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"Parasitismo, depredación y comportamiento de ataque de *Haplogonatopus hernandezae* (Hymenoptera: Dryinidae) y su efecto como agente de control biológico de *Tagosodes orizicolus* Muir (Homoptera: Delphacidae)"

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar por el grado de
Magister Scientiae

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DEDICATORIA

A mis papás y Diana
por sus consejos, apoyo
y fe en mis sueños

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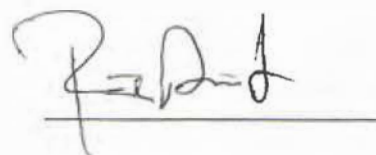
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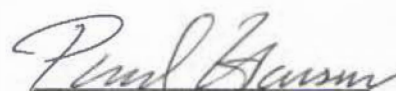
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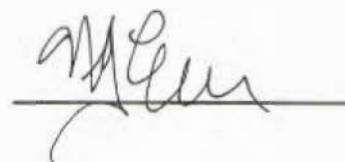

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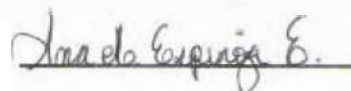Representante del Decano del Sistema
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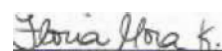Dr. William Eberhard Crabtree
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RESUMEN

Mora-Kepfer Floria

Parasitismo, depredación y comportamiento de ataque de *Haplogonatopus hernandezae* (Hymenoptera: Dryinidae) y su efecto como agente de control biológico de *Tagosodes orizicolus* Muir (Homoptera: Delphacidae)

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El delfácido *Tagosodes orizicolus* (Muir), es una plaga monófaga del arroz, *Oryza sativa* (Lin) y el vector del virus de la hoja blanca (RHBV), el cual puede causar hasta un 100% de pérdida en el cultivo. Este saltahojas se distribuye en todas las regiones arroceras en América y el Caribe.

Las poblaciones de *T. orizicolus* han sido atacadas con insecticidas sintéticos. Sin embargo, esta estrategia no ha sido efectiva y ha contribuido a la selección de insectos resistentes, resurgencia de plagas y la eliminación de enemigos naturales.

Por estos motivos, ha sido necesario explorar estrategias alternativas para controlar este insecto plaga, como lo es el control biológico utilizando enemigos naturales. *H. Hernandezae* actúa como depredador al alimentarse de ninfas de este delfácido para obtener energía y posteriormente utiliza otras ninfas para ovipositar y asegurar el desarrollo de su progenie. Esta avispa perteneciente a la familia Dryinidae ha sido encontrado en Costa Rica, Colombia y Argentina, utiliza exclusivamente a *T orizicolus* como hospedero y se reproduce por partenogénesis telytokia, generando una progenie de solo hembras.

En el presente estudio se analizó el efecto como controlador biológico de esta avispa sobre individuos *de T. orizicolus* en condiciones controladas, su factibilidad de cría y si la proporción de parasitismo y depredación variaba con la edad del dryinido.

También se avaluó el éxito en el desarrollo del parasitoide de acuerdo con el sexo del hospedero.

El porcentaje de parasitismo y depredación fue similar, 37% y 36.5% del total de potenciales hospederos que estuvieron en contacto con una hembra del parasitoide. La proporción de huéspedes que fueron depredados, parasitados y que no presentaron daño evidente no varió según la edad de la avispa. Del total de hospederos parasitados, 69% eran hembras y el restante 42% fueron machos, proporciones que fueron similares a lo largo de la vida de la avispa.

La sobrevivencia en las etapas de desarrollo del parasitoide también fue mas alta a partir de hospederos hembra, tanto desde la etapa de larva hasta emergir del hospedero, pupar en las plantas de arroz y alcanzar la etapa adulta. Los resultados indican una preferencia de *H. Hernandezae* a ovipositar en hospederos hembra y alimentarse de hospederos macho

Posteriormente, se describió la secuencia de comportamiento y tiempo de manipulación de ataque de *H. hernandezae*, tanto para ninfas que fueron depredados y parasitadas en hospederos de diferente etapa de desarrollo (ninfas de tercer, cuarto y quinto estadio). Los ataques para ovipositar fueron menos exitosos en ninfas de quinto estado. El tiempo de manipulación también presentó el mismo patrón, incrementó en ninfas mayores. Estas ninfas se defendieron del ataque al patear y sacudirse para liberarse del agarre del parasitoide. El tiempo que la avispa efectuó las categorías de paralización, anclaje y

taladraje fue mayor en hospederos mayores, sin embargo, el tiempo de oviposición fue similar en los diferentes estadios ninfales.

En el caso de ataques con la finalidad de alimentarse de una ninfa, el tiempo de manipulación no varió según la edad del hospedero. La hembra pinchó al hospedero repetidamente mientras se alimentaba.

También se encontraron diferencias en el manejo de la ninfa al finalizar el ataque. La hembra colocó cuidadosamente sobre las hojas de arroz a la ninfa en la que anteriormente había ovipositado. Al contrario, la manipulación de las ninfas que fueron depredadas fue sin cuidado y a menudo estas cayeron sobre el sustrato.

Los resultados de este estudio indican que la biología, tipo de reproducción por partenogénesis y eficiencia como parasitoide y depredador apoyan el uso de *H. Hernandezae* como agente de control biológico de *T. orizicolus*. Sería necesario evaluar el porcentaje de parasitismo y depredación con liberaciones masivas en regiones arroceras.

CAPITULO 1
ANTECEDENTES Y METODOLOGIA

PRESENTACION DEL TEMA Y REVISION BIBLIOGRAFICA

I. ANTECEDENTES

1.1. Método tradicional para el control de *Tagosodes orizicolus*

La estrategia de combate más utilizada para controlar las poblaciones de *T. orizicolus* es la aplicación de insecticidas sintéticos que atacan el sistema nervioso de este delfácido. Estos insecticidas organosfosforados y carbamatos inhiben la enzima acetilcolinesterasa, la cual propicia la acumulación de la acetilcolina en las sinapsis y como consecuencia se producen descargas nerviosas repetidas y un bloqueo de la transmisión nerviosa. Esto ocasiona la pérdida de coordinación muscular, convulsiones, y finalmente la muerte del insecto (Cremlyn 1978). Es importante indicar que las nuevas formulaciones de insecticidas sistémicos se redujo la toxicidad a mamíferos y por consiguiente su impacto en el ambiente (Hama 1980).

1.2 Efectos del uso indiscriminado de insecticidas sintéticos

El abuso en la aplicación de insecticidas ha sido un grave problema en Costa Rica, especialmente en las zonas arroceras de siembra ininterrumpida. En Guanacaste se han reportado casos de hasta ocho aplicaciones por ciclo de cultivo del arroz para el control de T. orizicolus (Espinoza com pers.). Lo anterior trae consigo problemas en los sistemas de producción agrícola sostenible, en el ambiente y en salud pública.

El resurgimiento como consecuencia de las aplicaciones indiscriminadas de insecticidas ocasiona incrementos significativos en las poblaciones del insecto blanco. Los factores determinantes en dicho resurgimiento son el resultado de la eliminación de enemigos naturales de la plaga y la selección de insectos resistentes a los insecticidas

(Benrey y Lamp 1994). Existen varios mecanismos bioquímicos, fisiológicos y de comportamiento de los insectos que influyen en la efectividad de los insecticidas (Corbett 1974). Un insecto puede, por ejemplo, evitar alimentarse o entrar en contacto con una planta tratada con insecticidas, o pueden seleccionarse de la población insectos resistentes. Un caso documentado es el del saltahojas del arroz *Nilaparva lugens*. La resistencia a organofosforados y carbamatos se debe a una detoxificación de estos insecticidas por esterasas hidrolíticas presentes en los insectos resistentes y a la insensibilidad a la acetilcolina (Heinrichs 1994).

Dadas las estrictas regulaciones en el uso de insecticidas sintéticos en relación a la contaminación del ambiente y a su toxicidad sobre otros organismos, es poco probable que se propicie el desarrollo de nuevos insecticidas que reemplacen a aquellos a los que los insectos se han vuelto resistentes (Hama 1980). Otros factores adicionales de riesgo son los residuos químicos que persisten en los alimentos y el efecto que puedan tener sobre las personas que realizan aplicaciones de estos productos en el campo.

1.3. Manejo integrado de plagas

Se desarrolló una estrategia alternativa para el control de *T. orizicolus*, el Manejo Integrado de Plagas (MIP), que tiene un enfoque ecológico como respuesta a los problemas asociados al uso frecuente de insecticidas que favorecen el resurgimiento de poblaciones. Se ha informado de casos de resurgimiento de las poblaciones de delfácidos en que lotes en los que se han utilizado insecticidas, teniendo como resultado densidades mayores de insectos que en lotes en los que no se aplicó (Kenmore *et al*. 1987).

El MIP hace énfasis en el uso de control biológico de las plagas, el uso de variedades resistentes y la implementación de prácticas culturales para mantener la plaga a

bajas densidades y el uso racional de insecticidas como último recurso (Schoenly et al. 1998). Esta estrategia requiere una forma más compleja de manejo de los insectos plaga por lo que es necesario investigar a fondo la dinámica de las poblaciones con el propósito de mantenerlas bajas (Cheng et al. 1994).

1.4. Uso de parasitoides como controladores biológicos

El estudio de enemigos naturales es una herramienta de gran utilidad en cuanto a la toma de decisiones respecto a estrategias de manejo de una plaga (Mattenson et al. 1994). La utilización de enemigos naturales para reducir el daño causado por plagas es conocido como control biológico (DeBach y Rosen 1991). El combate biológico de insectos depende de la supresión de poblaciones a través de la acción de enemigos naturales como lo son depredadores, parasitoides y patógenos (Smith 1919).

Dada la importancia de los mecanismos regulatorios naturales en la dinámica de las poblaciones de delfácidos en los trópicos, la conservación de las poblaciones de enemigos naturales debe ser un punto clave en la implementación de cualquier actividad de manejo utilizando el control biológico (Cheng et al. 1994). Análisis recientes de las tasas de establecimiento y éxito de controladores naturales han identificado patrones que pueden ser de gran utilidad para el desarrollo exitoso de estrategias para controlar las poblaciones de delfácidos (Benrey y Lamp 1994).

Aunque existe una gran diversidad de enemigos naturales de homópteros, la mayoría de intentos de control biológico se ha dirigido al parasitismo de los huevos (Benrey y Lamp 1994). Sin embargo, las ninfas y adultos son blanco de un extenso grupo de parasitoides y depredadores, pero el efecto de estos enemigos naturales en estos estadíos no se ha estudiado en detalle. Existe, asimismo, muy poca información respecto al

comportamiento de estos controladores biológicos, las interacciones con sus hospederos y su efectividad en control de plagas (Freytag 1985). Dentro de los órdenes Hymenoptera, Diptera y Strepsiptera hay especies con potencial para el control biológico de los delfácidos. La mayoría parasitan un espectro limitado de hospederos lo que representa una ventaja para su introducción en el cultivo y ya han sido reportadas para Costa Rica (Mora-Castro y Mora-Kepfer obs. pers).

1.5. Biología de la familia Dryinidae y su uso en control biológico

La familia Dryinidae perteneciente al orden Hymenoptera se distribuye en todos los hábitats terrestres desde la costa hasta los páramos en los Andes. Estos himenópteros parasitan ninfas y adultos de homópteros pertenecientes a Auchenorryncha. En la mayoría de los grupos las hembras, además de alimentarse de secreciones azucaradas, depredan a homópteros para obtener energía y posteriormente parasitan otros individuos para utilizarlos como hospederos para su progenie.

La hembra atrapa al hospedero por medio de sus patas anteriores modificadas en forma de quela y se alimenta de los tejidos y hemolinfa de este. En las subfamilias más especializadas (Gonatopodinae, Dryininae) los insectos depredados no sobreviven al ataque y la mortalidad por depredación puede igualar a la ocasionada por parasitismo (Olmi 1995).

Anteriormente, se han utilizado estos parasitoides en el control de poblaciones de delfácidos con resultados satisfactorios. *Haplogonatopus vitiensis* fue introducido exitosamente a Hawaii desde las islas Fiji para controlar poblaciones de *Perkinsiella saccharicida* Kirk., plaga severa de la caña de azúcar (Virla y Olmi 1998). En Cuba, el control biológico de *T. orizicolus* con el dryinido *Gonatopus sp.* ha reemplazado el uso de insecticidas sintéticos desde 1987 (Meneses com pers.).

1.6 *Haplogonatopus hernandezae*, su biología y potencial como agente de control biológico

Haplogonatopus hernandezae Olmi (Gonatopodinae: Dryinidae) actúa como parasitoide y depredador de las poblaciones de *Tagosodes orizicolus*. Este dryinido ejerce una doble función, las larvas son parasitoides de este delfácido y los adultos actúan como depredadores (Hernández y Belloti 1984). Varios autores han documentado que se observa una mayor mortalidad en este tipo de parasitismo (Olmi 1984).

H. hernandezae se reproduce por partenogénesis telytokia (Mora-Kepfer y Espinoza en prep), generando solo hembras en su progenie. Este tipo de reproducción tendría un buen efecto sobre el control natural de su hospedero ya que los adultos solo se dedican a buscar alimento y reproducirse y no invierte energía en copular. El potencial reproductivo es más amplio de lo que sería con la producción de machos.

En Colombia se realizaron liberaciones en campo de *H. hernandezae* en dosis de 100, 200 y hasta 300 adultos por hectárea de cultivo para controlar poblaciones de *T. orizicolus*, alcanzándose un 83.3% de parasitismo (Peñaranda et al.1999). Sin embargo, aunque Hernández y Belloti (1984) sugieren el potencial control que puede ejercer *H. hernandezae* como enemigo natural de *T. orizicolus*, se conoce poco sobre su impacto como parasitoide y depredador, y las posibles diferencias de comportamiento en tanto en ataques en que la avispa se alimenta o en los que oviposita. Tampoco existen estudios que evalúen el efecto del estadio de ninfal y sexo del hospedero sobre el éxito en el desarrollo del parasitoide.

II. OBJETIVOS

2.1 Objetivo general

Describir la biología, el comportamiento parasítico y predatorio, y el potencial reproductivo de la avispa *Haplogonatopus hernandezae* para determinar la posibilidad de su utilización en el control biológico del delfácido del arroz *Tagosodes orizicolus*.

2.2. Objetivos específicos

1. Analizar el efecto de *H. hernandezae* como depredador y parasitoide de *T. orizicolus*.

2. Explorar el efecto de la edad del parasitoide en la proporción de hospederos parasitados y depredados.

3. Documentar el éxito de desarrollo de la progenie de *H. hernandezae* según el sexo del hospedero.

4. Describir el comportamiento asociado a la oviposición y depredación por parte de *H. hernandezae*.

5. Comparar el periodo de paralización y posterior manipulación de *T. orizicolus* en ataques para parasitar y depredar.

6. Determinar si existen diferencias en el tiempo de manipulación de las ninfas parasitadas según su estadio de desarrollo.

7. Documentar el uso de la quela en las patas anteriores en el agarre y manipulación de las ninfas según su estadio de desarrollo y el resultado del ataque en individuos parasitados y depredados.

III. MATERIALES Y METODOS

3.1 Parasitismo y depredación de *Tagosodes orizicolus* Muir (Homoptera: Delphacidae) por *Haplogonatopus hernandezae*

Se establecieron colonias de *Tagosodes orizicolus* a partir de insectos colectados en arrozales en Guanacaste, Parrita y Turrúcares en Costa Rica. Los insectos con signos visibles de parasitismo, como un quiste en su tórax o abdomen se aislaron y criaron individualmente en jaulas (Chandra, 1980) para obtener nuevos parasitoides, cuando estos emergían para pupar en las hojas de arroz. Tanto los parasitoides como los delfácidos se criaron en un insectario bajo condiciones controladas de temperatura (25-30 °C) y humedad relativa 70-80% (Mora et al. 2005 en prensa).

Se observó diariamente cada una de las jaulas con una sola ninfa parasitada. Se registró el sexo del parasitoide que emergió, el sexo del hospedero y la duración cada estadio del ciclo de vida. También se registró la cantidad de progenie de cada hembra de *H. hernandezae*, el porcentaje de sobrevivencia, y la máxima etapa de desarrollo alcanzada.

Se introdujeron 10 ninfas de los estadios 3 y 4 diariamente en cada jaula con un parasitoide. Al día siguiente, la avispa se transfirió a una nueva jaula con nuevas ninfas, hasta que la hembra muriera. Se cuantificó la cantidad de ninfas depredadas y el resto fueron observadas por dos días para registrar el total de individuos que sobrevieron al ataque del dryinido. Para asegurar que el ataque había sido la causa de la muerte del hospedero, se observó a cada ninfa bajo un estereoscopio, ya que el daño físico es un claro indicador.

Se observaron las ninfas restantes por ocho días posteriores al ataque para detectar cualquier signo de parasitismo. Los insectos parasitados que alcanzaron la etapa adulta, se mantuvieron en la misma jaula que los no parasitados para determinar si los primeros

podían reproducirse. Se aisló cada uno de estos individuos de dos a tres días antes que la larva emergiera para pupar fuera del hospedero.

Se registró el número de días que tomó a cada ninfa llegar al estadio adulto y su sexo, ya que es muy difícil reconocer si una ninfa es macho o hembra. También se documentó la edad de cada parasitoide cuando ovipositó en el hospedero, el estadio de desarrollo máximo que este alcanzó y si este causó la muerte del hospedero. Para determinar la proporción de sexos de *T. orizicolus*, se cuantificó el número de adultos de ambos sexos en 9 jaulas de cría.

3. 2 Comportamiento de ataque y manipulación del dryinido *Haplogonatopus hernandezae* (Hymenoptera: Dryinidae)

Las 20 hembras de *H. hernandezae* que se utilizaron en este estudio eran hembras jóvenes de 2 a 4 días de emergidas como adulto. A cada hembra se le proporcionó ninfas de tercer estadio de *T. orizicolus* y miel diluida colocada en algodón. Para observar el comportamiento, se colocó cada hembra en una placa Petri transparente de 8.5 cm x 1.8 c m con hojas de arroz y cinco ninfas de tercer a quinto estadio. Cada parasitoide se introdujo en una placa Petri por una hora con ninfas de un estadio y luego fueron transferidas a otro recipiente con ninfas de otro estadio.

Se grabó el comportamiento de depredación y oviposición utilizando una cámara color CCD modelo VCC-3912 conectada a un estereoscopio. Los estadios ninfales se determinaron utilizando criterios morfológicos (Mora et. al, 2001).

Se determinó además la relación de tamaño entre el hospedero y el parasitoide en cada ataque utilizando la medida del ancho de la cabeza del dryinido (Yamada and Miyamoto, 1998) y el largo total del cuerpo de la ninfa en cada video.

Los ataques de cada hembra se grabaron por un periodo de dos a tres días. Cada ninfa manipulada por una hembra se transfirió a una jaula individualmente, para asegurar que cada delfácido estuviera en contacto con el parasitoide solo una vez.

El ataque se clasificó como exitoso si la hembra logró paralizar y luego alimentarse u ovipositar en el hospedero, como fracaso en el caso que no pudiera paralizar o dominar al hospedero. Para comparar la paralización de las ninfas y su manipulación en ataques exitosos de tanto de depredación, como de oviposición, se establecieron categorías de comportamiento y se registró el tiempo total y promedio de cada uno (seg ± SD).

Se registraron las siguientes categorías de comportamiento del parasitoide: a) búsqueda (cuando el parasitoide movía su ovipositor a lo largo de las membranas entre los segmentos abdominales de la ninfa), b) taladraje (el periodo en que la hembra colocó el ovipositor en un punto específico hasta el momento antes de ovipositar), y c) oviposición.

El comportamiento de la ninfa fue dividido en tres categorías: a) resistencia (el periodo en que la hembra se sacudió y pateó para liberarse del agarre), b) paralización (periodo en que la ninfa no mostró movimiento después de ser pinchada) y c) recuperación (el tiempo que la ninfa logró moverse de nuevo luego de ser paralizada). El tiempo de manipulación del parasitoide se dividió en: paralización (periodo en que la ninfa estuvo paralizada luego de ser pinchada con el ovipositor) y manipulación (periodo de manipulación posterior hasta que la hembra iniciara la alimentación u oviposición).

La manipulación para posteriormente alimentarse por parte de la avispa fue divida en dos: el periodo en que la hembra manipulaba al hospedero hasta colocar sus mandíbulas

en cierto punto específico y el tiempo que duraba alimentándose del tejido de la ninfa. El comportamiento del hospedero fue dividido en tiempo de resistencia y tiempo de paralización.

Se registró la duración y frecuencia de cada una de estas categorías de comportamiento con el programa JWatcher (Blumstein et al. 2000). El uso de la quela para manipular al hospedero fue dividido en dos categorías: ninfas que fueron colocadas suavemente en el sustrato luego la manipulación y ninfas que fueron soltadas sin ningún cuidado.

Las fotografías de los ataques de oviposición y depredación se tomaron con un microscopio electrónico al utilizar nitrógeno líquido. Los individuos fueron colocados en un congelador y luego se trasfirieron aún congelados a etanol absorbente a -20°C. Luego de ser fijados por una semana, se colocaron en temperatura ambiente y se cubrieron con oro para ser analizados mediante microscopía electrónica de rastreo.

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CAPITULO 2

Parasitism and predation of *Tagosodes orizicolus* Muir (Homoptera:Delphacidae) by a dryinid parasitoid

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ABSTRACT

We analyzed predation and parasitism by the dryinid *Haplogonatopus hernandezae* as a natural enemy of *Tagosodes orizicolus* under controlled conditions and whether the proportion of predation and parasitism varied according to the age of the wasp. The percentage of nymphs that were parasitized and preyed upon was similar, 37% and 36,5%. The proportion of hosts that were fed upon, parasitized and that showed no apparent damage did not vary according to the age of the dryinid. Of the total parasitized hosts, 69% were females and 42% were males; there was no change with dryinid age. Survival was higher in female hosts, both for larvae that emerged from the host and pupated, and for pupae that moulted to adults. Thus, female dryinids prefer to oviposit in female hosts and prey on males. The biology, reproduction by parthenogenesis and efficiency of *H. hernandezae* indicate that this dryinid wasp may be useful as a biological control agent of *T. orizicolus*.

KEY WORDS: rice, biological control, predator, *Haplogonatopus hernandezae*, parasitoid, *Tagosodes orizicolus*

INTRODUCTION

The delphacid *Tagosodes orizicolus* (Muir) is a monophagous pest of rice, *Oryza sativa* (Lin) and the vector of the hoja blanca virus (RHBV) (Everett 1961; Meneses et al. 1998). This insect can cause severe crop losses when the rice variety planted is susceptible to the insect and to the RHBV (Calvert and Reyes 1999, Peñaranda et al. 1999). This planthopper is found in all the rice growing regions in Tropical America and the Caribbean (Morales and Niessen 1985).

Populations of *T. orizicolus* have previously been attacked by using synthetic insecticides (Rey and Garcia 1980; Peñaranda et al. 1999). However, this strategy has contributed to resistant insect selection (Corbett 1974), the elimination of natural enemies (Mattenson 2000) and resurgence of the pest (Heinrichs 1994; Mattenson et al. 1994).

To date, the studies on natural enemies of *T. orizicolus* have included hymenopteran parasitoids belonging to Gonatopodinae (Hymenoptera: Dryinidae). Several species parasitize a specific group of hosts (Virla and Olmi 1998) with great potential use as biological control agents of delphacids (Chandra 1980a; Chua and Dyck 1982; Heong et al. 1982; Hernández and Bellotti 1984; Olmi 1984; Virla and Olmi 1998).

Previous studies have shown satisfactory results. *Haplogonatopus vitiensis* was successfully introduced to Hawaii from the Fiji Islands to control the populations of *Perkinsiella saccharicida* Kirk., severe pest of sugar cane (Clausen in Virla and Olmi

1998). Biological control of *T. orizicolus* using the dryinid *Gonatopus sp* has replaced the use of synthetic insecticides in Cuba since 1987 (Meneses com pers.). In Colombia, doses of 100, 200 and 300 individuals of *Haplogonatopus hernandezae* per Ha of the crop were released to control the populations of *T. orizicolus*, reaching a parasitism of 83% of the pest population (Peñaranda et al.1999).

Although Hernández and Belloti (1984) suggest the biological control potential of the species *H. hernandezae* as an enemy of *T. orizicolus*, little is known about its impact as a parasitoid and predator. There is also no present study that evaluates the effect of the sex of the host instar on the success in the development of the parasitoid.

H. hernandezae has been found in Costa Rica, Colombia and Argentina (Hernández and Belloti 1984; Olmi 1985; Virla and Olmi 1998). This species reproduces parthenogenetically (Olmi 1984, Hernández and Belloti 1984), generating only females, a process known as parthenogenesis telytokia (Suomalainen 1962; Johanowicz and Hoy 1998; Jervis and Kidd 1996).

Females feed from secretions that contain sugar, and also prey on nymphs. They paralyze and oviposit on other individuals where their larvae develop (Olmi, 1984; Hernández and Belloti 1984). The female captures the host with her anterior legs whose tarsi have been modified as quelae and feeds from the tissues and hemolymph. (Hernández and Belloti 1984). Predation on nymphs in addition to oviposition can lead to higher mortality and more effective biological control (Olmi 1984, Waloff 1974; Kidd and Jervis 1989; Jervis et al 1996).

This study analyzes the effect of *H. hernandezae* as a natural enemy of *T.orizicolus*, as both a predator and as a parasitoid. It also explores the effect of the parasitoid instar on

the proportion of hosts that are used to oviposit or for food, and its. developmental success in hosts of different sex.

MATERIALS AND METHODS

Colonies of *T. orizicolus* were established from insects collected in rice fields in Guanacaste, Parrita and Turrúcares in Costa Rica, during 2000 and 2001. Planthoppers with visible signs of parasitism, such as a dark cyst on their thorax or abdomen, were isolated and reared in a small rearing cage (Chandra, 1980b) to obtain the parasitoids that emerged from the host to pupate on the rice leaves. The planthoppers (Mora et al.2005 *in press*) and the dryinids were reared continuously in cages in an insectary under controlled conditions, humidity of 70-80% and temperature (25-30°C). In the *T. orizicolus* cages, the planthoppers fed from Blue Bonnet 50 rice plants that were placed on a flat tray filled with 10 cm of water to exclude ants and other insects. The dryinid cages consisted of a cylindrical container placed in a rice plant (Chandra, 1980b). Two or three rice leaves were placed inside the cage and held by foam taps in each extreme to avoid the escape of the dryinid. Individual wasps were kept in each container.

We observed each of the containers with a single parasitized planhopper on a daily basis. We recorded the sex of the parasitoid that emerged and the sex of the hosts that reached the adult stage, the duration of the complete life cycle and of each developmental stage. We also registered the number of progeny for each dryinid, their survival rate, maximum developmental stage reached, and the quantity of cysts in each host.

We introduced 10 host nymphs of instar 3-4 daily to each container with a single female dryinid. The next day the nymphs were kept in that container and the female was transferred to a new cage with 10 new nymphs until the wasp died.

Because wasps sometimes did not immediately kill nymphs on which they fed, we quantified the number of predated nymphs in each container and the remaining nymphs were observed for the next two days to register the total individuals that survived that had suffered predation. To ensure that this attack had caused the death of the host, we observed each nymph under a stereoscope, since the physical feeding is a clear indicator of the destructive host feeding.

We observed the remaining nymphs for eight days after they were exposed to the wasp to detect signs of parasitism (Fig 1a). Parasitized hosts that reached the adult stage were kept in the same container with other planthoppers of the opposite sex that were not parasitized to determine if these adults were able to reproduce and isolated only two to three days before the larvae emerged from the host

We registered the number of days it took each nymph to reach the adult stage, and its sex as an adult (since it is very difficult to distinguish the sex of the nymphs) . The age of each parasitoid at the moment it oviposited on the host, the developmental stage the parasitoid reached whether it caused the death of the host were also recorded (Fig 1.). To determine the control sex ratio of *T. orizicolus*, the number of female and male adults was counted as soon as they became adults in 9 rearing cages.

Statistical Analyses

To explore differences in the proportions of parasitism, predation and individuals with no apparent damage according to the age of the dryinid female, we performed

Repeated Measure ANOVAS. These proportions were analyzed after being expressed by using an arcosenic transformation (Zar 1996).

To determine if the sex proportion of *T. orizicolus* tends to a 1:1 relation, we performed a Wilcoxon's test for matched pairs. We also explored differences in the sex ratio in potential hosts in contact with a parasitoid with a Chi-square test.

RESULTS

We found planthoppers parasitized by *H. hernandezae* in all three rice fields that we visited. All the parasitoids collected from the rice fields (N=64) and the progeny that was reared in the lab (N= 228) were female, and virgin females in captivity produced fertile female offspring, indicating that this species reproduces by parthenogenesis.

A total of 228 descendants were obtained from females over seven generations. Each female produced an average of 12± 2.2 offspring of which an average of 9± 1.56 individuals reached the adult stage (N=47).

Of the hosts raised in the insectary, 22 were superparasitized, with two cysts in each individual. In 73% these, both larvae emerged from the host to pupate in the rice leaves: only one larvae survived in 18% , no larvae emerged from the host in 9%.

The duration of almost all the developmental stages of *H. hernandezae* raised in controlled temperature conditions decreased in comparison to those raised in natural conditions. However, the average duration of the adult stage in female wasps did not vary regarding the temperature they were raised in (Table 1.).

A total of 1099 potential hosts were contacted by 40 female adult wasps, of which 73,5% were attacked successfully (Fig.2), either parasitized (36.5%) or fed upon (37%). Of

the total parasitoid larvae, 87% emerged from the host and pupated on the rice leaves, causing the death of the host. The remaining 13% died as larvae inside the host, and 33% as pupae. Only 54% of the parasitoids that produced cysts were able to emerge from the host and reach the adult stage (N=407, Fig 3).

None of the parasitized hosts that reached the adult stage in contact with other non parasitized planthoppers of the opposite sex was able to reproduce. In contrast, we obtained progeny from individuals that were exposed to a female dryinid but were not parasitized in four cases.

The proportions of parasitized, consumed and unconsumed nymphs did not vary regarding the host age (F=0.008 d.f.=6, P>0.05; F = 0.0188, d.f.= 6, P> 0.05; F = 0.0188, d.f.= 6, P> 0.05, respectively, Fig 4.). The proportion of male nymphs was significantly higher than females, 57.44 ± 28.5 vs 45.22 ± 25.85, in rearing cages in which the nymphs had no contact with wasps (T=4.5, d.f.=8, P=0.03). In contrast, in the case of planthoppers that were in contact with a parasitoid during the nymphal stages, the proportion of female adults was 2 for each male that reached adulthood ($\chi2$= 94.88 , d.f.= 1, N= 643, P<0.05).

Female nymphs were also more parasitized than male nymphs ($\chi2$= 43.61 , d.f.= 1, P=0.001, Fig 5.) and wasps developed more successfully in female nymphs. Larvae in female hosts were more successful in emerging from the host to pupate. ($\chi2$= 10.57 , d.f = 1, P= 0.001, Fig 5.), so were pupae in females hosts that moulted into adults ($\chi2$= 8.74, d.f.= 1, P= 0.003). Parasitoids that emerged from female hosts (78,8%), were significantly more successful in completing their life cycle than those that emerged from male hosts ($\chi2$= 26.06, d.f.= 1, P= 0.00, N= 392). The tendency to preferentially attack female nymphs did not vary throughout the parasitoid age ($\chi2$= 11.43, d.f.= 8, P= 0.178, Fig 6.).

DISCUSSION

The relatively low number of *Haplogonatopus hernandezae* individuals found in rice fields may be due to the constant application of insecticides to control this planthopper. On the other hand, the wide distribution of *H. hernandezae* in rice fields in Costa Rica and its biology support the feasibility of massive rearing this parasitoid and its use as a biological control agent of *T. orizicolus*.

Reproduction parthenogenesis of this wasp is advantageous for rearing in captivity, as it eliminates possible problems regarding courtship, successful mating and sex ratios that many parasitoids suffer in massive rearing conditions (Ode and Heinz 2002). One possible explanation for the presence of female progeny from virgin females is the manipulation of reproduction by endosimbionts of the bacteria *Wolbachia* (Johanowicz and Hoy 1998; Huigens et al. 2000; Noda et al. 2001). Another advantage of using *H. hernandezae* as a biological control agent is that it is native to Costa Rica, avoiding all the problems associated with the introduction of exotic species, their success in establishing themselves and possible interference with other insect species (Jervis and Kidd 1996).

The establishment of this dryinid in controlled conditions of temperature and humidity was successful, and we obtained a stable number of progeny per female during several generations. The duration of each developmental stage decreased in higher temperature conditions. However, these conditions did not alter the duration of the adult life span or their effectiveness as a control agent. Under controlled conditions, the numbers of nymphs attacked for oviposition and feeding were similar.

Even though only about 50% of the dryinid progeny reached the adult stage, 73% of the larvae emerged from the cyst to pupate causing the death of the host. These results show the same tendency as previous observations of species of *Haplogonatopus* as the larvae leave their host (Hernández and Belloti 1984; Olmi 1984). None of the nymphs with visible signs of parasitism in which the parasitoid larvae did not survive and was not able to leave the host survived to adulthood. This result suggests that the death of the developing larvae caused the death of its host.

None of the parasitized planthoppers that reached the adult stage while parasitized and had contact with individuals of the opposite sex was able to reproduce. This could be the result of parasitic castration that has been observed on other parasitoids (Olmi 1984; Dutt and Giri 1978). A previous study reported that young nymphs of *Pocatello furrier* that were parasitized by *Haplogonatopus apicalis* were not able to reproduce (Kitamura 1988).

The proportion of parasitized, predated and undamaged hosts did not vary regarding the age of the adult individual of *H. hernandezae*. However, another study by Sahragard et al. (1991) indicated that the number of individuals used to feed upon increases as the dryinid *Dicondylus indianus* becomes older and the number of hosts parasitized decreased.

The dryinid age did not have any effect on the proportion of males and females that were predated or oviposited in. There was a bias towards more female hosts being parasitizing female rather than male hosts by wasps of different ages. This sex proportion suggests a higher predation of male nymphs and the use of female nymphs as hosts for the dryinid progeny. Visual differentiation of sex in *T. orizicolus* nymphs is not possible until the 5th nymphal instar, but there is a sexual dimorphism in size (Mora et al 2001). Adult females are larger, therefore female nymphs may also be larger than male nymphs. To

oviposit in a smaller host could have a negative effect on the developmental success of the progeny. Also, to feed from a small host could represent a less energetic cost regarding the capture, paralysis and host handling. Female *H. hernandezae* feed from the thorax or abdomen of the host causing the death of the host by destructive host feeding. However, they do not consume the entire host (Mora-Kepfer pers obs.).

Our results indicate that larvae that developed inside female hosts were more successful in reaching the adult stage than the ones developing in male hosts. Adult female *T. orizicolus* live an average of 31.1 days, while adult males live only an average of 14.6 days (Gómez-Sousa and Kamara 1980). Since males of *T. orizicolus* have a shorter lifespan , the longevity of the host could affect successful larval development.

Also, oviposition attacks of female *H. hernandezae* tend to be more successful in third and fourth instar nymphs (Mora-Kepfer and Espinoza in prep). Since a dryinid larvae can take up to 27 days to develop inside the host and emerge to pupate, to oviposit in third or fourth instar female nymphs would assure that a larvae will have both enough time to develop inside the planthopper.

The preference of attacking nymphal instars and acting as both as a predator and a parasitoid suggests the efficiency of *H. hernandezae* as a biological control agent of *T. orizicolus*. Its reproduction by parthenogenesis thelytokia also represents an advantage in the massive rearing of this wasp.

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LEYENDS TO FIGURES

Table 1. Duration of the developmental stages of the parasitoid *Haplogonatpus hernandezae* under greenhouse and insectary conditions

Fig 1. Developmental stages of *Haplogonatopus hernandezae*. a.) *T. orizicolus* nymph with a cyst caused by a dryinid larvae b.) Young pupae (3 days from emerging from the host. c.) Mature pupae about to emerge as an adult. d.) Female adult

Fig. 2. Percentage of *T.orizicolus* nymphs with signs of parasitism, predation undamaged after a parasitoid attack

Fig 3. Percentage of individuals of *H. hernandezae* according to the maximum developmental stage reached according to its host *T. orizicolus*

Fig. 4. Percentage of predated, parasitized hosts and individuals with no apparent damage regarding the age of *H. Hernandezae*

Fig. 5. Percentage of individuals of *H. hernandezae* that pupated and later emerged as adults regarding the sex of the planthopper host

Fig. 6. Percentage of parasitized males and females of *T. orizicolus* in relation to the age of its parasitoid *H. hernandezae*

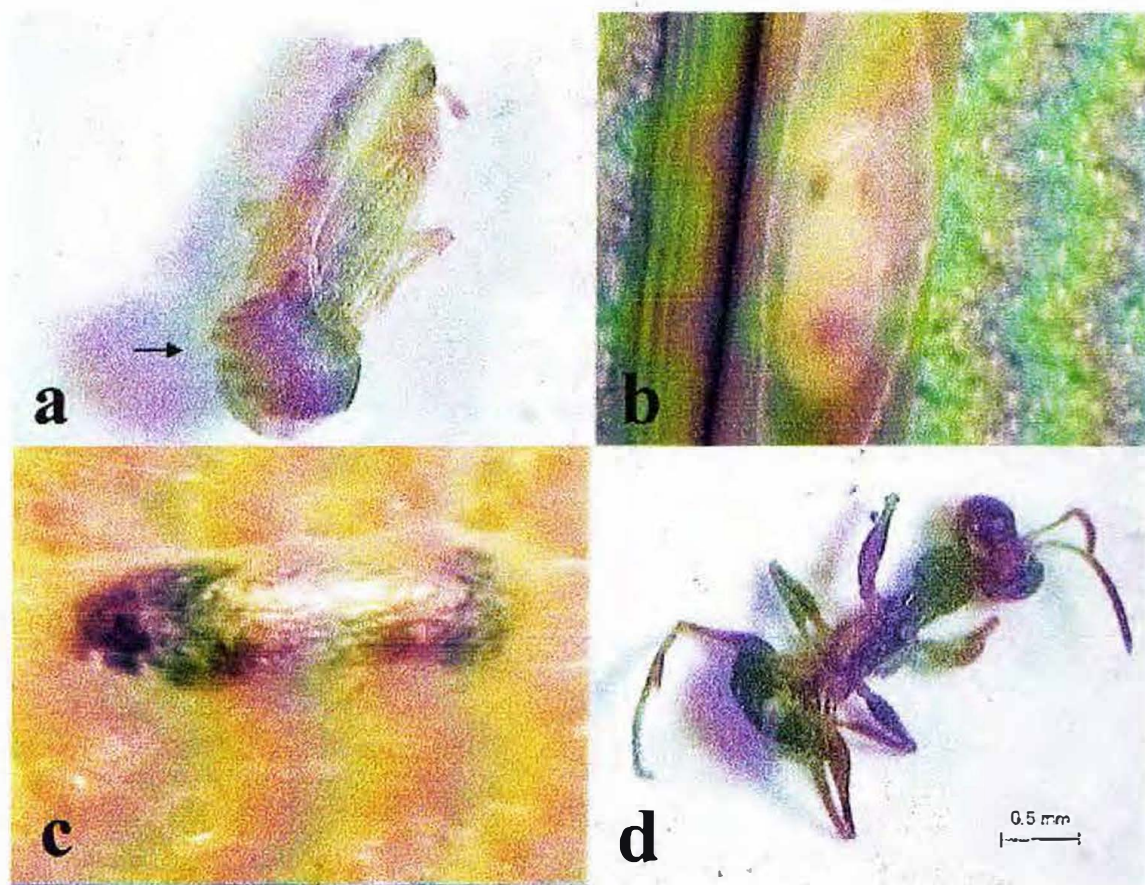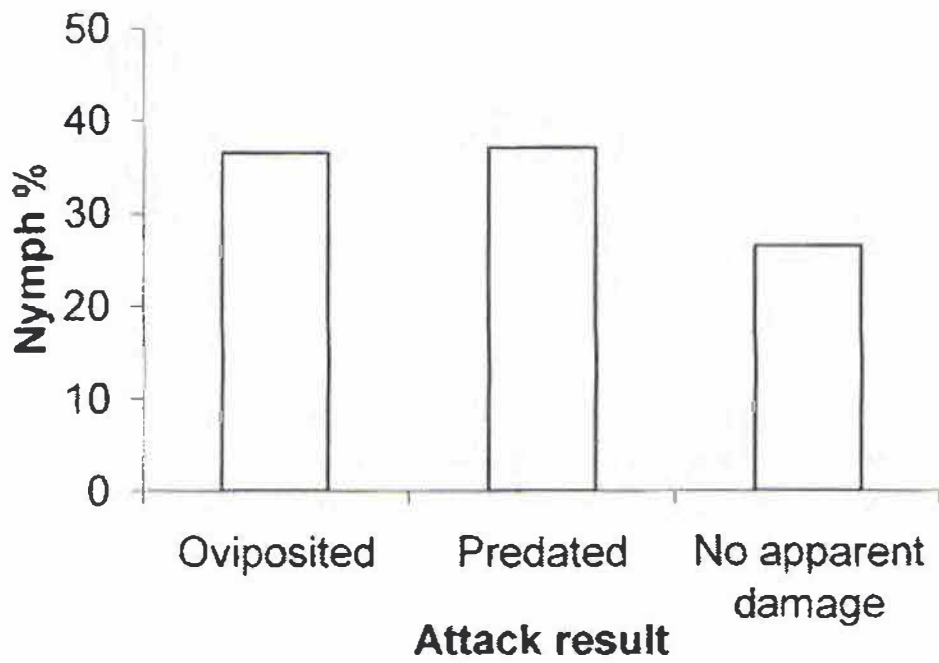| Developmental stage	Rearing conditions					
	Insectary		Greenhouse			
	Mean (Days)	SD	Mean (Days)	SD	T	P
Larva	15.78	3.78	27.49	4.61	12.91	0.00
	(N =41)		(N =47)			
Pupae	13.95	2.84	16.04	3.24	2.32	0.02
	(N =25)		(N =27)			
Adult	7.21	2.53	8.61	3.62	1.37	0.18
	(N =18)		(N =19)			
Complete	29.18	3.03	42.25	6.15	9.79	0.00
life cycle	(N =27)		(N =24)			

Table. 1

Fig 1

Fig. 2
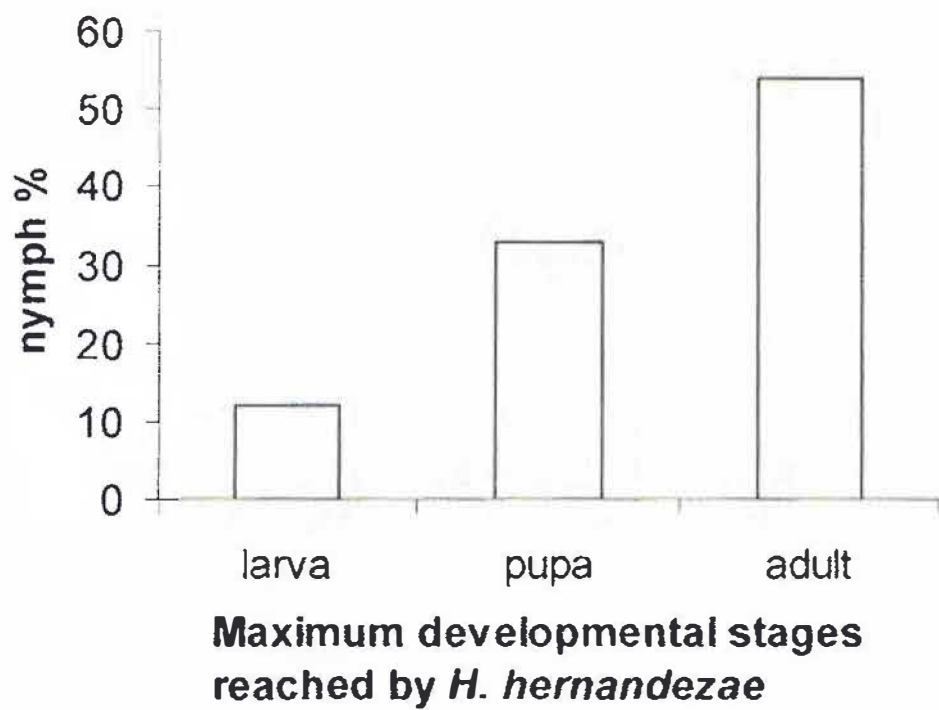
Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6

CAPITULO 3

Attack and handling behavior of the dryinid wasp *Haplogonatopus hernandezae* (Hymenoptera: Dryinidae)

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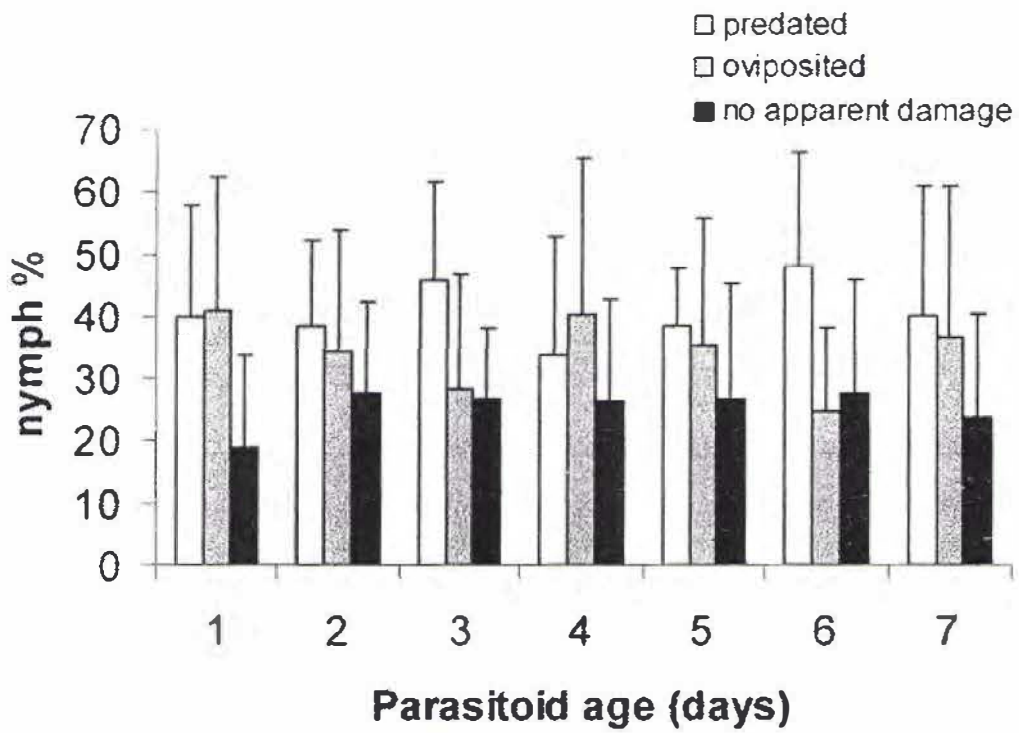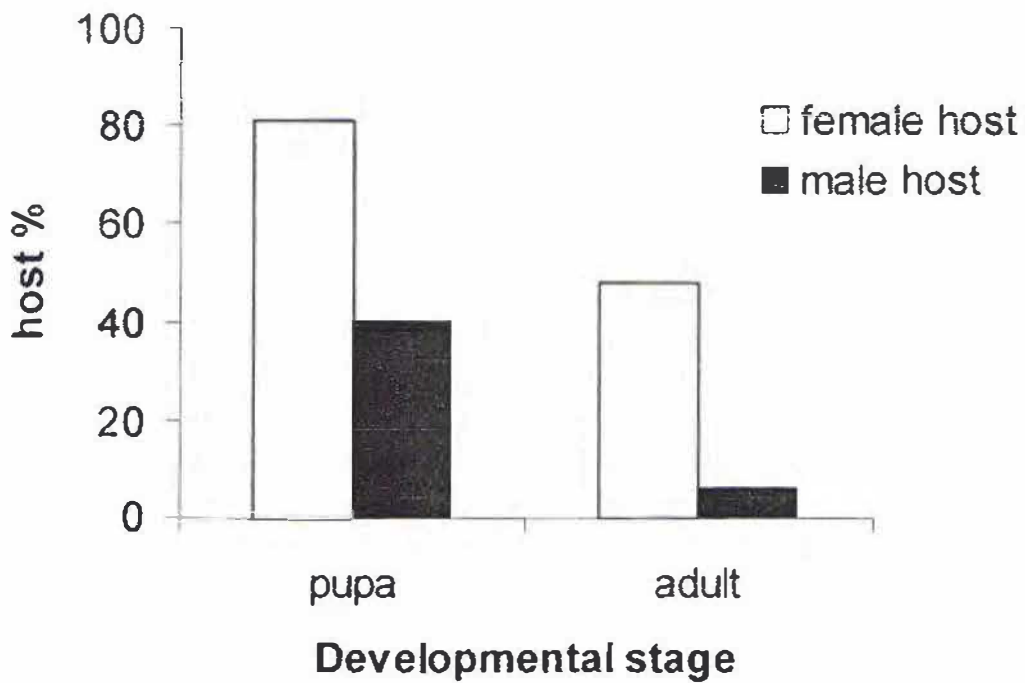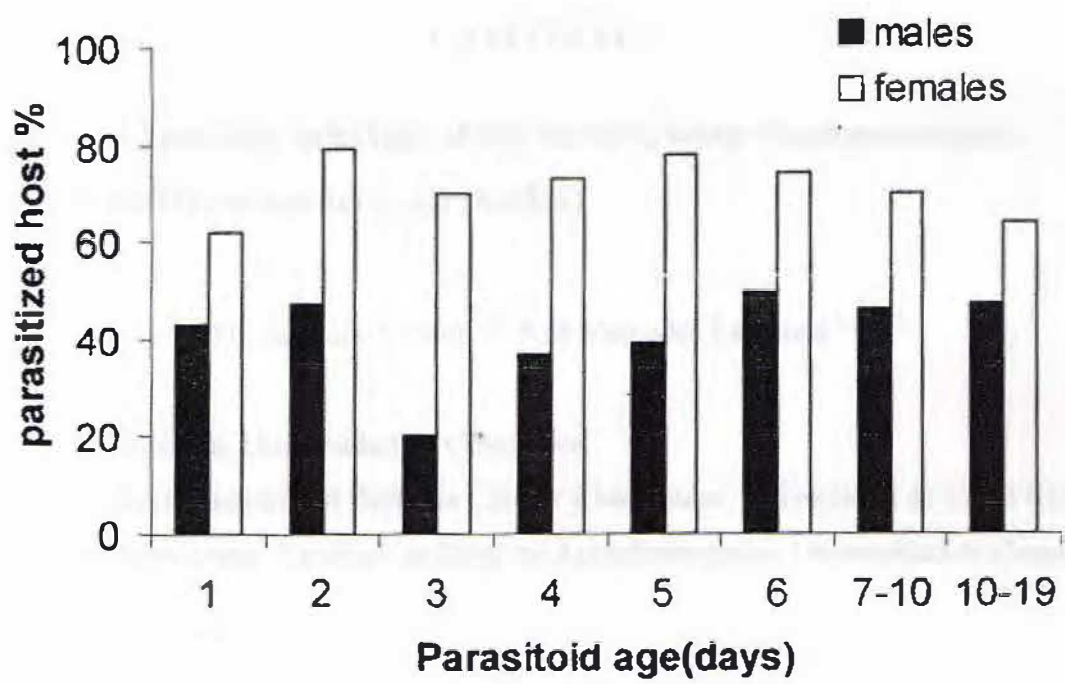
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ABSTRACT

We describe predation and oviposition behavior of *Haplogonatopus hernandezae* in hosts of different developmental stages of *Tagosodes orizicolus*. Oviposition attacks were less successful on older nymphs of *T. orizicolus*. Handling time during oviposition was higher with fifth instar nymphs than younger ones. Paralyzing, anchoring and drilling was also more time consuming in older hosts, but oviposition time was similar. When the wasp fed on a nymph instead of ovipositing, handling time did not differ regarding to host age. *H. hernandezae* stung the nymph repeatedly while feeding on it. Oviposition and feeding manipulation also differed in host grasping by the pincers and final manipulation. The clypeus was the host body structure the most frequently used for grasping in oviposition attacks. The particular pincer morphology of *H. hernandezae* facilitates the specific grasp of the clypeus and gena of *T. orizicolus*. Wasps carefully set nymphs back on the rice

leaves after ovipositing, but manipulated nymphs on which they fed on more carelessly and often allowed them to fall.

KEY WORDS

Oviposition, predation, handling time, *Haplogonatopus hernandezae*, *Tagosodes orizicolus*, pincer

INTRODUCTION

Host acceptance decisions by parasitoids are influenced by internal factors such as egg load and external factors as host abundance and host size (Jervis and Kidd, 1996; Kishimura, 1997; Barzman and Daane, 2001; King, 1998). It is common that parasitoids prefer to oviposit or feed on certain host stages and reject other (Baspinar et al. 1994; Jervis and Kidd, 1996; Kitamura 1988; Yamada and Watanabe 2002). Often, hosts are not passive victims of parasitoids and the proportion of oviposition attempts that are successful may be reduced by defensive behaviors such as vigorous squirming and jerking movements, kicking and jumping (Slansky, 1986; Gross, 1993; Stadler et al. 1994; Villagra, 2002). Handling time, which comprises the interval between the first contact with the host and the completion of the oviposition or host feeding event (Gross, 1993), may also be extended. Smaller hosts require less handling time and represent less injury risk. Behavioral defenses are frequently more effective in older host developmental stages (Gross 1993).

Many insect parasitoids use some potential hosts as food (Jervis and Kidd, 1986; Kidd and Jervis, 1989). Sometimes a significant proportion of the hosts are killed due to such feeding (Jervis and Kidd, 1986). Although the oviposition and host feeding behavior of parasitoids and the behavioral defenses of their hosts have been studied in different

groups of insects (Gross, 1993; King, 1998; Lauzière et al.; 2000; Rivero, 2000; Choi et al., 2001; Barzman and Daane, 2001), little is known about the differences in the handling time of hosts used for feeding or to oviposit and the possible effect of their developmental stage.

In this study, oviposition and host feeding behavior and their handling time is examined, for *Haplogonatopus hernandezae*, a solitary koinobiont parasitoid of the nymphs of the planthopper *Tagosodes orizicolus* Muir, found in rice fields (Olmi, 1984; Hernandez and Belloti, 1988). Female dryinids have chelate forelegs (Olmi, 1995), which they use to grasp and manipulate the host. Several studies have reported that the female dryinid uses her chelate pincers and mandibles to seize the host and sting the ventral side of the thorax in *Haplogonatopus hernandezae* (Hernandez and Belloti, 1988), *Echthrodelphax fairchildii* Perkins (Yamada and Imai, 2000), *Haplogonatopus atratus* Esaki and Hashimoto (Yamada and Kitashiro, 2002), and *Echthrodelphax fairchildii* (Yamada and Ikawa, 2005). However, little is known about the specific attack behavior and the functional morphology of the chelae of each of these species. The particular morphology of the pincers in each species serves as a key characteristic for the identification of adult females (Olmi, 1984). Currently, one poorly understood aspect of the pincer morphology is the significance of chelate foreleg variation, which is found even among closely related species.

Before laying an egg, the female probes several intersegmental membranes with her ovipositor and then inserts an egg between two abdominal segments on *H. atratus* (Yamada and Kawamura, 1999; Yamada and Miyamoto, 1998; Yamada and Watanabe, 2002). The parasitoid releases the host immediately after ovipositing.

In this paper, we describe oviposition and feeding behavior of *H. hernandezae*. We compare the period of host paralyzation and further manipulation in both the oviposition and host feeding attacks. We also explore the differences in manipulation time of the

parasitized hosts according to their nymphal instar. Finally, we document the use of the pincers of the foreleg in grasping and manipulating nymphs according to their developmental stage and the result of the attack, either feeding or ovipositing.

MATERIALS AND METHODS

Individuals of *H. hernandezae* and *T. orizicolus* were collected in Guanacaste, Costa Rica, during 2000 and 2001 and were reared in an insectary under controlled humidity (70-80%) and temperature (25-30°C). The twenty parasitoid females observed were young adults that had emerged 2 to 4 days previously in captivity and had been kept individually in small rearing cages (Chandra 1980b). Each female dryinid was provided daily with third instar nymphs of *T. orizicolus* and diluted honey placed on cotton for food.

To observe behavior, each female was placed in an 8.5 cm x 1.8 c m deep clear petri dish with rice leaves and five host nymphs of the same instar. Only 3rd- 5th-instars nymphs were used as instars 3 and 4 are the most frequently parasitized by *Haplogonatopus* species (Hernandez y Belloti, 1984; Yamada and Kawamura, 1999). The last (5th) instar was used to check whether the manipulation became more difficult because of the host size. Each parasitoid was introduced in a petri dish for one hour with nymphs of one instar, and then transferred to another container with nymphs of a different instar each day. We rotated the order in which the nymphs of different sizes were presented to the parasitoid.

Oviposition and predation behavior were recorded using a Color CCD Camera Model VCC-3912 coupled to the ocular of a dissecting microscope. The nymphal instars were determined using the morphological criteria (Mora et. al, 2001). The size relation between the host and the parasitoid in each attack was measured using the head width of

the dryinid (see Yamada and Miyamoto, 1998) and the total body length of the host nymph in the video images.

The attacks of each female wasp were recorded during a period of two to three days. Each nymph that had been manipulated by a dryinid female was transferred to an individual container, so each planthopper was encountered only once by a female parasitoid.

Each attack was classified: as successful (the female was able to paralyze the nymph and subsequently fed on it or oviposited) or unsuccessful (the parasitoid grasped but was not able to dominate and paralyze the host). To compare the paralyzation of the nymphs and their manipulation in successful oviposition and feeding attacks, we established behavior categories for both the host and the parasitoid. Total and mean durations (seconds ± SD) of the total handling time and each of the following behavior categories of the parasitoids were calculated: a) probing, when the parasitoid moved her sting several times along membranes between the abdominal segments of the nymph until she pressed against a point in the intersegmental membranes, b) drilling, as the period from pressing against this specific point to exposing her valvifers, c) oviposition, as the time between exposing the valvifers, retracting her sting into the third valvulae, pushing out her valvifers to the greatest extent possible and inserting the egg (see Yamada and Kawamura, 1999; Yamada and Imai, 2000) and d) recovery, as the period from which the female left nymph after ovipositing until the host without being manipulated was able to move again and walk.

We divided nymph behavior into three categories: resistance (the period in which the nymph offered resistance such as kicking, jumping and jerking after being caught by the parasitoid), paralyzed (the period in which the nymph showed no movement after being stung) and the recovery (the time between when the nymph was released after an

oviposition event until it recovered mobility. We divided the handling time of the parasitoid into two categories: paralyzation (period after seizing the host until stinging the host in the thorax to paralyze it) and manipulation (period of further handling until the achievement of ovipositing or host- feeding began). We recorded the duration and frequency of each of these behavior categories with the program JWatcher(Blumstein et al. 2000).

We divided the nymph manipulation with the chelae into two categories. Carefully set was established as when the wasp set the nymph gently on the rice leaf so the planhopper had its legs on the substrate. After placing the nymph, the wasp released its grasp simultaneously with both pincers, the nymph recovered and walked off. The second category was when the nymph was simply dropped. The dryinid released the nymph carelessly after feeding and the paralyzed nymph often fell lopsidedly or with its dorsal side towards the substrate.

Host feeding manipulation by the dryinid was divided into the period where the female grasped the host and directed her mandibles to press against a point (generally on the thorax) and, the feeding time, while the wasp fed from the nymph masticating with her mandibles into the thorax or abdomen tissue of the nymph. The host behavior was divided into resistance time and time paralyzed.

We took photographs with a scanning electronic microscope of specimens frozen during oviposition and feeding attacks using liquid nitrogen. These frozen specimens were placed in a -20°C freezer, then transferred still frozen to the -20°C absolute ethanol. After being fixed for more than one week, they were brought to room temperature, and then later dehydrated and sputter coated with gold.

Statistical analyses

We performed a Spearman correlation to determine if there was a relation between the host instar and its size. To analyze if the proportion of successful and unsuccessful attacks varied according to nymph developmental stage we used a Chi-square test. To compare the duration of each behavior category of the nymphs according to their developmental stages, we performed a Kruskal-Wallis test. The same test was used to explore differences among the nymphal stages in the handling time of the parasitoid and the time of occurrence of each category of oviposition and host feeding behavior of the female dryinid.

Differences between the final manipulation strategies of the hosts after the predation and oviposition events were analyzed by using a Chi-square test. To determine if the number stinging events varied according to the nymphal instars, we employed a G test.

Finally, the use of the pincers to grasp the host in oviposition and predating events was analyzed by using a Chi-square test. We also explored differences in the grasping with the pincers among nymphal instars with the same test.

RESULTS

A total of 199 attacks were recorded, 58.3% were successful oviposition events, 21.6% were successful predation events and the remaining attacks were unsuccessful (20.1%), either because the leafhoppers escaped from the grasp of the wasp (14%) or the leafhopper was seized but then rejected (6.1%). We found a positive relation between the host age and size (R= 0.06, T (n-2) = 10.28, N=165, P=0.00). The percentage of

unsuccessful attacks due to the nymphs escaping was higher for last instar nymphs. (χ^2 = 16.01, df= 6, P= 0.003, N = 189, Fig 1).

The handling time prior to oviposition were also higher as the nymphal instar increased (H= 20.60, P= 0.00, Fig 2a). In contrast, the handling time prior to feeding, did not change in relation to the host stage (H= 0.94, P= 0.62, Fig 2b).

The timing of each behavior category for also varied accordingly to whether the wasp fed or oviposited with respect to behavior of the nymphs. The resistance time was longer in older nymphs for attacks that led to oviposition. (H= 23.44, P= 0.00, Fig 3a). However, the resistance time did not differ for different host instars for attacks that ended in feeding (H= 0.32, P= 0.85, Fig 3b). The same tendency was found for the paralyzation time, older hosts took longer to be paralyzed in attacks which led to oviposition (H= 20.40, P= 0.00, Fig 3c). The time that hosts took to be paralyzed did not differ among the nymphal instars in attacks that led to feeding (H= 0.24, P= 0.88, Fig 3d). Finally, the recovery time was shorter for 5th instar nymphs following oviposition (H= 12.06, P= 0.02, Fig 3e).

The timing of different dryinid behavior categories showed similar tendencies. The time to paralyze a host was greater with older host instars in attacks that led to oviposition (H= 8.95, P= 0.01), but this trend was absent in attacks that led to feeding (H= 0.14, P= 0.93). Manipulation time was also higher for 5th instar hosts in those attacks that ended in oviposition (H= 20.97, P= 0.00), but not in those hosts that led to feeding (H= 20.98, P= 0.421).

The handling time of a female after paralyzing a host to later oviposit in it, was also higher in 5th instar hosts, both for the searching period and anchorage time (Table 1.). However, once anchoring had occurred, the time required for oviposition did not vary

among host instars (Table 1.). The handling time prior to feeding did not change among the host stages, nor did the duration of feeding itself (Table. 2).

The stinging events to paralyze varied according to the host stage in leafhoppers that were later parasitized. The number of 4th and 5th instar nymphs that were stung twice by the dryinid was significantly higher than for younger hosts (G = 30.47, df= 2, P= 0.00, N= 101, Fig 4a.). In feeding attacks, the most frequent behavior in all host stages was stinging continuously while the dryinid fed from the nymphs (G = 7.17, df= 2, P= 0.127, N= 41, Fig 4b).

Close up video images showed that the final manipulation of each host after being attacked differed regarding the decision to oviposit or feed. If the female decided to feed on a nymph, she paralyzed the host and fed on the dorsal side of the thorax or abdomen by masticating with her mandibles. The wound caused the death of the host, with a few exceptions in the case of fifth instar nymphs (Mora-Kepfer and Espinoza in prep, Fig 8d). The most frequent behavior of the parasitoid after feeding was to carelessly drop the paralyzed nymph (Fig 5.), which fell with its lateral or dorsal side on the substrate or rice leaf. In some cases, the parasitoid stepped on the nymph it had just fed on as it started to forage after the attack (N= 10), or released the grip on the nymph and started foraging while still dragging the nymph with the other pincer for a few seconds (N= 8).

In contrast, if the female finished ovipositing on a host, she carefully set the paralyzed host with its ventral side oriented towards the substrate (χ^2 = 69.13, df= 2, P= 0.00, N= 200, Fig 5). The wasp released the nymph simultaneously with both pincers and after it recovered from the attack, it simply walked or jumped when it became active again.

The video images also indicated that the quelate forelegs are used for grasping specific body parts of the host and this gripping varies according to an oviposition or

feeding attack. The female grasped the nymph either by its clypeus (holding the clypeus of the nymph with one pincer and legs or abdomen with the other pincer) or other body structures (holding the host with both pincers the side of the thorax or abdomen or legs, rather than the clypeus). The clypeus was grasped the most frequently in all nymphal stages after the host had been paralyzed and until the dryinid either dropped it or gently set in the substrate ($\chi^2 = 4.18$, df= 1, P= 0.123, N= 155, Fig. 6, Fig. 9.c). We also found that it is the relevant host structure used for grasping with the quelae in attacks that led to oviposition. It was gripped significantly more frequently than other host body structures after the host was paralyzed ($\chi^2 = 49.58$, df= 1, P= 0.00, N= 145, Fig, 7 Fig. 9.a, c, d). However, in attacks that led to feeding, the host gripping was not performed in specific body structures. The female used her pincers to grasp the clypeus or other body structures with similar frequencies (Fig. 7. Fig 9a, 9c).

DISCUSSION

Although a dryinid is capable of handling and attacking nymphs of all instars, the manipulation time increases with host age and size. The number of successful attacks that end in parasitism or predation events are also strongly influenced by the developmental stage and size of the leafhopper. Parasitoids in other families tend to have fewer successful ovipositions per encounter and a longer handling time with larger, better defended aphid instars (Gross, 1993).

Older *T. orizicolus* nymphs defend themselves more vigorously by shaking and kicking when held by a wasp causing the resistance time to increase, also observed in other homopterans (Bokonon-Ganta et al. 1995; Rivero, 2000). Our results indicate that the time

invested in handling an older, larger host increases in oviposition attacks. Stephens and Krebs (1986) stated that oviposition cost (including searching and handling the host) is quantified by the time and/ or energy expenditure to oviposit an egg. If different costs exist in laying eggs regarding the host stage, this suggests a trade off between difficulty of oviposition and offspring rearing conditions (Nishimura, 1997).

The same tendency was found in each of the behavior steps analyzed for both the host and the dryinid wasp. Handling time did not change regarding the stage in predation attacks, but time spent in each step previous to oviposition increased with host age. As our data indicates, resistance time was significantly higher in older nymphs. With smaller instars a dryinid may be able to approach the host closely to successfully oviposit while being less exposed to the range of the defensive leg kicking. However, in larger hosts a closer approach may be needed forcing the parasitoid to move within the "leg distance" of the host (Gerling et. Al 1990), making it more difficult to sting the host to paralyze it. The video images show this tendency, as older nymphs resist the parasitoid attack for a longer time by actively kicking the ventral side of the wasp body as it stings the thorax to paralyze the nymph. Body shaking and jumping is also more efficient in older nymphs as a strategy to escape than in younger nymphs.

Fifth instar nymphs were paralyzed for longer periods. During this time, searching for a site on the host body to parasitize also took longer in this instar. Previous studies demonstrate that females of *H. atratus* examine the membranes between the host abdominal terga with her ovipositor before ovipositing to detect if the host has been previously parasitized (Yamada and Kawamura, 1999; Yamada and Kitashito, 2002; Yamada and Sugaura, 2003; Yamada and Ikawa 2005). If a dryinid egg from a previous oviposition event from another parasitoid was located in this examination, the wasp stung and killed it.

This suggests that longer handling and inspecting time with a larger, older host serves to make sure it has not been previously parasitized.

Anchoring time (during which drilling took place) was longer for the oldest nymphs, suggesting a greater difficulty in penetrating the host cuticle. The ovipositor sheaths possibly serve as an anchor during the initial phases of the process (Vilhemsen 2003), and a strong correlation exists between the cuticle thickness of the host and oviposition success in some parasitoids (van Lenteren et al. 1998). However, the oviposition event itself did not vary according to the nymphal stage, probably laying an egg takes place only after an appropriate spot has been found and drilling is complete. The only time difference in oviposition reported for dryinids has been regarding to the sex of the egg (Yamada and Imai, 2003; Yamada and Kawamura, 1999). Female eggs tend to take longer to be laid, but in this study only female progeny (N=20) were observed.

Older hosts were frequently stung twice, prior to searching for a spot to oviposit. The resistance time for these hosts was longer and for 87% of the 5th nymphal instars more than one period of paralyzation was necessary to perform a successful oviposition. These nymphs also recovered faster than younger ones. Last instar nymphs become more costly in energy and time consuming to handle. They may also become adults too soon to assure that the larval parasitoid will have enough time to develop and leave the host to pupate. A female adult of *T. orizicolus* lives on average 31,1 days and males live less, an average of 14.6 days (Gómez-Sousa and Kamara 1980) and a larvae of *H. hernandezae* can take up to 27.7 days to emerge from the host and pupate (Mora-Kepfer and Espinoza in prep).

Feeding and oviposition attacks also differed in host grasping by the pincers and final manipulation. The clypeus was the host body structure the most frequently used for grasping in oviposition attacks (Fig 7c). The particular pincer morphology, the enlarged

claw with subapical tooth and 6 lamellae and segment 5 of the front tarsus with two rows of nearly eight lamellae (Olmi, 1984; Fig 7a), facilitates the specific grasp of the host clypeus and gena. This suggests the unexpected conclusion that function of the particular pincer morphology is to grasp this specific structure of *T. orizicolus*.

Our results indicate that the host handling is very different in oviposition and feeding attacks. When a wasp decided to feed on a nymph, the stinging used both to grasp and paralyze was continuous, causing damage to the host body. This suggests that the handling behavior in predation damages the host in contrast to the handling behavior in attacks that led to oviposition. The clypeus and other body parts were used frequently for grasping since the feeding did not need to be in a specific site, a wasp fed from the thorax or the abdomen. The host was subsequently killed by destructive feeding (Fig 7d.) and dropped carelessly to the substrate. As predation events are single encounters, the host age or the manipulation that a nymph receives has no further consequences on the future reproduction of the parasitoid.

However, when a female decides to lay an egg, she must manipulate this host carefully to assure that her progeny will be able to survive and develop successfully. Our observations showed that in oviposition attacks the wasp grasped the host precisely by the clypeus and either the tip of the abdomen in young nymphs or a leg in older instars. After the dryinid oviposited, she set the nymph carefully with its ventral side towards the rice leaf by releasing the grip of the quelate forelegs simultaneously. Rice fields are frequently flooded so this behavior might save the parasitized host from falling into the water while still paralyzed to assure the successful recovery of this nymph and the survival of the parasitoid egg that will develop inside the host.

ACKNOWLEDGEMENTS

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LEYENDS TO FIGURES

Table 1. . Occurrence time of each parasitism behavior category of *H. hernandezae* for each nymphal instar of its host *Tagosodes orizicolus*

Table 2. Occurrence time of each predation behavior category of *H. hernandezae* for each nymphal instar of its host *Tagosodes orizicolus*

Fig 1. Effect of host instar of *T. orizicolus* on attack success of *Haplogonatopus hernandezae*

Fig. 2. Effect of parasitoid handling time in relation to the host instar for oviposition and feeding in nymphs of *Tagosodes orizicolus*

Fig 3. Time of occurrence of each behavior category of the dryinid when attacking a host.
   a), c) and e): parasitized nymphs, b) and d): Predated Nymphs of *T. orizicolus*

Fig. 4. Number of times that hosts of different developmental stages of *T. orizicolus* were stung to be paralyzed by a female *H. hernandezae*

Fig. 5. Final manipulation of each host of *T. orizicolus* after being parasitized or predated.

Fig. 6. Pincer grasping of different nymphal instars of the host *Tagosodes orizicolus* by the dryinid *Haplogonatopus hernandezae*

Fig. 7. Host grasping with pincers performed by the dryinid *Haplogonatopus hernandezae* in hosts that were used for feeding or ovipostion

Fig. 8. (A), Open pincer of *H. hernandezae* (B) and closed pincer (C) Grasping of a host clypeus (cp) and gena (g) with a pincer (pi) (D) Dryinid feeding on the abdominal segments of a 5th instar nymph
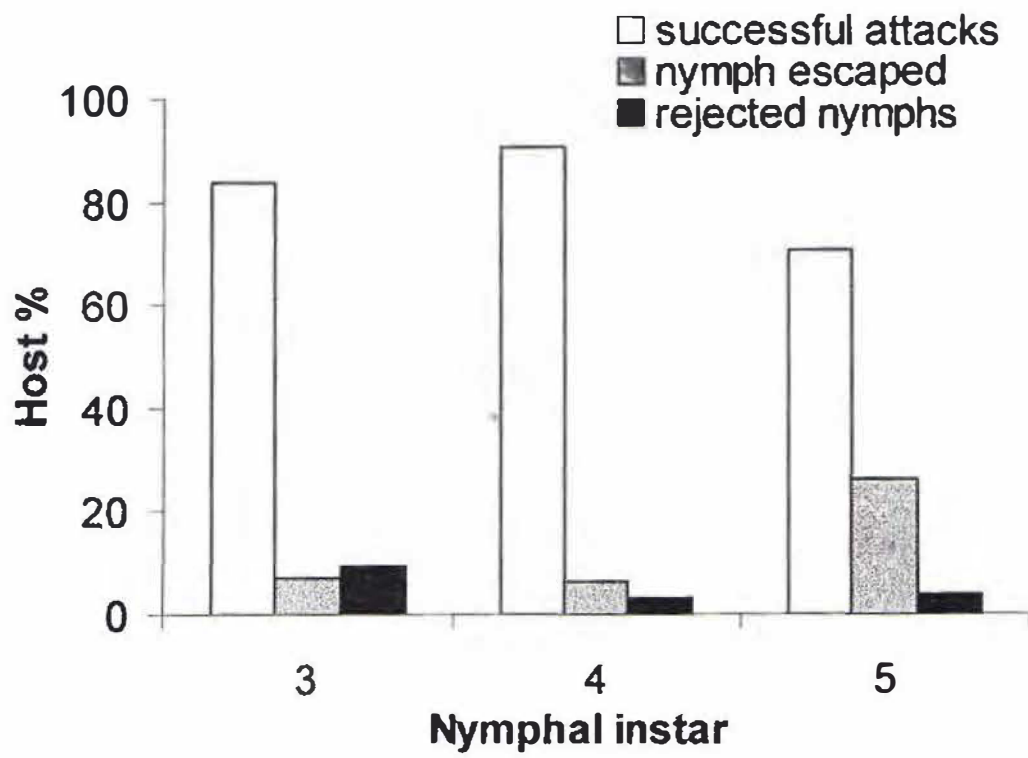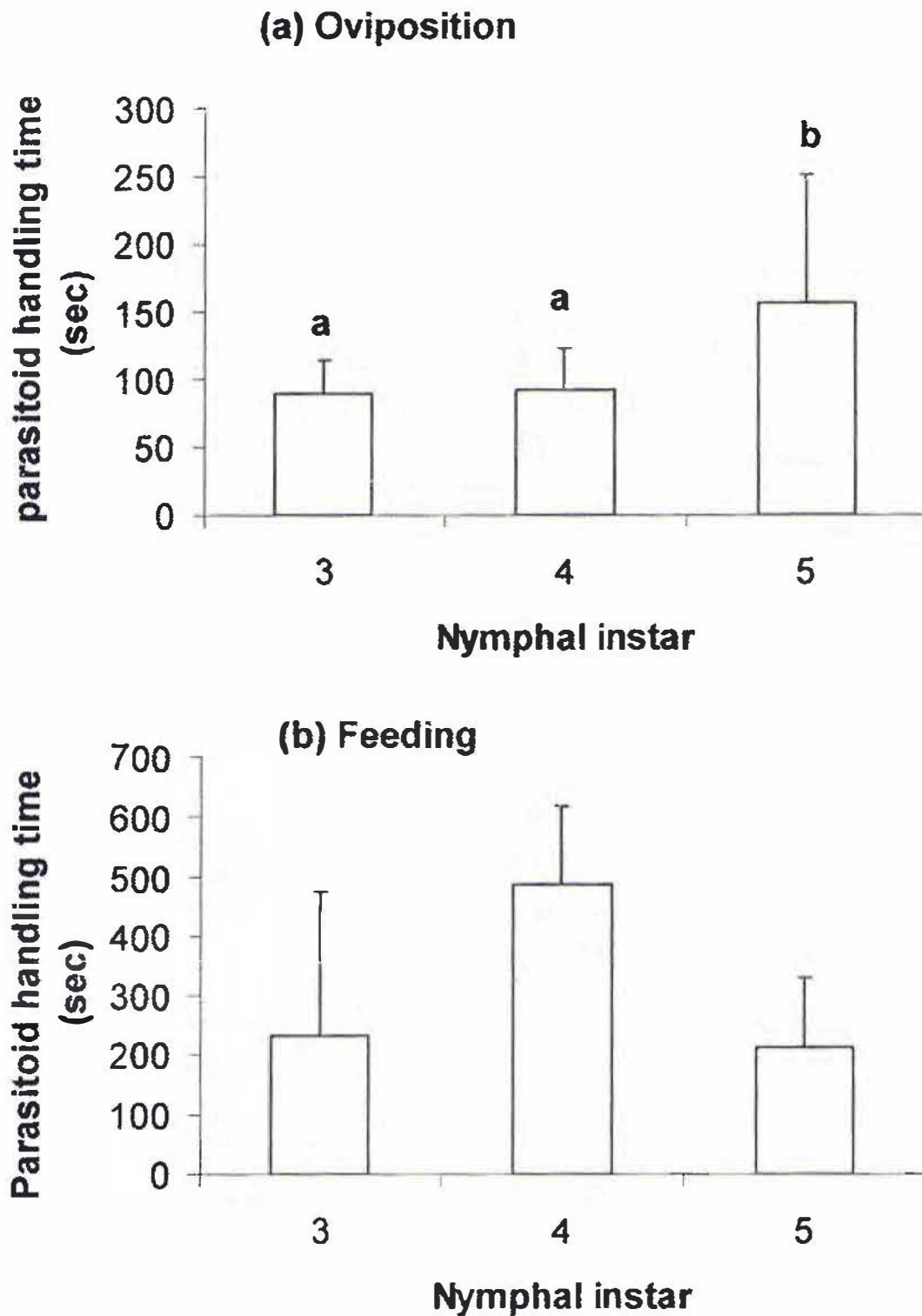
Fig.1.

(a) Oviposition

(b) Feeding

Fig. 2.
Kruskal-Wallis Test. Significant differences among nymphal instars are indicated with different letters
(P<0.05)
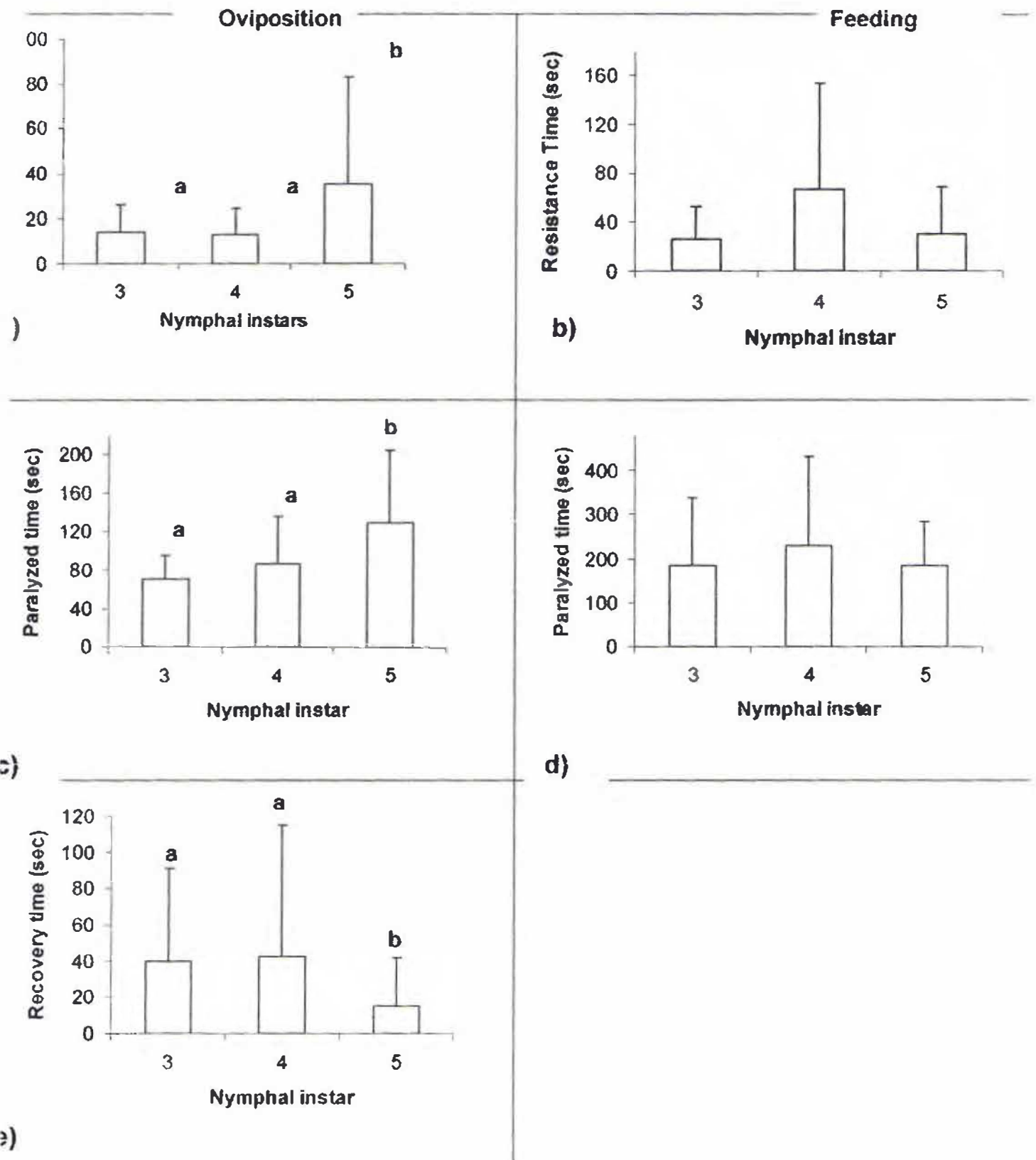
Fig 3.
Kruskal-Wallis Test. Significant differences among nymphal instars are indicated with different letters (P<0.05)

Table. 1

Behavior category (Sec)	Host instar							
	Third		Fourth		Fifth			
	Mean	SD	Mean	SD	Mean	SD	df	P
Probing	22.88 a	17.254	42.52 a	56.07	54.92 b	51.44	2	0.04
	(N =22)		(N =37)		(N =38)			
Drilling	41.97 a	14.10	37.277 a	14.18	64.93 b	35.55	2	0.04
	(N =24)		(N =34)		(N =11)			
Oviposition	73.93	61.07	17.31	42.33	10.02	14.63	2	0.33
	(N =25)		(N =38)		(N =40)			

Table 2.

Behavior category (Sec)	Host instar							
	Third		Fourth		Fifth			
	Mean	SD	Mean	SD	Mean	SD	df	P
Handling prior to feeding	166.45	123.44	216.81	188.58	154.33	91.54	2	0.58
	(N =7)		(N =17)		(N =18)			
Feeding	158.45	136.98	226.18	206.26	153.79	95.09	2	0.51
	(N =5)		(N =13)		(N =15)			

Kruskal-Wallis Test. Significant differences among nymphal instars are indicated with different letters (P<0.05)

Oviposition a) Fig. 4. Feeding b)
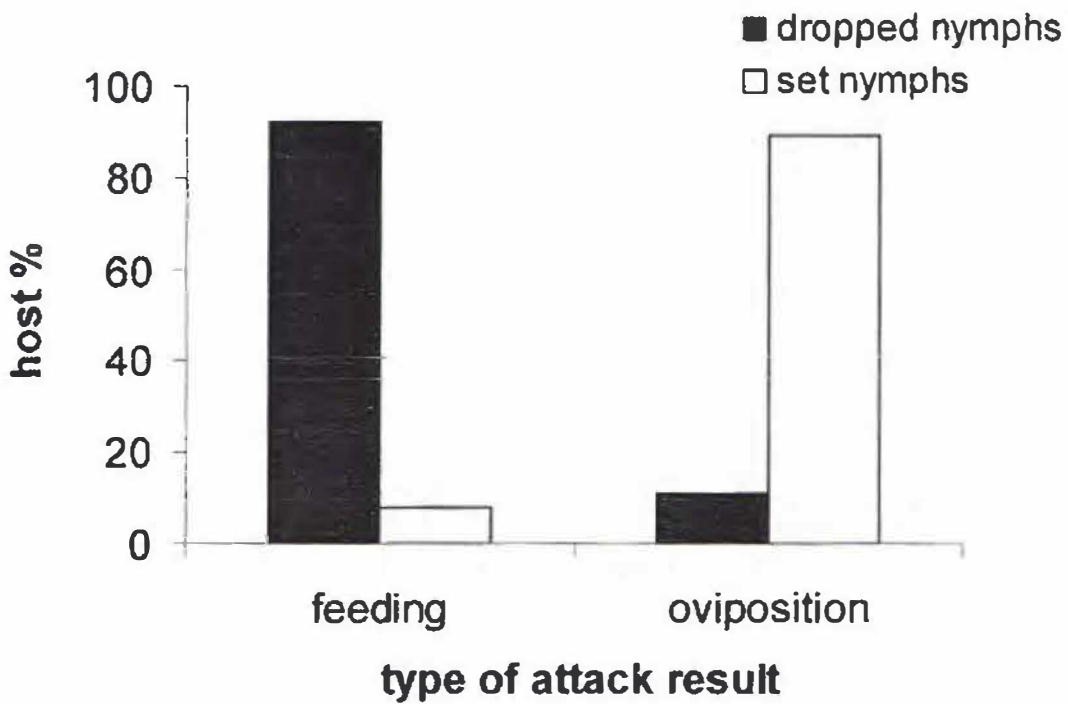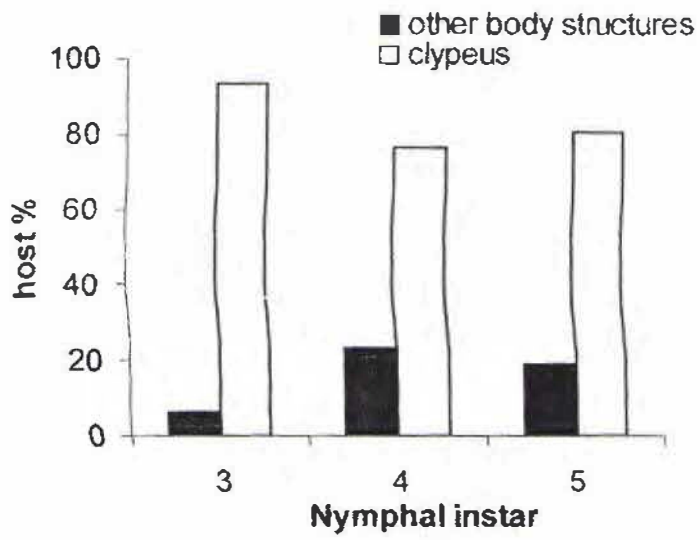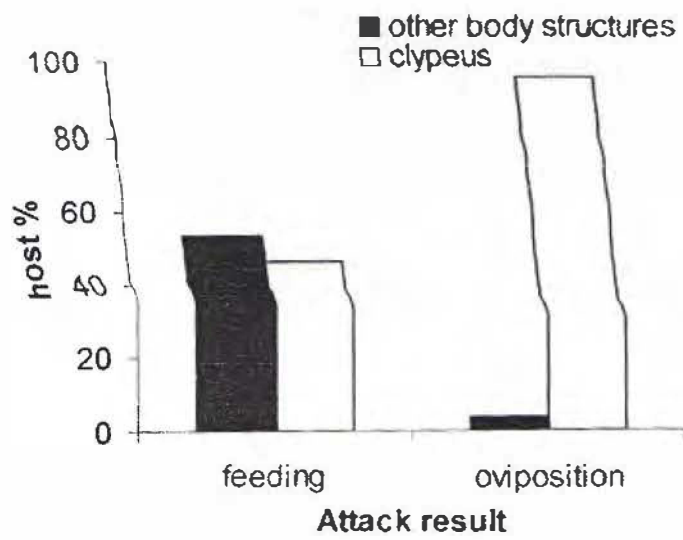

Fig. 5.

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Fig.6.

Fig. 7

Fig. 8.