviors, and the duration of the paralysis induced through parasitism.

The three studied species presented typical behavior of antennal contact with the surface of the oviposition unit during the process of searching for the host larvae, which is an important step for host detection (Leyva et al. 1991, Gonzalez et al. 2010). All of the species presented a similar foraging pattern, beginning the search for the host by walking and touching the oviposition surface with the antennae. Once a larva was detected, the females adopted an alert position that consisted of remaining immobile for some seconds with the antennae extended to the front, skimming the surface of the parasitization unit. On locating a larva, the females performed small turns on their axis until positioning their first pair of legs towards the front and arranging their ovipositor to form a 90° angle to the contact surface. On initiating the process of oviposition in the parasitization unit, as reported for *D. longicaudata* (Montoya et al. 2003), the females make various attempts to insert the ovipositor until contact was made with a host, which then was accepted or rejected.

According to our results, the three parasitoid species have a highdiscrimination ability in the form defined by van Alphen and Visser (1990) and Nufio and Papaj (2001), given that females rejected most of the previously parasitized hosts compared to those with no previous parasitization. However, *U. anastrephae* notoriously presented the highest frequency of rejection of parasitized hosts (79%). This suggests that this species possesses a high performance avoiding superparasitism and saving time and energy when foraging for their hosts (Godfray 1994, Mackauer 1990). This corroborate previous findings by Aluja et al. (2013), who showed that this species avoids ovipositing on previously parasitized hosts under conspecific and heterospecific situations, although it also has been

noted that superparasitized hosts yielded relatively more daughters (Alvarenga et al. 2016), as referred for *D*. *longicaudata* (Montoya et al. 2011). The host acceptance behavior presented by the three species was similar to that reported for *D*. *longicaudata* by Montoya et al. (2003), who observed that the previously parasitized hosts experienced a lower number of ovipositions than the hosts with no previous parasitization.

Doryctobracon crawfordi presented the longest time spent on oviposition compared to the other two species. Host acceptance may depend on extrinsic factors such as host availability and quality, as well as intrinsic factors such as the quantity of eggs in the females, the age and their nutritional state (Vet et al. 2002, Bernstein and Jervis 2008). In the case of *D*. *crawfordi,* availability of eggs in the females can be an important limiting factor (Iwasa et al. 1984). Females of this species may tend to be more selective, avoiding oviposition on previously parasitized hosts or those considered to be of poor quality (Rosenheim 1996, Ayala et al. 2014). This could explain the large numbers of oviposition attempts (host probing) observed and the greater time on selection of non-parasitized hosts. The native parasitoids *D. crawfordi*, *U*. *anastrephae* and *O*. *hirtus* invest more time in the process of oviposition (2.2 ± 0.8, 1.7 ± 0.1 and 1.52 ± 0.75 min, respectively) compared to the time invested by exotic species such as *D*. *longicaudata* (0.49 ± 0.2 min; Montoya et al. 2003) and *D*. *tryoni* (0.69 ± 0.065 min; Ramadan et al. 1994) under laboratory conditions, which could be related to the level of host discrimination ability, since *D. longicaudata* has a strong tendency to superparasitize (Montoya et al. 2003) while the native species here studied seem to avoid superparasitism. In *O. hirtus*, the duration of oviposition when parasitizing previously parasitized hosts was significantly smaller (1.2 ± 0.1 min) than with non-parasitized l hosts (1.6 ± 0.1). The time invested in oviposition can vary according to the particular species and host size (Rivero 2000). In our study, females with experience that had contact with previously parasitized hosts, proved to be the most insistent and inserted their ovipositor a second time in order to conduct contact (*D. crawfordi* 16/30, *U*. *anastrephae* 22/30 and *O*. *hirtus* 16/30).

Though koinobionts do not arrest host development, some species can induce transient host paralysis (temporary paralysis after being stung by the female wasp; e.g. Desneux et al. 2009, Chau and Maeto 2009). Our data show that the three braconids studied here temporarily paralyzed their hosts, with 100 percent of hosts undergoing transient paralysis. Interestingly, the duration of paralysis was species specific. Larvae parasitized by *U. anastrephae* remained immobile for 13 ± 1 minutes, and thus presented this state for the shortest time, compared to the immobility presented by host larvae parasitized by *D. crawfordi* and *O. hirtus* (20.9 ± 1.1 and 23.8 ± 1 min, respectively). The duration of immobility caused by oviposition of the native parasitoids exceeds the time of immobility experienced by larvae parasitized by *D. longicaudata* (4.8 ± 27 min; Montoya et al. 2003).

The factors associated with host immobility are toxic substances in a mixture such as venom, as well as polydnaviruses (PDVs) that function as regulatory elements and disrupt the host metabolism (Moreau and Guillot 2005, Kaeslin et al. 2009), affecting the immune system (Richards and Parkinson 2000, Cai et al. 2004). Two hypothesis have been advanced to explain the adaptive value of transient host paralysis: 1) facilitation of oviposition by interfering with host defensive behaviors; and 2) self-superparasitism avoidance. Support for the latter hypothesis comes from the work of Desneux et al. (2009) with two species of aphidiine braconids of the genus *Binodoxys* that attack aphids, and from the work of Chau and Maeto (2009) with *Meteorus pulchricornis* also a braconid (Euphorinae) that attacks a wide range of lepidopteran larvae. Transient paralysis caused by *Binodoxys* spp. lasted up to 15 min and paralyzed aphids were accepted at a significantly lower rate than control aphids (Desneux et al. 2009). Likewise, in *M. pulchricornis*, host movements remained at a low level for approximately 1h after oviposition, and additional ovipositions on paralyzed hosts were not observed (Chau and Maeto 2009). We further hypothesize that transient host paralysis may also be a means to avoid host detection by conspecifics and heterospecific competitors, reducing the risk of larval competition not only from superparasitism but also from multiparasitism. In solitary endoparasitoids only one

adult emerges per host, all other larvae are eliminated through direct (intrinsic) competition. Intrinsic competition in the guild of opine braconids that attack *Anastrepha* spp. has been demonstrated in *U. anastrephae* and *D. areolatus,* with the first instar larva of *U. anastrephae* being a superior competitor (Aluja et al. 2013). The duration of paralysis of the host in the three species studied here may allow some advantage to the developing embryo, delaying additional attacks. The first eclosed first instar larva might have more chances to win when competing with second laid individuals.

There are few studies regarding the oviposition behavior of opiine parasitoid species native to the Neotropical region, which makes our data of valuable importance. Our study reveals that behavioral differences among the studied parasitoid species lay mainly in the time of parasitization and in the time for which the parasitized hosts remained immobile, which could delay or minimize superparasitism. The three species were significantly capable of discriminating previously parasitized hosts, suggesting that this strategy is commonly present in the guild of fruit fly parasitoids attacking larvae in the Neotropics. Finally, our data also suggest that the studied species have the potential to be considered as suitable biological control agents. However, more studies are necessary to better understand the advantages and limitations that each one presents as natural enemies of fruit fly pests under field conditions.

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Tables

Table 1. Definitions of the different behaviors exhibited by *Utetes anastrephae*, *Doryctobracon crawfordi* and *Opius hirtus* while foraging for host larvae.

Behavior	Description
1. Walking	Female walking on the oviposition unit surface, antennae not directed to the substrate
2. Searching for a host	While walking the female touches the surface of the oviposition unit with the antennae
3. Detection of a host	The female stays immobile over a host larva
4. Oviposition attempt	Insertion of the ovipositor in order to have contact with the host. The latter is very mobile
5. Oviposition	Oviposition, the female remains immobile during a certain period of time with the ovipositor inserted in the interior of the host larva
6. Rejection	The female inserts the ovipositor in the host for a few seconds, but withdraws the ovipositor without actually laying an egg.
7. Failure	When the female inserts the ovipositor in the oviposition unit without having contact with some host, mainly by the escape of the larvae

Table 2. Average values (±SE) of number of ovipositions and attempts at oviposition on host larvae parasitized by conspecifics and non-parasitized host larvae.

	Number of ovipositions		Number of oviposition attempts (rejections)		
Species of parasitoid	Non-parasitized larvae	Parasitized larvae	Non-parasitized larvae	Parasitized larvae	*N*
Doryctobracon crawfordi	2.7±0.23[ab]	0.6±0.15*	56.9±5.1[a]	42.4±7.5[a]	30
Utetes anastrephae	3.3±0.25[a]	1.3±0.23*	12.2±2.1[b]	14.7±2.2[b]	30
Opius hirtus	2.3±0.32[b]	1±0.16*	15.9±2.2[b]	12.26±2.3[b]	30

Different letters indicate statistically significant differences (Anova, α=0.05) between species of parasitoids.

*Indicates a statistically significant difference between parasitized and non-parasitized hosts

Table 3. Average values (±SE) of duration of oviposition, vibration of the abdomen of the females, and immobility of the host after stinging (all in minutes) in non-parasitized and parasitized host larvae of *A. ludens*.

Species	Duration of oviposition		Vibration of the abdomen		Host immobility		
	Unparasitized host	Parasitized host	Unparasitized host	Parasitized host	Unparasitized host	Parasitized host	N
Doryctobracon crawfordi	2.2±0.1[a]	2.2±0.1	0.35±0.01[a]	0.35±0.03	21.3±1.2[a]	20.5±3.4	30
Utetes anastrephae	1.6±0.1[b]	1.9±0.1	0.28±0.01[ab]	0.26±0.01	13.4±0.6[b]	12.1±1	30
Opius hirtus	1.5±0.1[b]	1.2±0.1*	0.26±0.01[b]	0.3±0.01	23.8±1.2[a]	24.5±2	30

Different letters indicate a statistically significant difference per columns for each parameter (Anova, α=0.05). *Indicates a statistically significant difference between parasitized and non-parasitized hosts for each species per parameter.

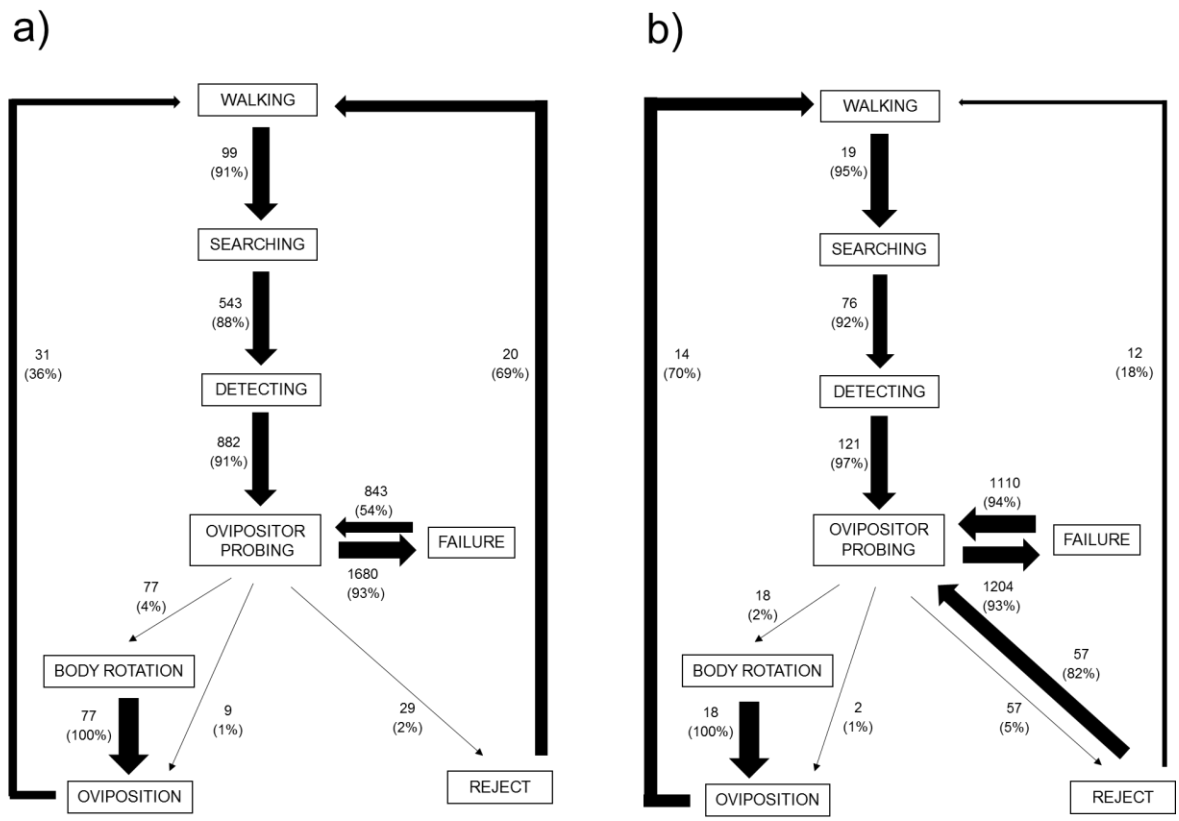

Figure 1. Ethogram of oviposition of females of *Doryctobracon crawfordi* on non-parasitized larvae (a) and larvae previously parasitized by conspecifics (b) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (the proportions are indicated in parentheses).

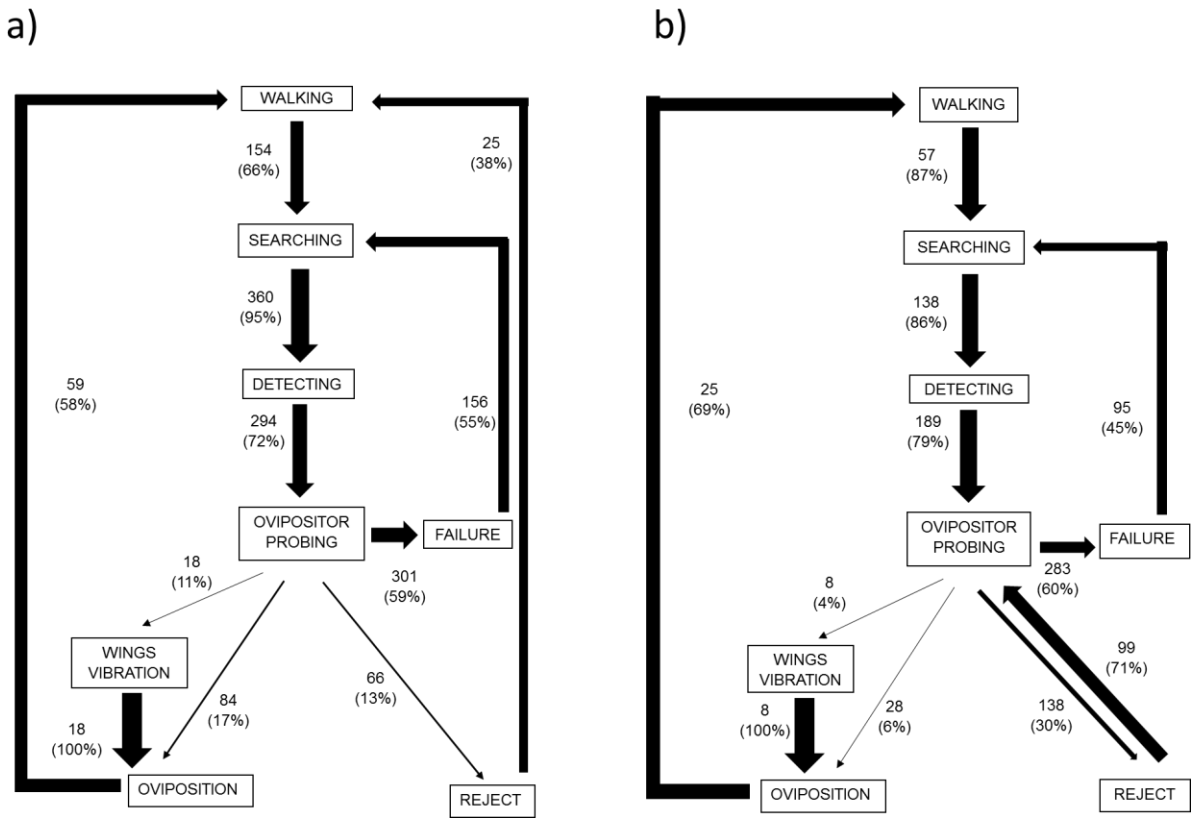

Figure 2. Ethogram of oviposition of females of *Utetes anastrephae* on non-parasitized larvae (a) and larvae previously parasitized by conspecifics (b) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (the proportions are indicated in parentheses).

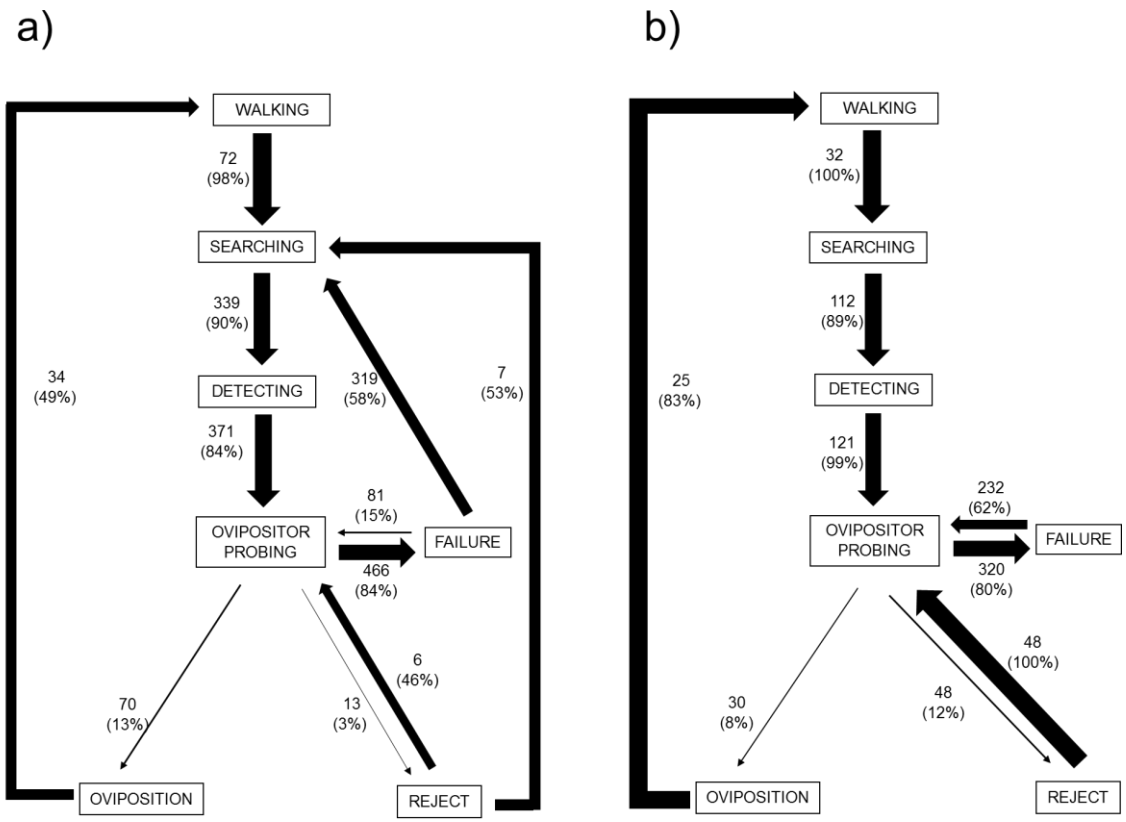

Figure 3. Ethogram of oviposition of females of *Opius hirtus* on non-parasitized larvae (a) and larvae previously parasitized by conspecifics (b) under laboratory conditions. The width of the arrow is proportional to the relative frequency of transition. The numbers associated with the arrows represent the observed frequencies of the successive behaviors of a complex sequence of behavior (the proportions are indicated in parentheses).

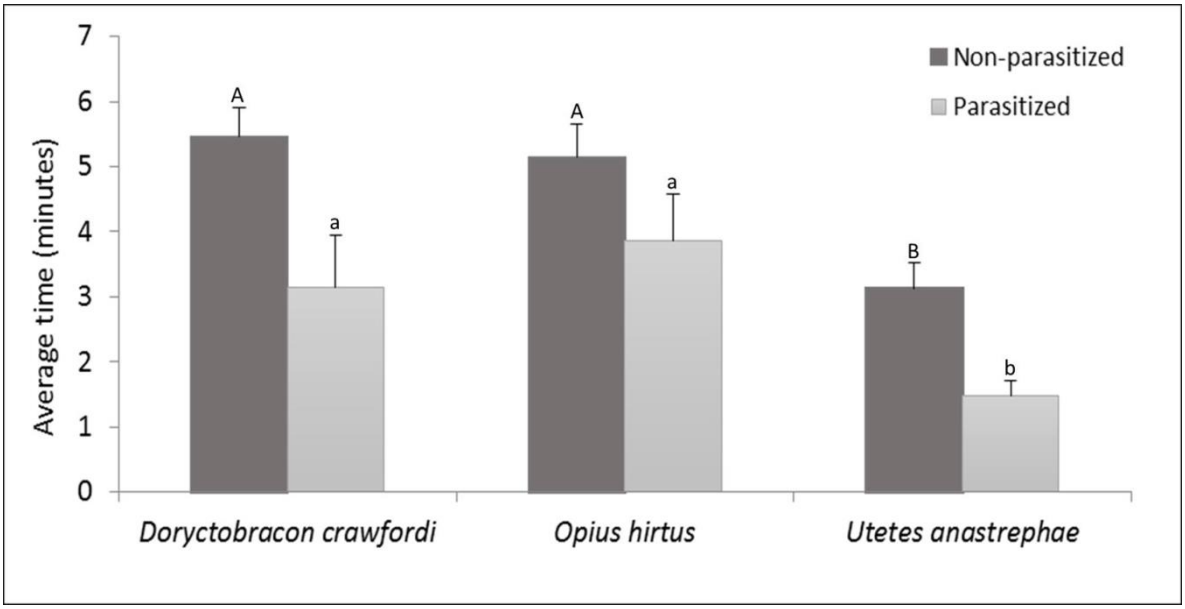

Figure 4. Latency (average ± SE, in minutes) between ovipositions of three native opine parasitoids attacking non-parasitized and previously parasitized *Anastrepha ludens* larvae. Different capital letters, indicate statistically significant difference between the bars. Different letters, indicate statistically significant difference between the bars. Different lower case letters, indicate statistically significant difference between species.

CAPITULO 3

Superparasitism and fitness parameters in three native parasitoid species of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae)

Amanda Ayala[1], Jorge Toledo[1], Gabriela Pérez-Lachaud[2], Pablo Liedo[1], Pablo Montoya[3]

[1] El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, 30700 México.

[2] El Colegio de la Frontera Sur, Avenida Centenario Km 5.5, Chetumal, Quintana Roo, 77014 México

[3] Programa Moscafrut SAGARPA-SENASICA. Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, 30860 México.

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Superparasitism and fitness parameters in three native parasitoid species of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae)

Abstract

Superparasitism refers to the action of a female parasitoid that oviposits in or on previously parasitized hosts. The objective of this study was to determine the presence of superparasitism in three species of native larval parasitoids that attack *Anastrepha* Shiner species (Diptera: Tephritidae), and its possible effect on wasps fitness parameters. The species evaluated were *Doryctobracon crawfordi* (Viereck), *Utetes anastrephae* (Viereck) and *Opius hirtus* (Fischer). The experiments were conducted under laboratory conditions with females acting alone (self-superparasitism), groups of five females of the same species (conspecific superparasitism) and groups of three females, one of each species (multiparasitism). Our results showed that, contrary to the introduced *Diachasmimorpha longicaudata* and *D. tryoni*, self-superparasitism is not common in the three evaluated species and that it is also rare under conditions of intraspecific competition. In the multiparasitism bioassays, a higher quantity of immature stages of *U. anastrephae* was observed, compared to those of *D. crawfordi* and *O. hirtus.* Most of the females of the native species under study appear to avoid superparasitism suggesting a high discrimination ability, which is probably result of a close relationship and evolutionary history maintained with *Anastrepha* hosts.

Key words: Self-superparasitism, multiparasitism, *Doryctobracon crawfordi*, *Utetes anastrephae*, *Opius hirtus*

Introduction

Reproductive success in parasitoid wasps depends to a large extent on the decisions female make during their foraging activities, depending on the ecological context in which they are found (Outreman and Pierre 2005). In this sense, acceptance of a host for oviposition becomes the most important decision once the host has been found, and this is dependent upon the quality of the host (Visser et al. 1992). One determining factor in acceptance of a host is its condition of being previously parasitized or not (Ueno 1994), since an already parasitized host will be of lower quality for the foraging female (Nelson and Roitberg 1995). In solitary parasitoids, where only a single individual develops per host, supernumerary individuals are normally eliminated through competition or physiological suppression (Bakker et al. 1985; Hubbard et al. 1987; Mackauer et al. 1992).

Superparasitism is a biological behavior that occurs when a female parasitoid parasitizes a host that has been previously parasitized, either by herself or by another female of the same species (van Alphen and Visser 1990); however, many species of solitary parasitoids have the ability to distinguish between parasitized and non-parasitized hosts, known as discrimination capacity (van Lenteren 1981; Mackauer 1990). This ability to discriminate previously parasitized hosts depends on the capacity of the parasitoid females for identifying external or internal marks on the hosts (Vinson 1976; Roitberg and Mangel 1988).

Superparasitism occurs at different levels: 1) self-superparasitism, when a female oviposits more than once on or in the same host; 2) conspecific superparasitism, when two or more females of the same species parasitize the same host (van Dijken and Waage 1987) and 3) multiparasitism, when two or more females of different species parasitize the same host (Vinson and Ables 1980; Pschorn-Walcher 1987; Pijls et al. 1995). Superparasitism is common in nature and, while initially considered a failure on the part of the female (Salt 1961), it has now been recognized as conferring an adaptive advantage under specific circumstances (van Lenteren 1981; Janssen 1989; van Alphen and Visser 1990).

Superparasitism can also be present under massive rearing conditions and

is, in some cases such as the introduced parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) attacking fruit flies of the genus *Anastrepha* Schiner (Diptera: Tephritidae), related to a sexual proportion favorable to females, without causing other fitness parameters to become unfavorable (González et al. 2007, Montoya et al. 2011). Superparasitism has been investigated in this new association, throughout conducting different studies under both laboratory and field conditions (González et al. 2007, 2010; Montoya et al. 2011, 2013); however, this phenomenon has been little studied in the guild of native parasitoids of the Neotropical region that attack flies of the genus *Anastrepha.*

In the Neotropics, the guild of the native parasitoids associated with fruit flies of this genus comprises mainly members of the family Braconidae (Hymenoptera), for the most part Opiinae solitary, larval-pupal endoparasitoids (Ovruski et al. 2000). The most important of these species include *Doryctobracon crawfordi* Viereck, *Utetes anastrephae* Viereck, and *Opius hirtus* Fischer (López et al. 1999; Ovruski et al. 2000), which has a wide distribution across this biogeographic zone. All of these species are polyphagous and associated with various species of *Anastrepha*. *Doryctobracon crawfordi* is distributed from central Mexico to Argentina (Ovruski et al. 2005) and is associated mainly with larvae of *Anastrepha ludens* Loew, *A. fraterculus* Wiedemann, *A. striata* Schiner, *A. serpentina* Wiedemann and *A. obliqua* Macquart (Aluja et al. 1990; López et al. 1999). The distribution of *U*. *anastrephae* extends from northern Florida to northern Argentina (Sivinski et al. 1997; Ovruski et al. 2000), while *O. hirtus* has a more limited distribution (Mexico, Costa Rica and the Dominican Republic) (Ovruski et al. 2000) and is related to species of *Anastrepha* that are not attacked by the other two species (López et al. 1999; Sivinski et al. 2000), as is the case in *Anastrepha cordata* Aldrich when it develops in *Tabernaemontana alba* Mill (Apocynaceae) (Hernández-Ortiz et al. 1994).

The objective of this study was to determine the presence of superparasitism of the three aforementioned species of native parasitoids of flies of

the genus *Anastrepha*, and its plausible effect on fitness parameters such as sex ratio, survival and fecundity of the progeny. These data can be of great value to fruit fly control programs that imply the massive rearing and release of these natural enemies.

Materials and Methods

Biological material. The experiments were carried in the Biological Control Laboratory of the Methods Development Unit of the Moscafrut Program SAGARPA-SENASICA, located in Metapa de Domínguez, Chiapas, Mexico. The environmental conditions were: temperature of 25 ± 1 °C, relative humidity of 75 ± 5% and photoperiod of 12:12 h (light: darkness). Eight-day-old host larvae of *A. ludens* provided by the Moscafrut plant were used, along with adult parasitoids of the species *D. crawfordi*, *U. anastrephae* and *O. hirtus*, reared using the methods described by Aluja et al. (2009).

Determination of superparasitism.

1) Self-superparasitism. Plexiglass cages (30 × 30 × 30 cm) were used, into which one mated, five-day-old female parasitoid was placed. This female was sexually mature and had no previous experience of oviposition. Twenty larvae of *A. ludens* mixed with artificial diet were exposed to a female parasitoid in a Petri dish type oviposition unit (8.5 x 1.5 cm), covered with mesh secured by an elastic band to avoid escape of the larvae. After two hours of exposure, the larvae were removed and placed in plastic containers (~ 100 ml) with a lid in order to continue their development to the pupal stage. After 24 hours, the diet was removed by washing with water and substituted by a moist substrate of coconut fiber in order to facilitate pupation. After 72 hours, the exposed hosts were observed under a stereoscopic microscope Discovery V8 Karl Zeiss (Göttingen, Germany) in order to quantify the number of oviposition scars presents on the pupae as an indicator of superparasitism (as in Montoya et al. 2000, 2003). A total of 10% of the pupae were dissected to corroborate and correlate the presence of immature stages of

parasitoids with the number of scars present on the host. The non-dissected pupae were placed on plastic containers (~100 ml), labeled according to the number of scars, to await the adults emergence and determine the relationship between the sexual proportion presented and the number of scars. Fifteen replicates were conducted per species of parasitoid female.

2) Conspecific superparasitism. Groups of 5 mated females of the same species and age, with no oviposition experience, were placed in the Plexiglass cages described above. Twenty larvae were then placed inside the oviposition unit. The methodologies described in the previous section were also followed for these tests.

In order to determine the effect of superparasitism on the longevity and fecundity of the progeny, pairs were formed with the adults emerged from hosts with different numbers of scars (1 scar, 2-3 scars and >3 scars). Survival was evaluated under two conditions: a) without food or water and b) with food (bee honey) and water. For the first condition, mortality was only recorded every day, while in the second condition, mortality was recorded daily and fecundity evaluated exposing 20 eight-day-old non-irradiated larvae of *A. ludens* from the fifth day, to each adult pair for 2 hours per day, until the female died. The experiment was conducted for each parasitoid species, with 15 replicates per species. Only data for females were analyzed.

3) Multiparasitism. Groups of 3 females (one female of each species) were introduced to the Plexiglass cages described above, where they were exposed to 20 larvae of *A. ludens* in a Petri dish type oviposition unit. The behavior of the females was observed during two hours in order to determine if any competitive interference behavior was presented among the females. The Petri dishes with the larvae were then removed and the larvae placed, with diet, in a plastic container (~100 ml) with a lid, in order to continue their development to the pupal stage. The diet was removed after 24 h of exposure and substituted with a moist substrate of

coconut fiber. The pupae were maintained thus until emergence of the adults, in order to determine the percentage of emergence (parasitism) and the sexual proportion of each species. After three days of exposure, 10% of the parasitized pupae were selected at random in order to corroborate the presence of immature stages of the parasitoid species in competition. The larvae were identified by morphological differences among species, according to Murillo et al. (2016). A total of 15 replicates were conducted.

Statistical analysis

A simple linear regression was performed in order to relate the number of oviposition scars per pupa with the number of immature stages found within each pupa. A logistic regression was conducted in order to relate the sexual proportion to the number of scars on the pupae. A comparison of curves through the log-rank test was conducted to analyze the survival and fecundity. Fecundity was estimated from the number of daughters emerged per female per day for each species of parasitoid. The multiparasitisim data were analyzed using a one-way ANOVA, with Tukey test. The logistic regression was conducted using the statistical package Statgraphics ver. XV (2008). In other cases, was used the program JMP version 7.0.1 (2007).

Results

1) Self-superparasitism. A total of 29% of the larvae exposed to *D. crawfordi* presented oviposition scars (87 larvae), of which 38% (33 larvae) had more than one scar. There was no relationship between the number of scars present per host and the number of immature stages found within the host (0-1 parasitoid larva/host pupa) (R^2= 0.0295, d.f.= 1, F= 0.75, P= 0.38, Fig. 1a), the percentage of emergence of *D. crawfordi* was 33%. In pupae parasitized by *U. anastrephae*, 49% (147 larvae) had scars, of which 50% (73 larvae) had more than one scar. No relationship was observed between the number of scars and the quantity of immature stages (R^2= 0.0149, d.f.= 1, F= 0.4, P>0.05, Fig. 1b). The percentage of

emergence in this species was 34%. A similar tendency was observed for *O. hirtus*, where the relationship between the number of immature stages and the number of scars on the pupae was also not significant (R^2= 0.0267, d.f.= 1, F= 0.082, P= 0.37, Fig. 1c), with 27% of the larvae with scars (81 larvae), of which 42% (34 larvae) had more than one scar. A percentage of adult emergence of 23% was observed. Sex ratio was not influenced by the number of scars per pupa in any of the three species: *D. crawfordi* (χ^2= 0.16, d.f.=1, P = 0.68), *U. anastrephae* (χ^2= 0.82, d.f.= 1, P= 0.82) and *O. hirtus* (χ^2= 0.0086, d.f.= 1, P= 0.93).

2) Conspecific superparasitism and fitness of the progeny. A total of 73% (219/300) of the larvae exposed to *D. crawfordi* recorded oviposition scars, of which 79% (173/219) had more than one scar. The relationship between the number of scars present on the hosts and the number of immature stages within them was low but significant (R^2= 0.25, d.f.= 1 F= 4.3, P= 0.05, Fig. 1d), the number of immature stages was never greater than four and the percentage of adults that emerged was 27%. In *U. anastrephae*, 58% (174/300) of the pupae presented oviposition scars, of which 84% (146/174) presented more than one scar. In this species, a positive and significant relationship was observed between the number of scars and the number of immature stages within (R^2= 0.77, d.f.= 1, F= 7.27, P<0.0001, Fig. 1e); the emergence of adults was 36%. In the case of *O. hirtus,* 57% of pupae (170/300) presented scars, with 80% of the pupae presenting more than one scar (135 pupae); however, was not a relationship between the number of scars per pupae and the number of immature stages found within (R^2= 0.06, d.f.= 1, F= 7.63, P= 0.83, Fig. 1f). The percentage of emergence of adults in this species was 13%.

Sex ratio. The probability of an emerging parasitoid being a female was not associated with the number of scars present on the host for any of the species evaluated: *D. crawfordi* (χ^2= 1.97, d.f.= 1, P= 0.16), *U. anastrephae* (χ^2= 0.96, d.f.= 1, P= 0.32), and *O. hirtus* (χ^2= 0.035, d.f.= 1, P =0.85) (Figs. 2a, 2b, and 2c respectively).

Survival. The number of oviposition scars on the host (1 scar, 2-3 scars and >3 scars) had no effect on the survival of the females of the three parasitoid species

when they were maintained without food and water: *D. crawfordi* (Log-Rank χ^2= 9.20, d.f.= 8, P= 0.32), *U. anastrephae* (Log-Rank χ^2= 17.95, d.f.= 10, P= 0.05) and *O. hirtus* (Log-Rank χ^2= 7.24, d.f.= 6, P= 0.29). However, on comparison among species, *O. hirtus* was the species that survived longest under these conditions (5.26 ± 0.24 days) (mean ± ES) (Log rank χ^2= 12.81, d.f.= 2, P= 0.0001), while *D. crawfordi* and *U. anastrephae* lived for shorter times 4.43 ± 0.25 days (±SE) and 4.38 ± 0.17 days (±SE), respectively.

When food and water and the possibility of egg-laying were provided, there were not significant differences observed in the survival of females of the three species obtained from hosts with 1 scar, 2-3 scars and >3 scars on its cuticle: *D. crawfordi* (Log-Rank χ^2= 5.78, d.f.= 2, P= 0.06), *U. anastrephae* (Log-Rank χ^2= 4.64, d.f.= 2, P= 0.09) and *O. hirtus* (Log-Rank χ^2= 0.71, d.f.= 2, P= 0.7) (Fig 3a, 3b and 3c). The number of scars present on the pupae had no effect on the survival of the emerged females.

Fecundity. Females of *D. crawfordi* that emerged from hosts with a lower number of scars produced a significantly higher number of daughters (Log rank χ^2=14 d.f.= 7, P= 0.05) compared to those that emerged from pupae with more than three scars (Table 1). In contrast, the number of daughters in the progeny of *U. anastrephae* females was unaffected by the number of scars present on the host from which they emerged (Table 1).

3) Multiparasitism. In the dissected pupae, a greater number of immature stages of the parasitoid *U. anastrephae* was observed (1±0.06) (±SE) (F= 22.7, d.f.= 2, P<0.01), followed by *D. crawfordi* and *O. hirtus*. Only one pupa was found with three immature stages in its interior, one of each species. The combination of immature stages with greater frequency observed was *D. crawfordi* and *U. anastrephae* (4 pupae) (Figure 4). Of the non-dissected pupae, it was observed that the emergence of adults presented no significant difference between *U. anastrephae* and *D. crawfordi* (F= 2.15, d.f.= 1, P= 0.13). However, the number of adults emerged from the species *O. hirtus* (0.58±0.22) (±SE) was significantly

lower than the numbers emerged from the species *D. crawfordi* and *U. anastrephae* (3.08±0.86 and 2.83±0.78 respectively) (±SE) (F=4.04, d.f.= 2, P=0.03).

Discussion

Our results show that in the three species of native parasitoids studied, superparasitism does not appear to be an adopted adaptive strategy, since when the species acted alone this phenomenon was practically non-existent and, under intraspecific competition conditions, it occurred only infrequently. Furthermore, multiparasitism of the host was observed only in nine instances.

When the females of the three studied species foraged alone, 40% of the hosts provided to *D. crawfordi* presented more than one scar, followed by *O. hirtus* (42%) and *U. anastrephae* (50%) though unparasitized hosts were available. This high percentage of pupae with more than one scar can be mainly attributed to the probing activity of females during host discrimination and not to an act of superparasitism, since no correlation was observed between the number of scars present on the pupae and the number of immature stages found within them when the females foraged alone. This is in contrast to what has been reported in the introduced species *D. longicaudata* (Montoya et al. 2000; González et al. 2007) and *Diachasmimorpha tryoni* (Ayala et al. 2014).

The finding described above gains particular importance since we can infer that the females of these species have an intrinsic high capacity for discrimination that makes them very selective, and they do not appear to be influenced by external factors such as the number of non-parasitized hosts available. Egg load in parasitoid females is a factor that normally correlates with the discrimination capacity (Godfray 1994), where a limitation in the production of eggs makes females to be more selective. However, the females of the species here studied are sinovigenic and were categorized by Cicero et al. (2011) as species limited by time. Such species often have a high number of eggs or the ability to replace them quickly, so their reproductive success can be proportional to the number of hosts

they can attack during their life-time (Stephens and Krebs 1986, Charnov and Stephens 1988). This type of species can use the strategy of superparasitizing their hosts or can reabsorb their eggs, which acts to increase their life expectancy (Rivero-Lynch and Godfray 1997). In a broad sense, it is assumed that the production of eggs represents a high cost to parasitoid females, for which reason when there is a variety in the quality of hosts, the females will select the best host (Iwasa et al. 1984; Mangel 1992).

Chemical signals such as marking pheromones, or different chemical secretions that accompany the process of oviposition, can actively participate in the process of host selection and facilitate the recognition of previously parasitized hosts (Vet and Dicke 1992). The solitary endoparasitoid *Venturia canescens* Grav. (Hymenoptera: Ichneumonidae) has the ability to detect concentrations of secretions of the Dufour gland injected into previously parasitized hosts (Howard and Baker 2003). The selectivity observed in the females in this study could be related to the detection of marking pheromones or substances injected during the process of oviposition (e.g., venoms and associated viruses) that diminish the quality of the host by causing internal changes (Mackauer 1990; Gauthier and Monge 1999; Outreman et al. 2001). The effects of the substances introduced into the hosts during oviposition play a principal role in conditioning the physiology of the host in order to facilitate development of the parasitoid (Moreau and Asgari 2015). Another effect of these substances is the permanent or temporary paralysis of the host (Vinson and Iwantsch 1980), as is the case with the species under study (Ayala et al. 2018). This facilitates oviposition without the parasitoid wasps being subjected to the defense activities of the host (Vinson and Iwantsch 1980), although it could also be a strategy to avoid competition by delaying the moment at which a host can be attacked again.

It is possible that the experience of first contact of oviposition with a non-parasitized larva, and subsequently with a previously parasitized larva, was another factor that favored the capacity for discrimination observed in the females, since it was demonstrated in *D. longicaudata* that females with oviposition

experience presented reduced oviposition on previously parasitized hosts (Montoya et al. 2003). There are even cases where the female, in addition to discriminating previously parasitized host, has the capacity to identify the sex of the previously oviposited egg and assign the sex of her progeny accordingly, as is the case with the species *Anisopteromalus calandrae* Howard (Hymenoptera: Chalcidoidea) (Lebreton et al. 2010).

Competition for hosts in the presence of conspecifics seems to promote superparasitism. Species such as *D. longicaudata* and *D. tryoni* increase their superparasitic activity when faced with conditions of competition (Montoya et al 2000; Ayala et al. 2014), with no adverse effects on the longevity and fecundity of their progeny (González et al. 2010; Ayala et al. 2014). In the case of the native species studied here, the presence of conspecific females foraging in the same patch had no effect on the superparasitism in *O. hirtus*; however, in *D. crawfordi* and *U. anastrephae*, a direct relationship was observed between the number of scars and the number of immature stages within the pupae of *A. ludens*, which suggests an increase in the propensity to superparasitize hosts when conspecifics are present in these two species.

According to King et al. (1993), sex ratio in parasitoid wasps can vary depending on three conditions: a) host size, b) superparasitism and c) incidence of various females foraging the same site. There are cases where superparasitism favors the sexual proportion towards the production of females, which is mainly explained by the premise that the female larvae have higher possibilities of winning in the case of larval competition, such as in the cases of *Eupelmus veulleti* (Hymenoptera: Eupelmidae) (Darrouzet et al. 2003) and *Anaphes victus* Huber (Hymenoptera: Mymaridae), where the females were shown to be better competitors that the males under conditions of superparasitism (van Baaren et al. 1999). For the exotic species *D. longicaudata* and *D. tryoni*, superparasitism has been positively related to the production of females (González et al. 2007; Montoya et al. 2011), while in the three native species here studied the observed superparasitism has no effect on the proportion of females. Our results also show

that, in the cases of *O. hirtus* and *U. anastrephae*, the number of oviposition scars on the pupae had no effect on the longevity and fecundity of the progeny, unlike in the case of *D. crawfordi* where lower longevity and lower fecundity have been reported in females emerged from pupae with more than one scar (Ayala et al. 2014).

Interspecific competition in parasitoids can arise as a result of exploitation of a common resource (May and Hassell 1981; Schoener 1983; Bográn et al. 2002; Wang et al. 2008). The greater similarities existing among the foraging habits, oviposition and feeding, increase the probability of competition. According to our multiparasitism test, the species apparently most competitive was *U. anastrephae*, since the highest percentage of dissected pupae that contained a single immature stage corresponded to this species, although similar percentages of subsequent emergence of adults of *U. anastrephae* and *D. crawfordi* were observed.

The first larval instar of *U. anastrephae* presents larger mandibles than the other two species, an adaptation that could allow this species to resist competition (Aluja et al. 2013, Murillo et al. 2016). For its part, *O. hirtus* seems to be the least competitive species, since it presented lower adult emergence than in the other two species. According to Harvey et al. (2013), the conditions of multiparasitism are more advantageous for species with a more rapid development, or species that attack their hosts in earlier stages of development.

Even when superparasitism in parasitoid wasps is common in nature and can be adaptive under specific conditions, the native species under study tended to avoid it, probably as a result of the close relationship they maintain with hosts of the genus *Anastrepha*. The tendency to superparasitize has also been observed in species such as *Trichogramma* spp. under mass rearing conditions, where the high proportion of females leads to superparasitism and the sexual proportion of the progeny favors the production of males (Smith 1996).

The origin of superparasitism in each species could be the result of different factors, such as host availability, previous experience of oviposition in the females, physiological resistance of the host, mechanisms of adaptation exhibited by the

parasitoids and host defense mechanisms (van Alphen and Visser 1990; Godfray 1994; Rosenheim and Hongkham 1996). However, none of these factors appear to cause this phenomenon in the native parasitoid species here studied. This may represent an adaptive strategy of importance in a scenario of high intra and interspecific competition, where the capacity to effectively discriminate already parasitized hosts can be of vital importance.

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Tables

Table 1. Number total of daughters per female (mean ± SE). Females emerged from pupae with a different number of scars under the condition of conspecific superparasitism.

Number of scars	**Parasitoid species**		
	D. crawfordi	*U. anastrephae*	*O. hirtus*
1	4.7 ± 0.37a	3.02 ± 0.6a	2.3 ± 0.40a
2-3	3.5 ± 0.60ab	2.02 ± 0.3a	1.6 ± 0.2ab
> 3	2.8 ± 0.43b	2.1 ± 0.5a	1.1 ± 0.17b

Different letters in a single column indicate a statistically significant difference (ANOVA, $P < 0.05$)

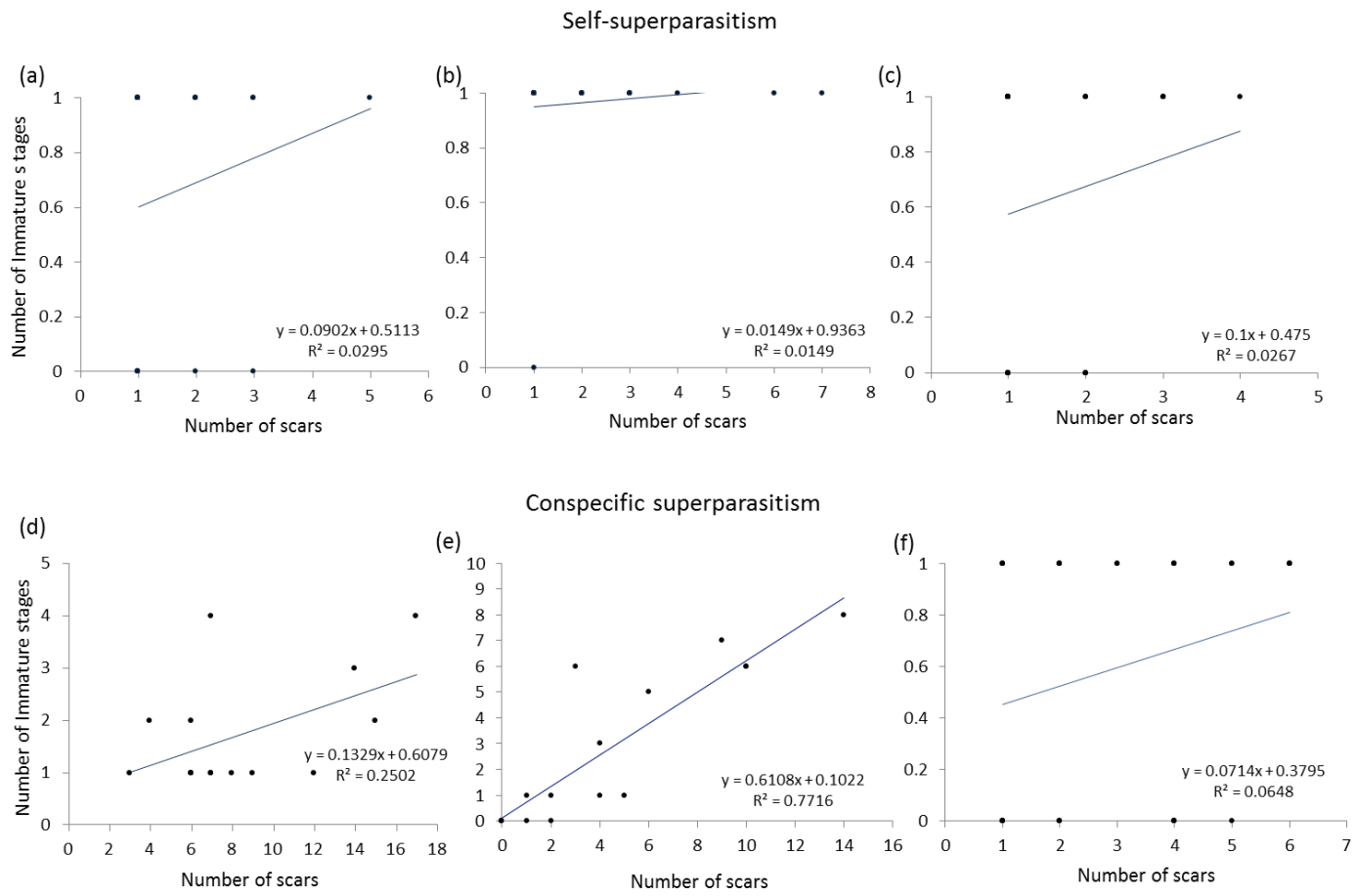


Figure 1. Relationship between the number of oviposition scars per pupa and the number of immature stages in self-uperparasitism and conspecific superparasitism situations in *Doryctobracon crawfordi* (a,d), *Utetes anastrephae* (b,e) and *Opius hirtus* (c,f).

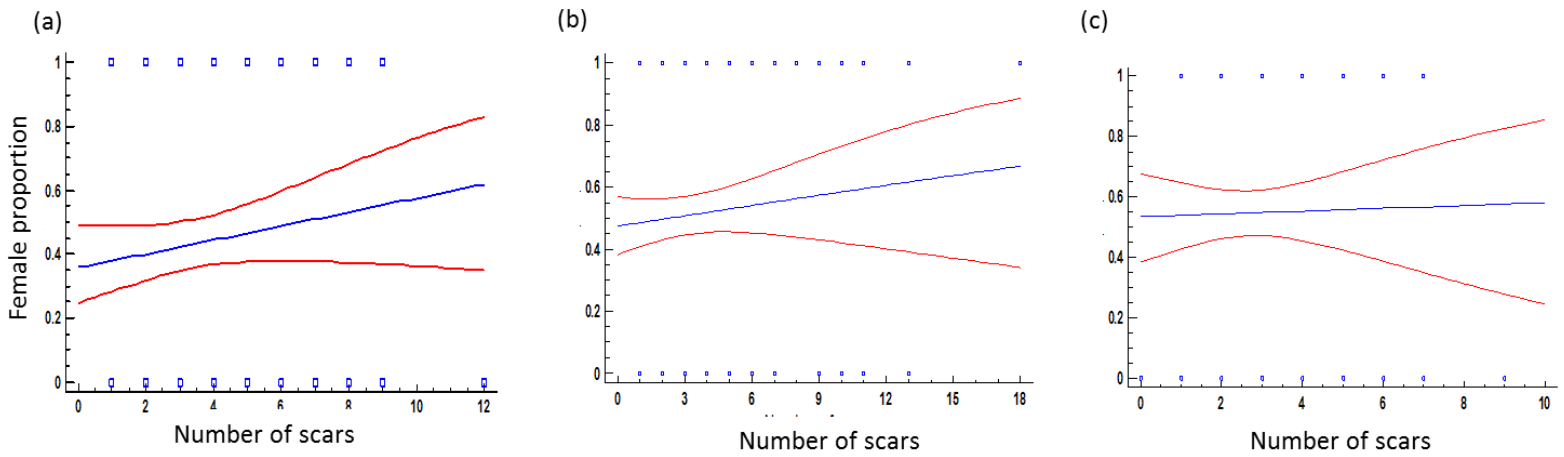
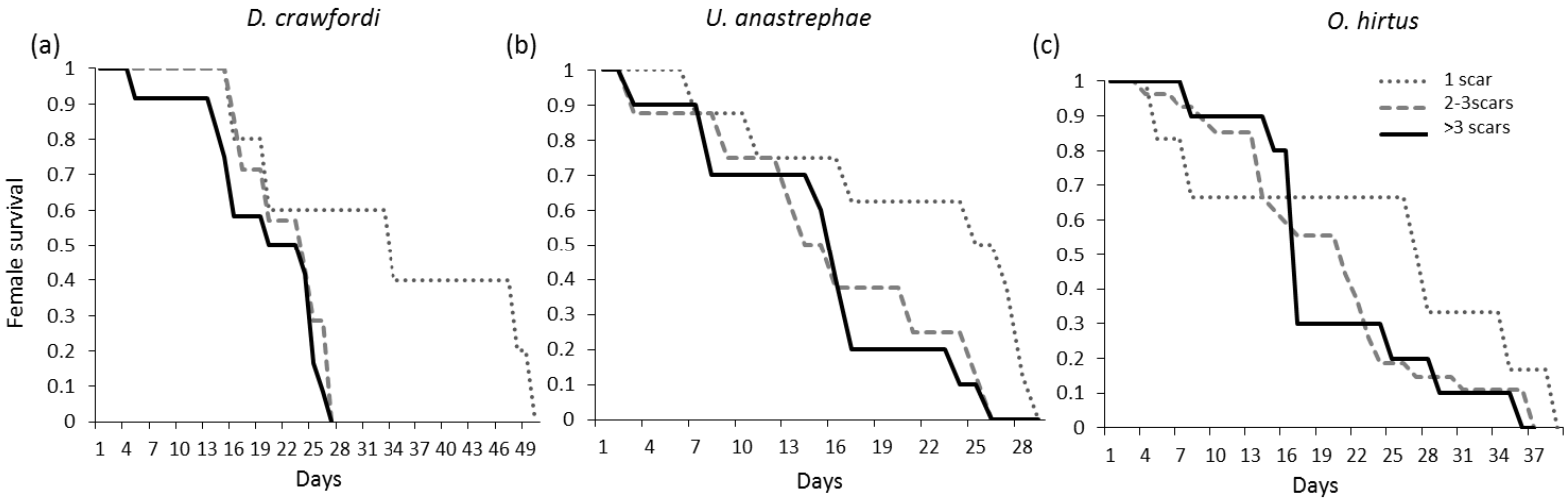


Figure 2. Logistic regression between the sexual proportion (proportion of females) and the number of oviposition scars per pupa in *D. crawfordi* (a), *U. anastrephae* (b) and *O. hirtus* (c) when the females forage with conspecifics. Binary data (1= females, 0= males).



Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	5.7783	2	0.0556
Wilcoxon	4.5379	2	0.1034

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	4.6421	2	0.0982
Wilcoxon	5.9146	2	0.0520

Test	ChiSquare	DF	Prob>ChiSq
Log-Rank	0.7112	2	0.7008
Wilcoxon	1.2910	2	0.5244

Figure 3. Survival of females of *D. crawfordi* (a), *U. anastrephae* (b) and *O. hirtus* (c) emerged from pupae with different numbers of oviposition scars. Females had access to food and water and were provided with hosts.

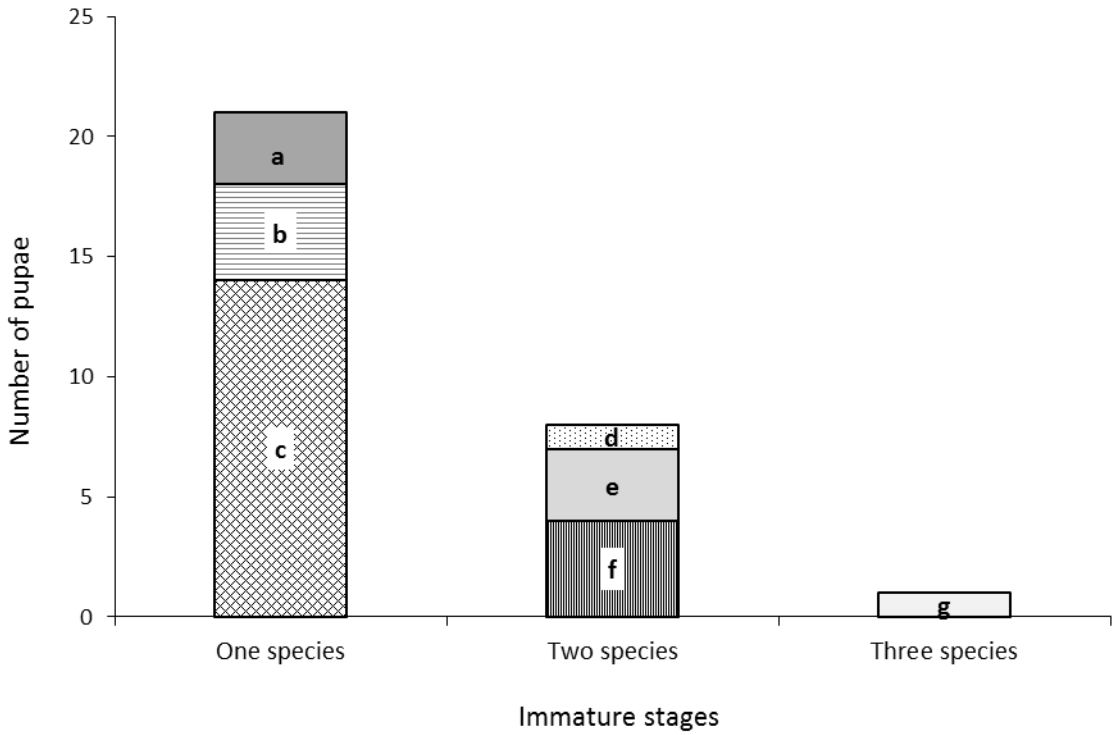


Figure 4. Number of dissected pupae (*n*=30) that contained immature stages of one, two and three species under multiparasitism condition. Species: *Opius hirtus* (a), *Doryctobracon crawfordi* (b), *Utetes anastrephae* (c), *Utetes anastrephae/Opius hirtus* (d), *Doryctobracon crawfordi/Opius hirtus* (e), *Doryctobracon crawfordi/Utetes anastrephae* (f) and *Doryctobracon crawfordi/Utetes anastrephae/Opius hirtus* (g).

CAPITULO 4

Discusión general y conclusiones

Discusión general

Durante el proceso de localización de hospederos, las hembras parasitoides se encuentran bajo una presión de selección para ser eficiente en términos de tiempo, localización y explotación de recursos. Para ello las hembras responden a una gran cantidad de estímulos ambientales entre los que se encuentran las señales químicas que proveen sus hospederos y que juegan un papel fundamental (Vet y Dicke 1992, Vinson 1998). Las especies bajo estudio presentaron un comportamiento similar en el forrajeo y detección de hospedero, pues las hembras de las tres especies mantuvieron un contacto constante de las antenas con la superficie de la unidad de oviposición, el cual ya había sido reportado en el parasitoide braconido de moscas de la fruta *D. longicaudata* (Leyva et al. 1991, González et al. 2010).

El patrón general de comportamiento exhibido por las hembras parasitoides consistió principalmente en la búsqueda y detección del hospedero, el intento de oviposición, y rechazo o aceptación del hospedero. Fue común observar también la falla de las hembras a causa del movimiento o escape de las larvas hospederas. Generalmente cuando las hembras detectaron a su hospedero, realizaron una serie de intentos de oviposición hasta que lograron un contacto que les permitió elegir o rechazar. También en *D. longicaudata* se ha observado este comportamiento (Montoya et al. 2003).

En parasitoides solitarios la aceptación de hospederos depende en gran medida del tamaño del hospedero y si está o no parasitado, éstos factores favorecen la aceptación (van Alphen y Visser 1990). Cuando se elige un hospedero que no ha sido previamente parasitado, el desarrollo de la progenie no se ve comprometido debido a que no existe una competencia por el recurso. Sin embargo, cuando una hembra elige un hospedero que ha sido previamente parasitado por hembras conespecíficas o heteroespecíficas, la supresión de los estados inmaduros supernumerarios es inevitable (Salt 1961, van Lenteren 1981, González et al. 2007), casi siempre a favor de la primera especie en parasitar.

El grado de la capacidad de discriminación puede variar entre especies de parasitoides (van Lenteren 1981). En algunas especies esta capacidad puede ser innata y casi total, como el caso de *Cephalonomia stephanoderis* (Hym.: Bethylidae) atacando estados inmaduros de la broca del café (Barrera et al. 1994, Cabrera y Barrera 1998). Las especies aquí estudiadas mostraron una buena capacidad de discriminación exhibiendo una notable preferencia por larvas sin parasitar. La especie *U. anastrephae* fue la que rechazó más larvas previamente parasitadas, comparada con las otras especies.

El rechazo de las larvas previamente parasitadas puede explicarse bajo la consideración de que las especies bajo estudio paralizan temporalmente a sus hospederos durante el proceso de oviposición (Michaud y Mackauer, 1994), y que la presencia de un huevo del parasitoide produce un cambio en la fisiología y desarrollo del mismo, convirtiéndolo en un hospedero de menor calidad. Cuando las hembras parasitoides ovipositan sobre hospederos previamente parasitados existe una repercusión en la progenie del parasitoide afectando su desarrollo, longevidad y fecundidad (Roitberg et al. 2001, Ayala et al. 2014).

Existen especies en donde la aceptación de hospederos previamente parasitados puede no ser una limitante, dependiendo de la disponibilidad de hospederos y de los tiempos mediados entre las oviposiciones. La especie *D. longicaudata* posee una buena capacidad de discriminación, sin embargo, estudios previos han demostrado que las hembras con frecuencia superparasitan a sus hospederos a pesar de disponer de un gran número de larvas no parasitadas, de las cuales optimiza recurso y se obtiene una progenie con una proporción sexual inclinada a hembras (Montoya et al. 2000, 2003), lo cual sugiere que para esta especie el superparasitismo puede ser una estrategia adaptativa. Se considera que este fenómeno (el superparasitismo) generalmente se presenta cuando existe una limitación de recursos (hospederos), alta competencia o la oviposición requiera de un proceso muy desgastante entre otros (van Alphen y Visser 1990, Weisser y Houston 1993). En las especies nativas bajo estudio se observó un comportamiento diferente, pues cuando las hembras actuaron en

solitario, el superparasitismo fue casi nulo, y bajo competencia conespecífica se observó de manera intermitente y con poca frecuencia.

Por otro lado, los efectos del superparasitismo sobre la progenie de parasitoides van desde la reducción del tamaño de los adultos emergidos de hospederos superparasitados hasta un efecto en la proporción sexual (Wylie 1965, King 1993), la cual puede variar dependiendo de ciertas condiciones externas como el tamaño del hospedero, el nivel de superparasitismo (González et al. 2007, Montoya et al. 2011) y la presencia de un gran número de hembras sobre un mismo parche de hospederos (King 1993). En las especies evaluadas, el nivel de superparasitismo no tuvo un efecto sobre la proporción de sexos, a pesar de considerarse como un factor que incide en la asignación del sexo como resultado de una mayor capacidad competitiva de las larvas que darán origen a hembras (Darrouzet et al. 2003).

La supervivencia sin agua y sin alimento tampoco se vio afectada cuando las hembras provinieron de pupas con una, dos o más de tres cicatrices. Con respecto a la fecundidad, solo en el caso de *D. crawfordi* se observó una disminución en éste parámetro cuando las hembras emergieron de pupas con más cicatrices. El número de cicatrices no tuvo efecto sobre la fecundidad de las hembras de *U. anastrephae.* En el caso de *O. hirtus* se observó una diferencia en la progenie de hembras emergidas de una cicatriz comparada con la progenie de aquellas hembras emergidas con más de tres cicatrices.

Las especies parasitoides que comparten hospederos pueden entrar en competencia por el recurso, favoreciendo la presencia de multiparasitismo (Godfray 1994). El multiparasitismo puede estar presente cuando los hospederos son escasos y la aceptación de los mismos puede variar dependiendo de la experiencia de oviposición de la hembra ya que la mayoría de las especies no son capaces de reconocer hospederos previamente parasitados por heteroespecíficas (Klomp et al. 1980, van Alphen y Visser 1990, Godfray 1994). Bajo condiciones de multiparasitismo *U. anastrephae* fue la especie que mayor número de estados inmaduros presentó al interior de los hospederos, mientras que *O. hirtus* presentó

el menor número, así como una menor emergencia de adultos. Se considera que la progenie de la primera hembra que parasita al hospedero es la que tiene mayor probabilidad de completar su desarrollo al inducir las respuestas fisiológicas del hospedero (Strand 1986, Godfray 1994, De Moraes et al. 1999, Pexton y Mayhew 2004).

Conclusiones:

- Podemos concluir que las tres especies de parasitoides nativos estudiadas mantienen un comportamiento de forrajeo similar.
- Presentan buena capacidad para discriminar a hospederos previamente parasitados por conespecíficos.
- Esto sugiere que al inmovilizar a los hospederos durante el proceso de oviposición inyectan sustancias que pueden ser reconocidas por las hembras conespecíficas.
- El superparasitismo, fue poco frecuente en las tres especies, aún bajo condiciones de competencia conespecífica.
- La longevidad no se vió afectada en parasitoides provenientes de pupas con una, dos o más cicatrices.
  La fecundidad
- Se requieren más estudios para comprender mejor las ventajas y limitaciones que cada especie presenta como enemigo natural.

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ANEXOS

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RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH

Host acceptance by three native braconid parasitoid species attacking larvae of the Mexican fruit fly, *Anastrepha ludens* (Diptera, Tephritidae)

Amanda Ayala[1], Gabriela Pérez-Lachaud[2], Jorge Toledo[1], Pablo Liedo[1], Pablo Montoya[3]

1 *El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, CP 30700, México*
2 *El Colegio de la Frontera Sur, Avenida Centenario km 5.5, Chetumal, Quintana Roo, CP 77014 México*
3 *Programa Moscafrut SAGARPA -IICA. Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, CP 30860 México*

Corresponding author: *Amanda Ayala* (apayala@ecosur.edu.mx)

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Abstract

We studied the oviposition and host acceptance behavior of three braconid parasitoid species native to Mexico, *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fischer), and *Utetes anastrephae* (Viereck), with potential to be considered as biocontrol agents against tephritid fruit fly pests in the Neotropics. Third instar larvae of *Anastrepha ludens* (Loew), with and without previous parasitization by conspecifics, were simultaneously offered to females of each species, and the individual behavior was video recorded to construct oviposition flow diagrams. The patterns of foraging and host acceptance were similar in the studied species; all rejected mostly parasitized hosts suggesting that this strategy is common in the guild of larval parasitoids attacking *Anastrepha* spp. The complete searching and host acceptance process took 2.2 ± 0.1 min (mean ± SE) in *D. crawfordi*, 1.7 ± 0.1 s in *U. anastrephae* and 1.5 ± 0.1 s in *O. hirtus*. Notably, because of toxins injected by parasitoid females during oviposition, the parasitized hosts experienced a transient paralysis of variable duration. Hosts attacked by *U. anastrephae* remained immobile for the shortest time (12.5 ± 1 min) (mean±SE), followed by *D. crawfordi* (20.5 ± 3.4 min) and *O. hirtus* (24.1 ± 2 min). Our data revealed a notable discrimination ability in all three species, and that behavioral differences lay

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Subject: BICO-D-18-00134: Submission Confirmation
To: Amanda Pricila Ayala <apayala@ecosur.edu.mx>

Dear Mrs Ayala,

Thank you for submitting your manuscript, Superparasitism and fitness parameters in three native parasitoid species of the Mexican fruit fly Anastrepha ludens (Diptera: Tephritidae), to BioControl.

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Tapachula, Chiapas, 4/April/2018

Amanda Pricila Ayala Ayala

We are pleased to inform you that your abstract submitted to the 10th International Symposium on Fruit Flies of Economic Importance has been accepted to be presented as a poster.

Title	Superparasitism and fitness parameters of three native parasitoid species (Hymenoptera: Braconidae) of the genus *Anastrepha* (Diptera: Tephritidae)
Session	7 Natural Enemies and Biological Control
Date	Thursday 26, April, 2018

We would appreciate if you can confirm your participation to flies@mail.ecosur.mx, before Tuesday 10/April. For poster guidelines please login at our web site.

Posters will be in exhibition during the whole week, so please bring your printed version on Sunday 22 between 10:00 and 18:00 hrs, or Monday 23 between 7:30 and 8:30, to be placed. Do not forget to upload the electronic version of your poster in our web site before April 13th.

Sincerely yours

Pablo Liedo
Chair Local Organizing Committee

10th International Symposium on Fruit Flies or Economic Importance
Tapachula, Chiapas, April 23-26, 2018
Tel:(962) 628 9800, ext. 5471, 5472,5762 Fax: (962) 628 9800, ext 5006

Superparasitism and fitness parameters of three native parasitoid species (Hymenoptera: Braconidae) of the genus *Anastrepha* (Diptera: Tephritidae)

Amanda Ayala[1,3], Jorge Toledo[1], Gabriela Pérez-Lachaud[2], Pablo Liedo[1] & Pablo Montoya[3]

[1]El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, 30700 México. Email: apayala@ecosur.edu.mx; [2]El Colegio de la Frontera Sur, Avenida Centenario km 5.5, Chetumal, Quintana Roo, 77014 México; [3]Programa Moscafrut SAGARPA - IICA. Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, 30860 México

Background: Superparasitism refers to the action of parasitoids ovipositing eggs in hosts that were already parasitized; these phenomenon has been associated with failures of parasitoid females in the discrimination ability recognizing hosts previously parasitized by conspecifics or by themselves. However, several authors consider that under specific conditions this strategy can be adaptive. Here we investigated the superparasitism performed by three of solitary larval parasitoids of Anastrepha ludens (Loew; Diptera: Tephritidae). The native braconid parasitoid species were *Utetes anastrephae* (Viereck) *Doryctobracon crawfordi* (Viereck) and *Opius hirtus* (Fischer).

Methods: Tests were conducted under laboratory conditions evaluating the behavior of females acting alone (self-superparasitism), in groups of five females (conspecific superparasitism) and simultaneously groups of three females by each species in a multiparasitism condition.

Results: Under self-superparasitism conditions, the females of the three species did not superparasitize; the number of first instar larvae found in each dissected host pupa was never greater than one regardless the number of oviposition scars observed per pupa; this finding is the opposite than that observed with the exotic parasitoids *Diachasmimorpha longicaudata* and *D. tryoni*. In the conspecific superparasitism test, *U. anastrephae* presented was the only species with a significant relationship between the number of scars and the number of immatures inside the pupa. There was no effect of scars number and sex ratio. The survival of adults coming from superparasitized pupa was negatively affected just in *D. crawfordi*. The fecundity of the three species was higher in females emerging from pupae with one scar. In the multiparasitism test, we observed a greater number of first instar larvae of *U. anastrephae* than the other species, but the adult emergence was similar in the three species, being *O. hirtus* the species with the lowest emergence of adults.

Conclusions: Our results suggest that native species attacking the *Anastrepha* fruit fly genus, have developed a high discrimination ability avoiding to fall in the superparasitism condition, since the fecundity of the emerged females was negatively affected. This is of greater importance in those species with potential as biocontrol agents against this type of pests.

Key Words: Self-superparasitism, multiparasitism, *D. crawfordi*, *U. anastrephae*, *O. hirtus*

Session 7. Natural Enemies and Biological Control

10th International Symposium on Fruit Flies of Economic Importance
Tapachula, Chiapas
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This is to certify that

The work titled:

Superparasitism and fitness parameters of three native parasitoid species (Hymenoptera: Braconidae) of the genus *Anastrepha* (Diptera: Tephritidae)

has been presented by:

Amanda Ayala, Jorge Toledo, Gabriela Pérez-Lachaud, Pablo Liedo & Pablo Montoya

as POSTER presentation in the
10th International Symposium
on Fruit Flies of Economic Importance
held at Tapachula, Chiapas, México; from 23th to 27th april 2018

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