UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSGRADO

ASPECTOS ECOLÓGICOS, ENEMIGOS NATURALES Y MANEJO DE *Bagrada hilaris* (HEMIPTERA: PENTATOMIDAE)

Tesis

Que presenta MOISÉS FELIPE VICTORIANO

Como requisito parcial para obtener el grado de:
DOCTOR EN CIENCIAS EN PARASITOLOGIA AGRICOLA.

Saltillo, Coahuila Julio 2019

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Dr. Sergio René Sánchez Peña
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Saltillo, Coahuila

Julio 2019

ASPECTOS ECOLÓGICOS, ENEMIGOS NATURALES Y MANEJO DE *Bagrada hilaris* (HEMIPTERA: PENTATOMIDAE)

Tesis

Elaborada por MOISÉS FELIPE VICTORIANO como requisito parcial para obtener el grado de Doctor en Ciencias en Parasitología Agrícola con la supervisión y probación del comité de asesoría.

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Julio 2019

DEDICATORIA

A DIOS:

Gracias por la familia, mis hijas Sofía y Karla, mi esposa Martha y por todos los amigos que obtuve durante el programa doctoral.

A MI FAMILIA

A mis hijas Sofia y Karla Itzayana
A Martha mi esposa

Quienes son parte fundamental en mi vida.

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INTRODUCCION

Bagrada hilaris (Hemiptera: Pentatomidae), comúnmente se le llama chinche pintada (del inglés painted bug), o chinche Bagrada (Bundy *et al.*,2012). Este insecto es nativo de África a partir de donde se distribuyó a Asia, Europa y posteriormente en 2008 al continente americano (Howard, 1906).

El primer reporte de este insecto en América del norte se dio en California, (USA). actualmente se encuentra ampliamente distribuido en Estados Unidos donde ya se le considera como una plaga principal en crucíferas (Palumbo y Natwick 2010; Bundy *et al.,* 2012; Huang *et al.,* 2014; Palumbo, *et al.,* 2016). En México se observó por primera vez en el año 2014, en el suroeste del estado de Coahuila (Sánchez- Peña, 2014), actualmente ya se encuentra ampliamente extendido en los estados de Aguascalientes, Coahuila, Guanajuato, Nuevo León y Zacatecas, cabe mencionar que los estados de Aguascalientes, Zacatecas y Guanajuato son de los principales productores de crucíferas tales como brócoli, coliflor, repollo, rábano entre otros.

El daño lo ocasionan ninfas y adultos, al alimentarse de los jugos celulares de sus hospederos, después de la alimentación las hojas desarrollan una clorosis (en forma de estrella), en daños severos se desarrolla una necrosis con retraso del crecimiento de los cultivos (Palumbo *et al.,* 2016). Diversos autores concuerdan en que el mayor daño lo ocasionan cuando el insecto migra de crucíferas silvestres hacia cultivos recién trasplantados (Palumbo y Natwick 2010).

Para el control de la chinche Bagrada normalmente se utilizan productos sintéticos químicos tales como Piretroides, Organoclorados y Organofosforados. Los principales productos empleados son el Endolsulfan, Malatión, Metil dimetoato, Monocrotofos, y

Fenitrotión que han probado ser efectivos en condiciones de laboratorio y en campo (Pasquale *et al.*, 2007; Joseph *et al.*, 2016; Bawaskar *et al.*, 2017).

Al ser una plaga exótica algunos autores como Palumbo *et al.* (2016), mencionan que en el continente Americano no existen enemigos naturales específicos que puedan hacer un control eficiente de este insecto. Sin embargo, posterior al primer reporte en México, Acosta *et al., (*2016), menciona que existen hongos autóctonos que pueden inducir epizootias naturales cuando las poblaciones de la chinche Bagrada es alta y las condiciones ambientales son favorables pueden desarrollarse infecciones fúngicas de microorganismos tales como *Beauveria bassiana*, *Metarrizium*, *Zoophthora* entre otros.

Respecto a parasitoides de huevo de la chinche Bagrada en países como la India, Paquistán, Japón y África (Scelionidae (*Hadrophanurus*)), ya se han reportado. En Pakistán Mahmood *et al.* (2015), menciona que en huevos centinela colocados en campo 4 días y posteriormente incubados en laboratorio han observado la emergencia de 3 parasitoides pertenecientes a las familias Platygastridae (*Trissolcus* sp y *Gryon* sp.) y *Encyrtidae* (Ooencyrtus sp.), a partir de estos resultados en Estados Unidos de América se estado realizando investigación con parasitoides de *Bagrada hilaris*, donde en condiciones de laboratorio se ha observado un parasitismo exitoso independientemente de la edad de los huevos empleados (Sforza *et al.*, 2017).

REVISIÓN DE LITERATURA

Generalidades de la chinche Bagrada

Bagrada hilaris (Burmeister, 1835) (Hemiptera: Pentatomidae), comúnmente se le denomina chinche Bagrada o chinche pintada (Bundy *et al.*, 2012). Este insecto es nativo de África oriental y meridional, así como de diferentes partes de Asia y Europa (Howard, 1906), en América se observó por primera vez en el estado de California, Estados unidos en el año 2008 a partir de donde comenzó a dispersarse a los estados de Nuevo México, Arizona y Texas (Bundy *et al.*, 2012; Palumbo *et al.*, 2016). En México se observó por primera vez en el 2014, en el municipio de Saltillo, suroeste del estado de Coahuila (Sánchez-Peña, 2014), y en 2018 se reportó en el municipio de Irapuato en el estado de Guanajuato, alimentándose de maíz blanco, (Hernández *et al.*, 2018). Posteriormente este insecto en el 2017, fue reportado causando daños en *Brassica rapa*, en Sudamérica en las provincias de Santiago, Qiolicura y Estero de las Cruces en Chile (Faúndez *et al.*, 2017).

En México este insecto ya se ha establecido en la zona del bajío, donde ya se considera una amenaza seria para el cultivo de brócoli y otras crucíferas, (Hernández *et al.*, 2018), por su rápida dispersión apunta a ser una plaga importante en crucíferas en los próximos años.

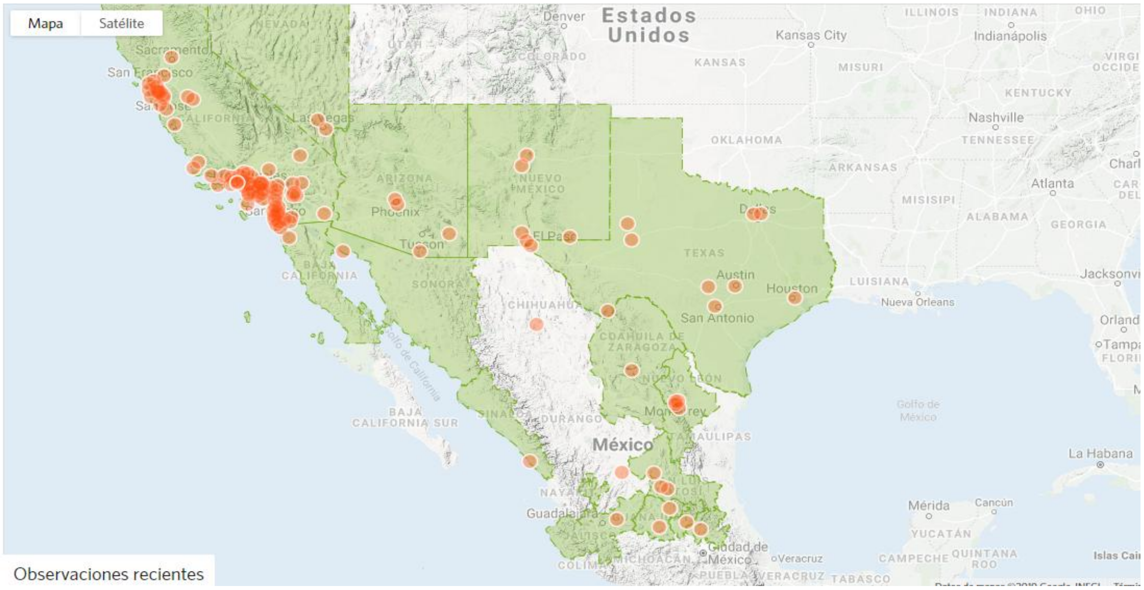

Figura 1. Distribución actual de *Bagrada hilaris*, en América del norte. Tomado de https://www.naturalista.mx/taxa/152131-Bagrada-hilaris

Ubicación taxonómica de la chinche Bagrada

Reino: Animalia
Filo: Arthropoda
Subfilo: Hexapoda
Clase: Insecta
Subclase: Pterygota (Insectos Alados)
Orden: Hemiptera (Chinches)
Suborden: (Heteroptera)
Infraorden: Pentatomomorpha
Superfamilia: Pentatomoidea
Familia: Pentatomidae
Subfamilia: Pentatominae
Tribu: Strachiini
Género: *Bagrada*- Especie: *hilaris*

Clasificación taxonómica Tomado de: https://www.naturalista.mx/taxa/152131-Bagrada-hilaris

Sinonimos

Bagrada cruciferarum Kirkaldy 1909
Bagrada picta Fabricius 1775

(Azim and Shafee 1986, Horvath 1936, Rebagliati et al. 2005)

Hospederos de *Bagrada hilaris*

Bagrada hilaris presenta una amplia gama de hospederos, afecta a 74 especies de 23 familias de las cuales 56 son cultivos, 13 malas hiervas y 5 ornamentales, (Palumbo *et al.*, 2016). La chinche Bagrada se le considera una de las principales plagas de crucíferas tales como repollos, col rizada, coliflor, col de Bruselas, arúgula, brócoli, rábanos entre otros (Palumbo y Natwick 2010; Huang *et al.*, 2014).

Daño causado por *Bagrada hilaris*

Bagrada hilaris se alimentan mediante un método denominado lacerado enjuague, este se lleva a cabo mediante la inserción repetitiva del estilete en las células epidérmicas de la hoja, lo que causa un daño mecánico del tejido celular (Reed *et al.,* 2013). Posteriormente inyectan enzimas salivales la cual produce muerte celular en hojas y tallos (Ahuja *et al.*, 2008), el daño en las hojas se observa como una clorosis en forma de estrella, la cual en daños severos se desarrolla una necrosis con una posterior caída de las hojas.

Figura 2. Daño de Bagrada hilaris por alimentación en discos de hojas de brócoli

El daño principal ocasionado por *Bagrada hilaris* se da posterior a la germinación del cultivo, generalmente cuando se comienzan a desarrollar las primeras hojas verdaderas, en esta etapa las plantas se encuentran en la fase de mayor susceptibilidad (Joseph *et al.*, 2017). En investigaciones realizadas en laboratorio se ha podido determinar que las plantas recién emergidas son más susceptibles a morir comparados con plántulas que presentan más de 4 hojas verdaderas, lo anterior indica que *B. hilaris* tiene preferencia a tejido joven en los cultivos, (Huang *et al.*, 2014).

Por el daño debido a la alimentación de estos pentatómidos en los cultivos, se ha observado una reducción en variables fisiológicas tales como: la tasa fotosintética, clorofilas totales, conductancia estomática, compuestos orgánicos volátiles, así como una reducción de variables agronómicas: área foliar, pesos secos, pesos frescos y bajos rendimientos, (Huang *et al.*, 2014; Guarino *et al.* ,2017). Un daño importante a resaltar en crucíferas es que cuando la chinche Bagrada se alimenta de brotes jóvenes de brócoli, induce el desarrollo de múltiples brotes adventicios florales lo que comúnmente se le denomina plantas siegas o en ocasiones no desarrollan la cabeza (Nyabuga, 2008; Palumbo y Natwick 2010).

Ciclo de vida de *Bagrada hilaris*

Se ha observado en pruebas de laboratorio que el ciclo de vida de la chinche Bagrada en promedio es completado en un periodo de 38 a 65 días bajo condiciones controladas de humedad, temperatura y con una fuente de alimento ininterrumpida, con presencia de alimento Halbert y Eger (2010) mencionan que se pueden obtener
múltiples generaciones por año (hasta 6 generaciones por año).

Huevos

Los huevos tienen forma de barril de color blanquecino de 0.87 a 1 mm de longitud, los cuales se tornan de un color rojizo a los 2 días después de la ovoposición, esta coloración está relacionado con el desarrollo del embrión (Reed *et al.*, 2013). La eclosión de ninfas se da después de 3 a 6.6 días después de la ovoposición de acuerdo a Halbert y Eger (2010). Sin embargo, de nuestras pruebas en campo la emergencia se observó después de 10 días en invierno y 5 a 7 días en verano (datos no publicados). En otros trabajos se menciona que existen 4 periodos: 1) pre-copulación que dura de 3.5 a 4.5 días, 2) periodo de copulación de 0.5 días, 3) periodo de pre-ovipocición 5 días y 4) periodo de ovoposición 5 días, (Ghosal *et al.*, 2006).

La chinche *Bagrada* depositan sus huevecillos individualmente o en pequeños grupos de 3 a 15 huevos entre las grietas del suelo (Verma *et al.*,1993), en ocasiones se pueden observar masas de huevecillos o solitarios sobre tallos, hojas y flores (Hutson, 1935). Taylor *et al.*, (2014) en su artículo comportamiento de oviposición inusual de la chinche apestosa *Bagrada hilaris* menciona 6 fases: 1) fase de búsqueda, que es pre-oviposición y las fases 2- 6, que son fases de oviposición (posicionamiento, estacionario, deposición de huevos, extracción de abdomen y cubierta de huevos)

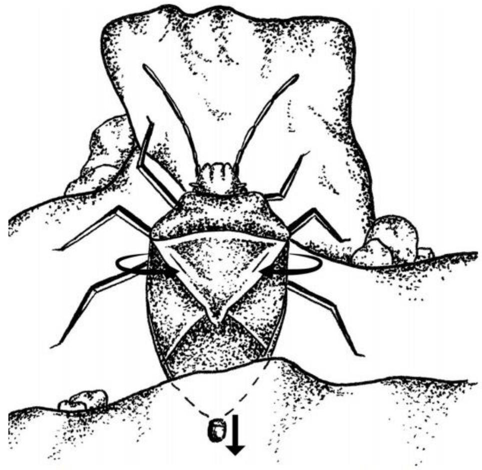

Fig. 5. Ovipositional behavior of *B. hilaris*, egg deposition phase, part 2 (dorsal view). Egg is deposited.

Figura 3. comportamiento de oviposición inusual de la chinche apestosa *Bagrada hilaris* (Hemiptera: Heteroptera: Pentatomidae)

Ninfas

La chinche Bagrada tiene cinco estadios ninfales, las recién emergidas son de color naranja a rojo, y en estadios posteriores la cabeza y tórax se oscurecen (Reed et al., 2013). En colonias en laboratorio se ha podido observar que el tamaño de las ninfas oscila entre 1 mm a 5 mm del primer al quinto estadio ninfal (Singh and Malik 1993; Verma *et al.*, 1993). Los sexos en las primeras etapas ninfales no se distinguen fácilmente hasta la edad adulta, donde se puede apreciar a la hembra de mayor tamaño. De acuerdo a Ghosal *et al.*, (2006), los estadios ninfales del 1-5 duran un periodo de 2.5, 4, 3, 4 y 4 días respectivamente, en total el ciclo en estado ninfal dura 17.5 días.

Adultos

El tamaño del insecto adulto mide de 5 a 7 milímetros de longitud (en promedio hembras 7 mm y machos 5.9 mm de longitud), tienen forma de escudo con marcas de color negro blanco y naranja, las hembras siempre son más grandes que los machos. Los adultos de *Bagrada hilaris* son de color negro con puntos naranjas, la mayor parte del tiempo se pueden observar en cópula la cual puede durar varias horas, (Huang *et al.*, 2013).

se ha observado que las hembras causan más daño en la alimentación comparado con los machos. Su longevidad en los adultos varia de 8 a 16 días dependiendo de la preferencia a la especie de la que se esté alimentado (Rakshpal, 1949; Verma *et al.*, 1993). Este insecto es muy activo, se pueden observar caminando en el suelo o en las plantas, aunque tienen alas es difícil verlo volar debido a que prefieren caminar. En etapas iniciales de infestación en los cultivos es difícil poder observarlos en el campo debido a su tamaño y principalmente por que se ocultan en el envés de las hojas cuando detectan el movimiento entre los surcos de los cultivos, la longevidad varía entre machos y hembras, en hembras es de 14.33 y en machos es de 15.83 días (Ghosal *et al.*, 2006).

Monitoreo

Actualmente no hay un método de muestreo o herramienta de monitoreo eficiente y aplicable en campo para *Bagrada hilaris*. Por lo que se recomienda que las plantas sean inspeccionadas para detectar la presencia del insecto desde la mitad de la mañana hasta la tarde, tiempo en el que el insecto es más activo, así mismo se recomienda iniciar la inspección inmediatamente después del trasplante del cultivo (Huang *et al.,* 2013; Reed *et al.,* 2013).

Control químico

Aun no se ha establecido un umbral de acción, por lo que con las primeras observaciones de insectos en las plantas se sugiere comenzar la aplicación de insecticida de contacto y sistémicos, en algunos trabajos se ha observado que un adulto por cada tres hileras de plantas en semilleros puede ocasionar daños irreparables, ocasionando una perdida total (Reed *et al.,* 2013).

Para el control químico de la chinche Bagrada, se han empleado insecticidas de amplio espectro tales como Piretroides, Organoclorados, Ciclodienos y Neonicotinoides (Palumbo *et al.,* 2016; Joseph, 2017). Los principales productos que se han utilizados para el manejo son: Clorpirifos, Profenofos, Endolsulfan, Malatión, Metil dimetoato, Monocrotofos, Fenitrotión, Bifentrina, λ-cihalotrina, c-cipermetrina (piretroides), dinotefuran, clotianidina, imidacloprid (neonicotinoides) ya que han probado ser efectivos en condiciones de laboratorio y campo contra la chinche Bagrada (Pasquale *et al.,* 2007; Palumbo *et al.,* 2013; Palumbo *et al.,* 2014; Palumbo *et al.,* 2015; Joseph *et al.,* 2016; Bawaskar *et al.,* 2017).

En california Estados Unidos en zonas productoras de crucíferas los productores usan productos de contacto altamente eficientes, se menciona que cerca del 90 % de la superficie ha sido tratada con insecticidas en un promedio de cuatro veces por ciclo de cultivo para control de la chinche Bagrada lo que incrementa los costos de producción, en otros trabajos se menciona que el 98 % de los agricultores dependen del uso de insecticidas sintéticos para control de la chinche Bagrada y otros insectos tales como *Plutella xylostella*, y *Brevicoryne barassicae* **(**Reed *et al.,* 2013)**.**

Control biológico

Parasitoides de huevos

La chinche Bagrada al ser una plaga exótica en América algunos autores como Palumbo *et al.,* (2016), mencionan que no existen enemigos naturales específicos que puedan hacer un control eficiente de este insecto. Actualmente diversos autores ya han reportado parasitoides de huevo de la chinche Bagrada en diversos países como la India, Paquistán, Japón y África (Scelionidae (*Hadrophanurus*)), dichos reportes de parasitoide son principalmente de huevo y corresponden a la familia Scelionidae (Chacko & Katiyar, 1961; Mahmood *et al.,* 2015)

En Pakistán Mahmood *et al.,* (2015), menciona que en huevos centinela colocados en campo 4 días y posteriormente incubados en laboratorio han observado la emergencia de 3 parasitoides pertenecientes a las familias Platygastridae (*Trissolcus* sp y *Gryon* sp.) y *Encyrtidae* (Ooencyrtus sp.). Para el caso de *Telenomus* se ha observado una tasa de parasitismo de 15 a 25 % en condiciones de campo (Samuel, 1942). A partir de los resultados anteriores en Estados Unidos de América se estado realizando investigación con parasitoides de *Bagrada hilaris,* donde en condiciones de laboratorio se ha observado un parasitismo exitoso independientemente de la edad de los huevos empleados (Sforza *et al.,* 2017).

Depredadores naturales

En campo también se han observado depredadores naturales (Taquinidos, arañas, mantis, hormigas, hemípteros entro otros), pero actualmente no se conoce la eficiencia de depredación de estos insectos (Taylor *et al.,* 2014; Mahmood *et al.,* 2015; Palumbo *et al.,* 2016). Scott *et al.,* (2012) mencionan que, en Nuevo México, USA, *Collops* spp (Coleoptera: Melyridae) y *Geocoris* spp, (Hemiptera: Geocoridae), se han observado alimentándose exclusivamente de ninfas de segundo instar. En México se observó a *Zelus longipes* (Hemiptera: Reduviidae) alimentándose de adultos de chinche Bagrada, en maíz (Hernández *et al.,* 2018).

Hongos entomopatógenos

Acosta *et al., (*2016), de pruebas en laboratorio de *Zoophthora radicans* contra *Bagrada hilaris,* observaron que con un tiempo de exposición de 24 horas (1665 conidias/mm2), se puede alcanzar una mortalidad del 90 % en *Bagrada hilaris,* Acosta menciona que la mortalidad es proporcional al tiempo de exposición de la chinche Bagrada al entomopatógeno a mayor tiempo de exposición mayor mortalidad. En trabajos posteriores Halder *et al.*, (2017), mencionan que *lecanicillium lecanii,* puede inducir una mortalidad de 69.05%, lo que lo hace altamente efectivo contra ninfas de chinches Bagrada, otros entomopatógenos como *Beauveria bassiana* y *Metarizium anisopliae* pueden ocasionar una mortalidad de 58.49 y 59.11% respectivamente. En trabajos no publicados Acosta *et al., (*2016), menciona que en campo pueden observarse epizootias naturales en la chinche Bagrada causados por hongos entomopatógenos tales *como Zoophthora radicans, Beauveria bassiana, Metarizium anisopliae, lecanicillium lecanii* y un tipo de fusarium entomopatógeno.

Semioquímicos para manejo de la chinche Bagrada.

El empleo de aceites naturales es poco estudiado en la agricultura respecto a productos químicos tradicionales, sin embargo, estos tienen un amplio potencial en campos como la biomedicina, (Royden *et al.,* 2018; Kumar *et al.*, 2018) y en la agricultura ya que inhiben el desarrollo de insectos, bacterias, hongos y virales.

El efecto insecticida o fungicida de los aceites esenciales se debe principalmente a la gran cantidad de compuestos volátiles y no volátiles que estos contienen. (Ramalakshmi y Sankar 2018; Royden *et al.,* 2018). Joseph, (2017)**,** probaron la repelencia de citronellal, lemongrass oil, geraniol, peppermint oil, thyme oil, rosemary oil, pine needle oil, and vetiver mediante un olfatometro contra *Bagrada hilaris*, de este ensayo se observó que el único aceite que indujo repelencia en la chinche Bagrada fue el geraniol, por ello es importante seguir trabajando en este campo desarrollando nuevos métodos de aplicación tales como la combinación de semioquímicos con hongos entomopatógenos.

La combinación de dosis bajas de semioquímicos e insecticidas con hongos entomopatógenos puede ser un método eficaz para el control de plagas y enfermedades, en estos tipos de evaluaciones se esperaría que haya una mayor progresión de la enfermedad causada por el entomopatógenos haciendo más susceptible la plaga al contacto con el insecticida o que el modo de acción del insecticida haga más susceptible al insecto a la infección causada por el al hongo entomopatógeno con lo que se incrementaría el sinergismo entre ambos, (Meyling et *al.*, 2018), sin embargo pueden observase otros efectos como antagonismo o aditivismo.

Compatibilidad de entomopatógenos con aceites naturales

En trabajos previos sobre compatibilidad de entomopatógenos/aceite esencial, se menciona que los aceites esenciales como el Neem y de *Cymbopogon citratus* reduce el crecimiento vegetativo y viabilidad de esporas de *Beauberia bassiana*, (Depieri et al., 2005) y otros hongos fitopatógenos como *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger* (Tzortzakis and Economakis, 2007). En otros trabajos el aceite de Neem se menciona que es compatible con entomopatógenos (*Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* y *Pseudomonas fluorescens*) (Halder *et al.*, 2017) y depredadores (orius sp) (Otieno *et al.*, 2017). Es importante realizar pruebas sobre la compatibilidad de aceites esenciales con hongos entomopatógenos con dosis que no causen mortalidad o una mortalidad muy baja, para evaluar el antagonismo, sinergismo o aditivismo. Por lo anterior en este trabajo se evaluó la compatibilidad del hongo entomopatógeno *Isaria farinosa* con aceites vegetales (Neem y zacate limón) y un metabolito microbial (Spinosad) para manejo de la chinche Bagrada. En pruebas de compatibilidad entomopatógenos-aceite natural de Neem contra la chinche Bagrada, se observaron mortalidades superiores al 93.08%, por lo que al aplicarse en combinación los insecticidas naturales muestran un efecto de sinergismo, (Halder *et al.*, 2017).

ARTÍCULO 1 (Journal of Hymenoptera Research-Bulgaria)

Records of Scelionidae (Hymenoptera) parasitizing eggs of *Bagrada hilaris* (Hemiptera: Pentatomidae) in Mexico

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Journal of Hymenoptera Research 30 de mayo de 2019, 13:06

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Dear Mr Moises Felipe-Victoriano:

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ARTÍCULO 1 (Journal of Hymenoptera Research-Bulgaria)

Records of Scelionidae (Hymenoptera) parasitizing eggs of *Bagrada hilaris* (Hemiptera: Pentatomidae) in Mexico

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Abstract

The painted bug or bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is a key pest of crops in the family Brassicaceae. In this work, three species of Scelionidae (Hymenoptera) are reported for the first time as parasitoids of painted bug eggs in Mexico, at Saltillo, state of Coahuila: *Gryon myrmecophilum* (Ashmead), *Trissolcus basalis* (Wollaston), and *Telenomus podisi* Ashmead. This is also the first report of a species of the widespread genus *Telenomus* as an egg parasitoid of *B. hilaris*. Total percent parasitism, high resolution images, and CO1 sequences are provided for each species. In the future, research in Mexico should be carried out on parasitoid species presented in this work to determine their potential as biological control agents and the feasibility of augmentative, classical or inoculative biocontrol strategies for integrated pest management.

Key Words: Heteroptera, stink bug, biological control, parasitoid, Mexico

Introduction

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae), known in Mexico with the common names of bagrada bug or painted bug, is a key pest of cole crops (family Brassicaceae) originally distributed in Africa and Asia (Howard 1906, Ahuja et al. 2008, Kavita et al. 2014). This pest first invaded California, USA, in 2008 (Palumbo et al. 2016) and in 2014 was detected in Saltillo, southeastern Coahuila, Mexico, causing economic damage in broccoli (*Brassica oleracea* L. var. italica), cabbage (*B. oleracea* var. capitata), and cauliflower (*B. oleracea* var. capitata) (Sanchez-Peña 2014; Torres-Acosta and Sánchez-Peña 2016).

The family Scelionidae is a cosmopolitan group of parasitoids that attack the eggs of a variety of arthropods, including Hemiptera. In the Old World, several authors have reported parasitoids of this family attacking painted bug eggs. In India, *Gryon karnalense* (Chacko & Katiyar) and *Telenomus samueli* Mani were reported from the eggs of *B. hilaris* (as *Bagrada cruciferarum* Kirkaldy) (Chacko & Katiyar 1961; Mani & Sharma 1982). In Pakistan, sentinel eggs of *B. hilaris* placed in the field for four days and subsequently incubated in the laboratory yielded three species of hymenopteran parasitoids: *Trissolcus hyalinipennis* Rajmohana & Narendran, *Gryon gonikopalense* Sharma and a species of *Ooencyrtus* Ashmead (Encyrtidae) (Mahmood et al. 2015, Sforza et al. 2019). In the USA, Ganjisaffar et al. (2018) reported *Trissolcus basalis* and *Tr. hyalinipennis* parasitizing painted bug eggs in California. The objective of this work is to determine the presence of parasitoids of painted bug through sentinel eggs in northwestern Mexico and facilitate future work in this line of research.

Materials and Methods

Field site

The work was carried out in the experimental fields of the Universidad Autónoma Agraria
Antonio Narro (UAAAN) in Saltillo, state of Coahuila, México (101° 2' 17.98" W, 25° 21' 15.80" N, 1746 meters above sea level) (INEGI 2018). The specific irrigated field (0.07 hectares) was planted with an assortment of Brassicaceae cultivars in equal numbers of the following plants: Arugula (*Eruca vesicaria* L. ssp. sativa), broccoli, cabbage, cauliflower, kohlrabi (*Brassica napobrassica* Miller. 1768), mustard (*Sinapis alba* L. 1753), radish (*Raphanus sativus* L. 1753) and turnip (*Brassica rapa* L. 1753 subsp. *rapa*).

Detection of parasitoids of painted bug through sentinel eggs.

Eggs were obtained by rearing field-collected mating pairs of painted bugs in the laboratory. Eight mating pairs were placed in Petri dishes at a temperature of 26–28 °C with diffuse overhead daylight. After 12 hours in the laboratory, mating pairs in the Petri dishes produced an average of 270 eggs (range of 85–580). The mating pairs were removed and the eggs (on the same uncovered Petri dish bottom they were laid on) were placed in the soil at a distance of approximately 5 cm from a broccoli stem. If it was necessary to handle the eggs, a soft number 2 brush (Pinceles Rex, Mexico City) was used.

The sentinel eggs tests were conducted monthly in the field from 25 November 2017–20 December 2018. The eggs were exposed 7–8 days in the field, and subsequently incubated
at 24–28 °C and a relative humidity of 60% in the laboratory until the emergence of parasitoids. The wasps that emerged were placed in 96% ethanol until their subsequent identification.

DNA analysis.

Specimens were softened in 70% ethanol for two hours, then DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen). DNA extracts were quantified using a NanoDrop
2000 spectrophotometer (Thermo Scientific). At least 20 ng of genomic DNA was used per PCR. The 5'-CO1 barcode region was PCR-amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994). PCRs were performed at 25 μl volumes using HiFi HotStart DNA Polymerase (Kapa Biosystems). PCR thermocycle conditions were: 1) initial denaturing at 95°C for 2:00 minutes followed by 32 cycles of steps 2–4, 2) 98°C for 30 seconds, 3) 50°C for 30 seconds, 4) 72°C for 40 seconds, and 5) final extension at 72°C for 7:00 minutes. PCR products were verified by gel electrophoresis and cleaned for
sequencing with QIAquick Gel Extraction Kits (Qiagen). Purified PCR products were Sanger sequenced in both directions using BigDye Terminator v3.1 (Applied Biosystems)
chemistry on a SeqStudio Genetic Analyzer (Applied Biosystems). Sequence reads were trimmed and sequence contigs were assembled in Sequencher 5.4.6 (Gene Codes Corporation). CO1 barcodes generated during this study were deposited in GenBank. Accession numbers for these sequences are presented in Table 1.

Morphological identification

Specimens of *G. myrmecophilum* and *Te. podisi* were identified to species using the keys of Masner (1980) and Johnson (1984), respectively. Specimens of *Tr. basalis* were identified using Talamas et al. (2015) and the description by Ganjisaffar et al. (2018) of morphological variation present in individuals that emerge from *B. hilaris* eggs.

Stacks of photographs were taken with a Macropod imaging system and rendered using HeliconFocus. Specimen collection data and host associations are deposited in the Hymenoptera Online Database (hol.osu.edu). Voucher specimens for all scelionid species are deposited at the Florida State Collection of Arthropods (FSCA), Gainesville, Florida, and the Entomology collection, Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico.

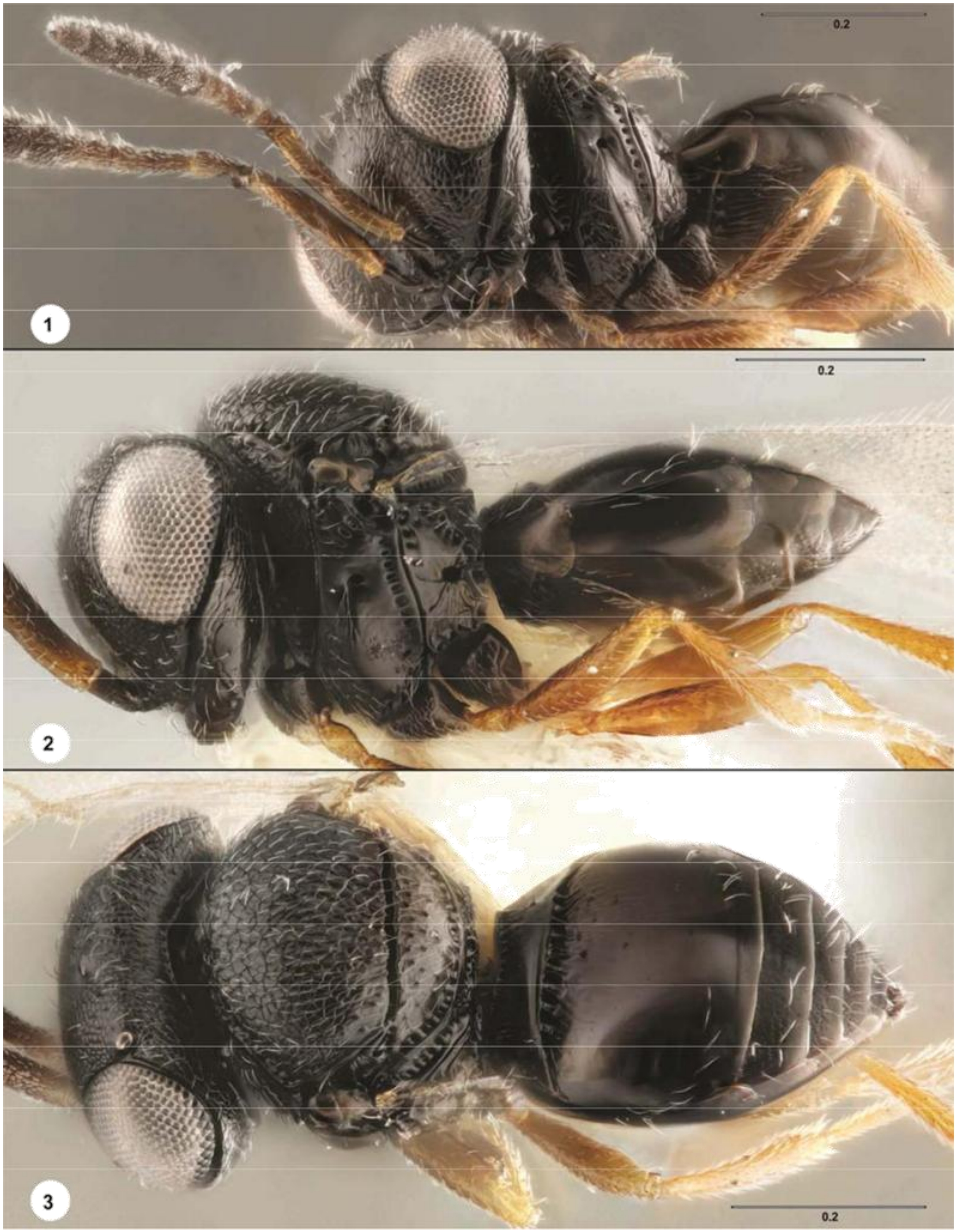
Results

Scelionid wasps were detected only in November 2017 and June, July and August 2018. In a subsequent paper we will discuss in detail the phenology of the parasitoid complex on painted bug eggs at this location in Mexico.

Trissolcus basalis **(Wollaston) Figs (1**–3**)**

As reported by Ganjisaffar et al. (2018), specimens of *Tr. basalis* that emerge from the eggs of *B. hilaris* have reduced episternal foveae and fainter striation on T2 relative to specimens that emerge from larger stink bug eggs (Figs 2–3). BLAST comparison of the CO1 sequence from specimen FSCA 00090267 retrieved a 100% match to a *Tr. basalis* sequence in Genbank from the USA (MK188338.1), providing confirmation of the morphological identification.

On the collection date of 25 November 2017, a total of 29 *Tr. basalis* were collected (this
date was the only time *Tr. basalis* emerged from sentinel eggs) and the percentage of parasitism was 12.4% (n= 384 eggs).

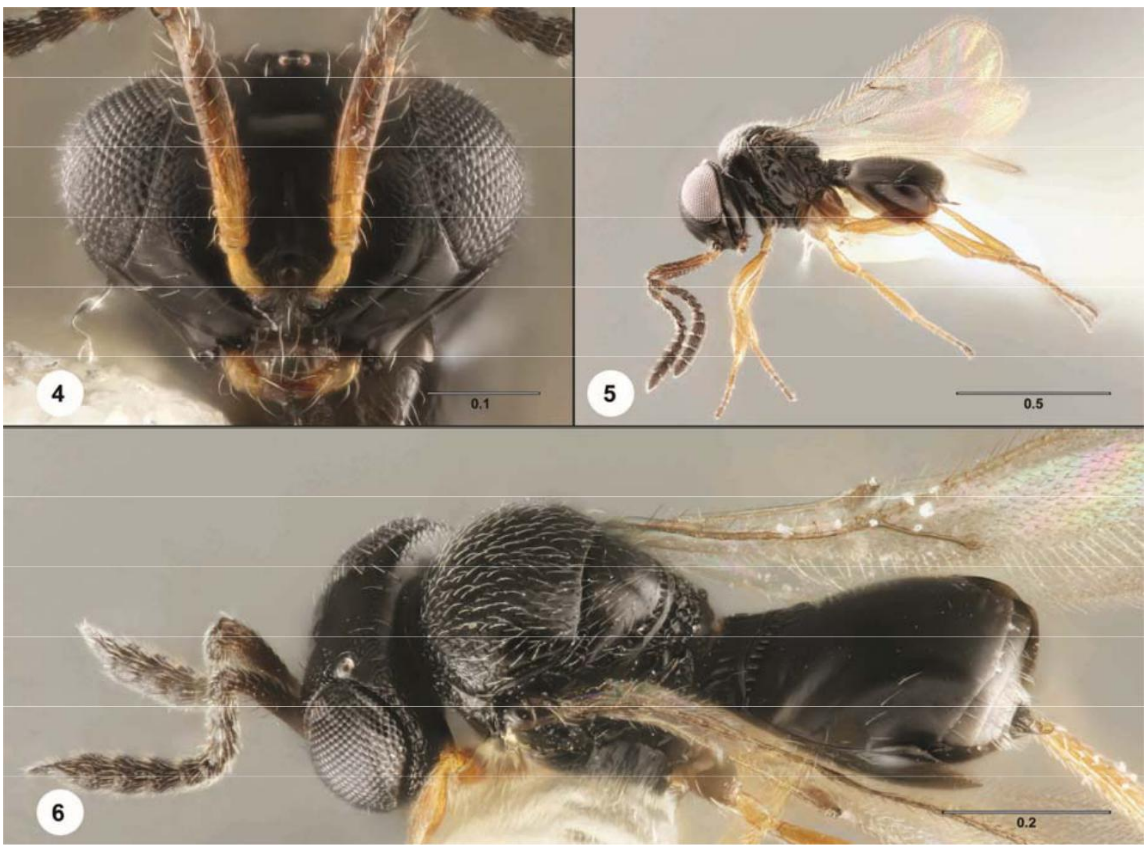

0.2
1
0.2
2
3
0.2

Trissolcus basalis female (FSCA 00090267) 1. head, mesosoma, metasoma, ventrolateral view 2 head, mesosoma, metasoma, lateral view 3 head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

Telenomus podisi **Ashmead Figs (4–6)**

The small size of *B. hilaris* eggs does not influence the diagnostic morphology of *Te. podisi* and no relevant differences were found between the specimens in this study and *Te. podisi* reared from other stink bug eggs. BLAST comparison of the CO1 sequence from specimen FSCA 00090266 retrieved a match of 98.9% sequence identity *Te. podisi* sequence KR870961.1 in Genbank.

On the monthly collection dates between January-December 2018, a total of 51 *Te. podisi* was collected in the months of June and July, resulting in 9.25% and 9.92% of parasitism respectively (n= 532 eggs).

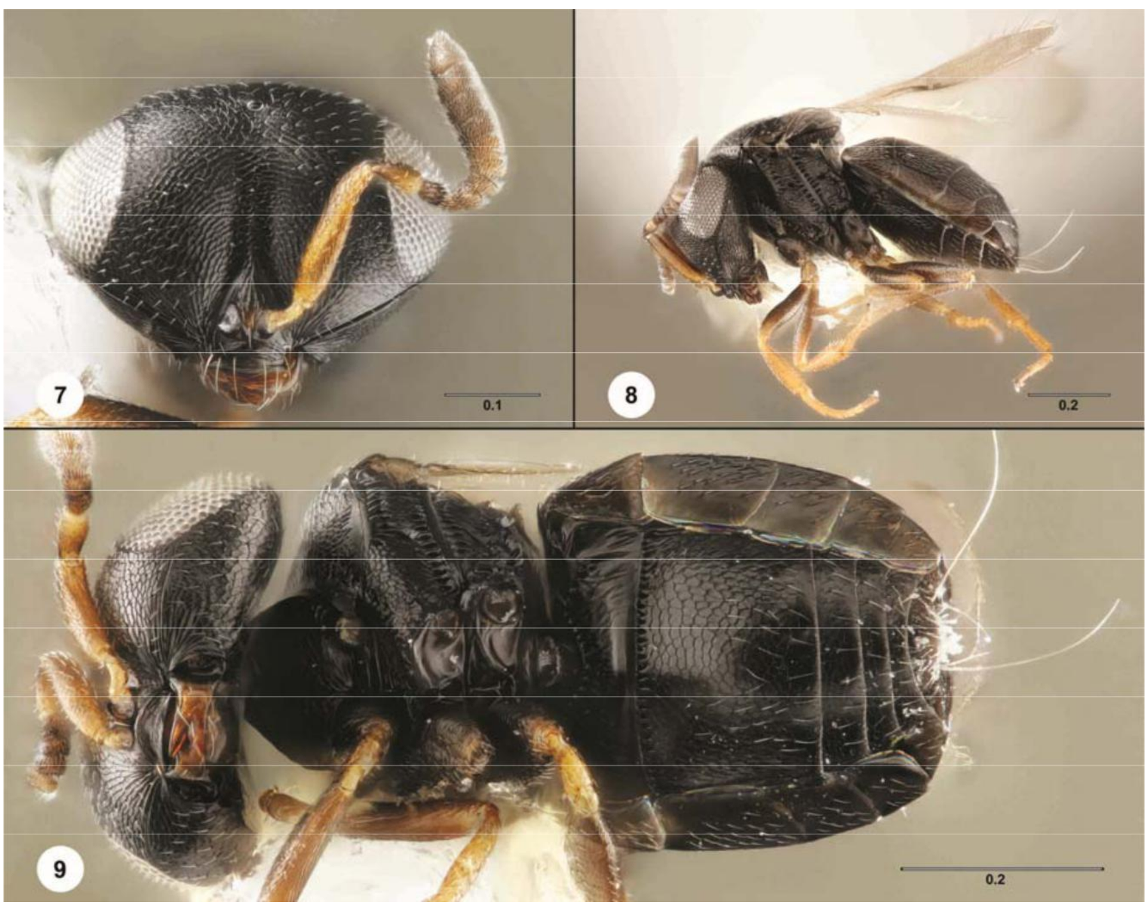


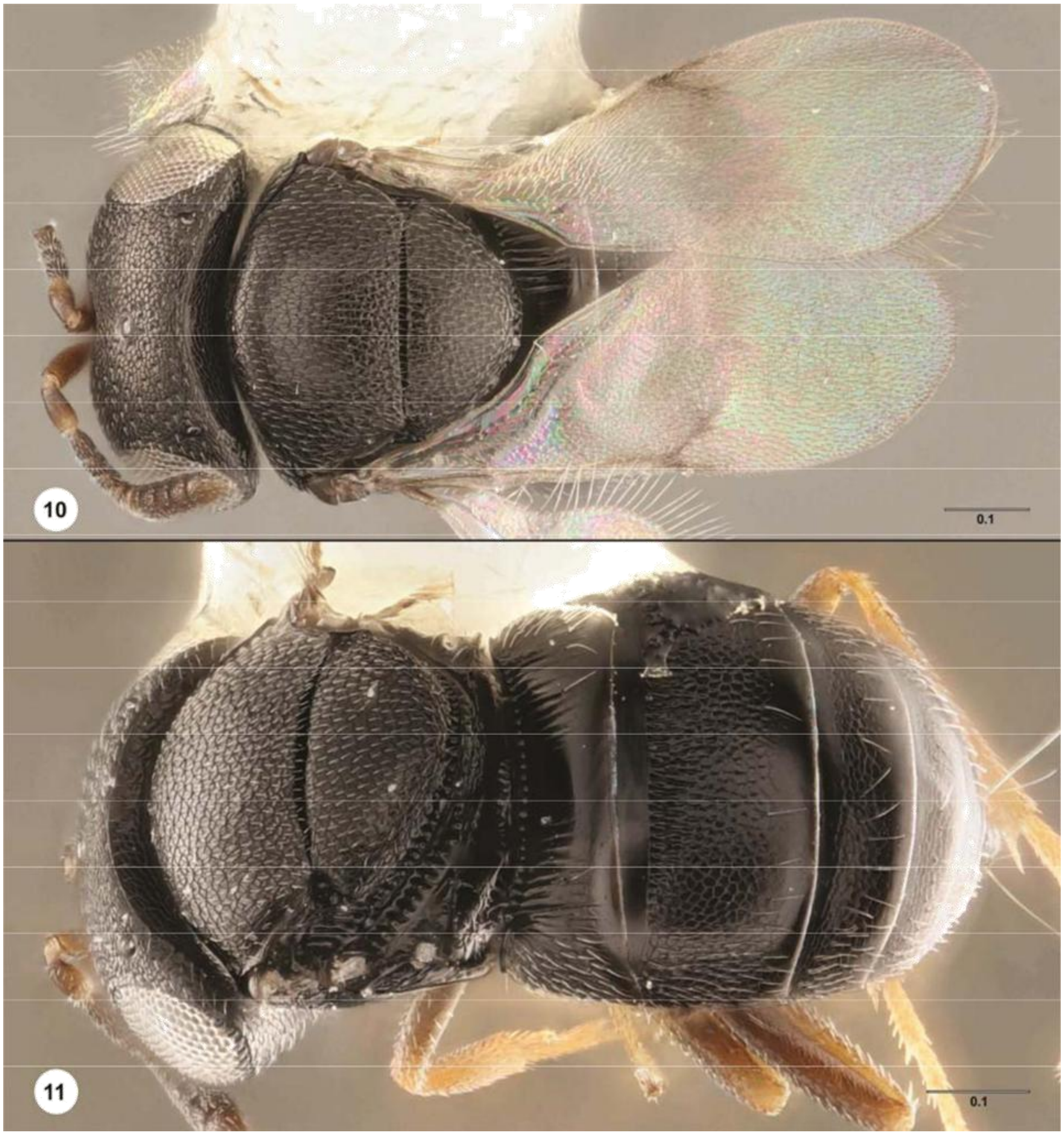
Telenomus podisi 4 female (FSCA 00033549) head, anterior view 5 female (FSCA 00090266) habitus, lateral view 6 female (FSCA 00033275) head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

***Gryon myrmecophilum* (Ashmead) Figs (7–11)**

Our identification of this species is based on the revision of the genus by Masner (1980) and the specimens in this study were compared to photographs of the holotype specimen made available by Talamas et al. (2017). The systematics of *Gryon* is currently under revision by the second author. Preliminary analysis indicates that *G. myrmecophilum* belongs to a cosmopolitan cluster of similar species, some of which may have intercontinental distributions. A specimen of *G. myrmecophilum* from New Jersey (FSCA 00090445) was sequenced to provide a comparison with a specimen closer to the type locality (Washington, DC). BLAST comparison of the sequences from Mexico and New Jersey yielded 88% sequence identity, indicating that *G. myrmecophilum* exhibits a high degree of variability in this gene region, or it is possibly a cryptic species complex.

On the monthly collection dates between January-December 2018, a total of 115 *G. myrmecophilum* were collected in the months of June, July and August, resulting in 3.0, 7.63 and 43.25% of parasitism respectively (n=786 eggs).





Gryon myrmecophilum 7 female (FSCA 00090446) head, anterior view 8 female (FSCA 00090447) habitus, lateral view 9 female (FSCA 00090446) head, mesosoma, metasoma, ventral view. Scale bars in millimeters.

Gryon myrmecophilum, female (FSCA 00090447) 10 head and mesosoma, dorsal view 11 habitus, dorsal view. Scale bars in millimeters.

Species	Locality	Collection Unit Identifier	GenBank Accession Number
Trissolcus basalis	Saltillo, Mexico	FSCA 00090267	MK720829
Telenomus podisi	Saltillo, Mexico	FSCA 00090266	MK720830
Gryon myrmecophilum	Saltillo, Mexico	FSCA 00090442	MK720831
Gryon myrmecophilum	Saltillo, Mexico	FSCA 00090443	MK720832
Gryon myrmecophilum	Rutgers, NJ, USA	FSCA 00090445	MK937524

Table 1. Accession numbers for specimens of Scelionidae used for DNA sequencing.

Discussion

In 2017, *Tr. basalis* emerged only in the month of November (29 specimens), for 12.4% of egg parasitism. This wasp is a near-cosmopolitan parasitoid of stink bug eggs for which one widespread host is the southern green stink bug, *Nezara viridula* (L.) (Powell and Shepard 1982).

During the 2018 monthly sampling dates, scelionid wasps were detected only in June-August. The percentages of egg parasitism by all Scelionidae in the months of June, July and August 2018 correspond to 12.2, 17.4 and 49.6% respectively (total of 166 wasp specimens).

Regarding total monthly percent parasitism, *G. myrmecophilum* (115 specimens) contributed with 24.2, 43.5 and 100% (in June, July and August respectively); *Te. podisi* (51 specimens) contributed with 75.8 and 56.5% (for the months of June and July respectively). Species of *Telenomus* have been reported as a parasitoids of other stink bugs, including *Euschistus heros* (F.), *Halyomorpha halys* (Stål), *Oebalus insularis* Stål, *Piezodorus guildinii* (Westwood) and *Tibraca limbativentris* (Stål), among others.

In Pakistan, Mahmood et al. (2015) reported that *Tr. hyalinipennis* and *G. gonikopalense* had a combined parasitism rate of 32–38%. This level is similar to the parasitism level obtained in our work. In California, USA, Ganjisaffar et al. (2018) reported that *Tr. Basalis* and *Tr. hyalinipennis* parasitized 4.0-20.0% of *B. hilaris* sentinel eggs in January of 2018. We did not observe parasitism by scelionids in January, but it should be noted that study includes only a small number of sampling dates. Additional sampling is required to describe the phenology of these wasps on *Bagrada* eggs.

To our knowledge, this is the reports the highest percentage of field parasitism of painted bug eggs in the USA and Mexico. It is also the first report of *Te. podisi* parasitizing painted bug eggs. We continue studying the identity, distribution and population fluctuation of beneficial wasps associated with painted bug eggs at selected localities in Mexico. Future research should be carried out on these species, and possibly others that have yet to be detected, to determine their potential as biological control agents. In particular, there is a need for critical comparative analysis of the different modalities of biological control (classical, augmentative or inoculative) to utilizing parasitic wasps in the integrated pest management of the painted bug in Mexico.

Acknowledgments

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ARTÍCULO 2 (The Pan-Pacific Entomologist)

Population Fluctuation of the Parasitoid Guild on *Bagrada hilaris* eggs in northeastern México

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Abstract

Bagrada hilaris (Burmeister, 1835) or painted bug, is a primary pest of crucifers throughout the world. There is a lack of information on biological control agents of this recent invader of North America, first detected in the United States in California, in 2008. The insect was detected in Mexico in 2014. Reports from the United States indicate an absence or very low levels of egg parasites of this pest, with occasional, single individuals being reported. In this work we report on the population fluctuation of a guild of painted bug egg parasitoids at Saltillo, Mexico, using the sentinel eggs technique. Painted bug eggs glued to paper squares were placed on the foliage and on the ground they were placed in petri dishes at a distance of approximately 5 cm from the stem in the Brassica plots in Saltillo during November 2017, through December 2018.

We detected egg parasitism on several exposure dates and a maximum of 49.6% parasitism. The primary parasitoid guild consisted of species of the family Scelionidae (*Gryon myrmecophilum*, *Telenomus podisi* and *Trissolcus basalis*) and Encyrtidae (*Ooencyrtus californicus* and a yet unidentified genus of this family). We did not detect putative hyperparasitoids. To our knowledge this is the highest level (49.6%), of consistent primary parasitism in the field of painted bug in North America. Further studies should evaluate the feasibility of manipulating these parasitoids as a biological control agent of painted bug through introductions or augmentative releases.

Key Words: Heteroptera, stink bug, biological control, Mexico

Running Title: Painted bug egg parasitoids from Mexico

Introduction

The stink bug, *Bagrada hilaris* (Burmeister, 1835) (Hemiptera: Pentatomidae), known as painted bug, is a key pest of (mainly) cruciferous crops originally distributed in Africa and Asia. It has colonized southern Europe and was detected in North America (California) in 2008, where it severely impacted Brassica production (Palumbo et al. 2016; Guarino et al. 2017). In 2014, it was detected in Saltillo (southeastern Coahuila state), Mexico, causing economic damage in broccoli (*Brassica oleracea* L. var. italica), cabbage (*B. oleracea*. var. capitata), and cauliflower (*B. oleracea.* var. botrytis) (Sánchez-Peña 2014). As mentioned, this insect is a primary pest of crucifers (Palumbo et al. 2016): for example, in 2009 in California, this insect caused an estimated 35% yield reductions on cabbage and 20% on broccoli (Palumbo 2010). From 2010 to 2014, up to four applications had to be made for painted bug control on broccoli in California (Palumbo 2015).

In North America, where painted bug is a recent invader, specific natural enemies that attack this pest should not exist. Since its colonization of the United States, generalist predators including spiders (Aranea), soft-wing flower beetles (Coleoptera: Melyridae), predaceous stink bugs (Hemiptera: Pentatomidae), and praying mantids (Mantodea) among others have been observed attacking painted bugs under high populations of the pest (Grasswitz 2016; Palumbo et al. 2016). However, as usual for invasive, exotic pests, there is an apparent paucity of specific natural enemies capable of successfully exploiting painted bug as a food resource and of exerting significant control in the invaded areas (Palumbo et al. 2016). since its colonization of the United States, generalist predators including spiders (Aranea), soft-wing flower beetles (Coleoptera: Melyridae), predaceous stink bugs (Hemiptera: Pentatomidae), and praying mantids (Mantodea) among others have been observed attacking painted bugs under high populations of the pest (Grasswitz 2016; Palumbo et al. 2016).The painted bug is unusual among the Pentatomidae in that a significant amount of eggs is laid in soil cracks, as opposed to the typical behavior of oviposition on foliage. Several authors have reported egg parasitoids of painted bug in its native range in the Old World. In India, egg parasitoids have been reported for painted bug (as *Bagrada cruciferarum* Kirkaldy): *Hadrophanurus karnalensis* Chacko & Katiyar, 1961 and *Telenomus samueli* Mani, 1942 (both Scelionidae) (Chacko & Katiyar, 1961; Mani & Sharma, 1982). In Pakistan, sentinel eggs of B. hilaris placed in the field for four days and subsequently incubated in the laboratory yielded three species of hymenopteran parasitoids, in the genera, *Trissolcus* Ashmead, 1893, *Gryon* Holiday, 1833 (Scelionidae) and *Ooencyrtus* Ashmead, 1900 (Encyrtidae) (Mahmood et al. 2015). In the United States, laboratory research showed that the wasps, *Trissolcus hyalinipennis* and *Gryon* sp., successfully parasitized 1- to 4-day-old eggs (Sforza et al. 2017).

Sentinel egg studies in California yielded very low numbers of parasitoids: one individual of each of three species in central California, and unspecified numbers in southern California (Grettenberger et al. 2018). The economic impact of this insect dictates that it is critical to introduce and establish specific natural enemies of painted bug in its invasive range of North America. There are many cases where invasive pests are attacked in the introduced range by parasitoids that aren’t purposely released biological control agents. These parasitic interactions can result from a) generalist, native parasitoids that are able to use the invasive insect as a larval food source de novo, or b) adventive parasitoids from the geographic origin of the invasive pest (Herlihy et al. 2016). The objective of this study was to detect parasitism on painted bug sentinel eggs at Saltillo, Mexico, and to make initial comparisons of parasitism levels on soil and on foliage in patches of cultivated crucifers.

Materials and Methods

Field site

The work was carried out in the experimental fields of the Universidad Autónoma Agraria Antonio Narro (UAAAN) in Saltillo, Coahuila, México. The coordinates of the specific field are 101° 2' 17.98" W, 25° 21' 15.8" N at an altitude of 1,746 meters above sea level (INEGI 2018). This field was planted to an assortment of Brassicaceous cultivars: arugula (*Eruca vesicaria* L. ssp. sativa), broccoli (*Brassica oleracea* L. var. italica), cabbage (*B. oleracea* var. capitata), cauliflower (*B. oleracea* var. capitata), kohlrabi (*Brassica napobrassica* Miller. 1768), mustard (*Sinapis alba* L. 1753), radish (*Raphanus sativus* L. 1753), and turnip (*Brassica rapa* L. 1753 subsp. rapa). Painted bug populations were very high, exceeding 200 insects (nymphs and adults) per plant on highly preferred (mustard) and an average of 35 insects/plant on arugula.

Detection of insect parasitoids painted through sentinel eggs placed in foliage and soil

Eggs were obtained by incubating field-collected, mating pairs of painted bugs in the laboratory. Mating pairs were placed in Petri dishes at a temperature of 26-28 °C and a 12:12 photoperiod. Eggs (12-24 h old) were manipulated with a soft paint brush. Groups of ten eggs were glued on fifteen squares of brown blotting paper measuring 3x3 cm, using white non-toxic glue (Resistol 850, Henkel Mexicana, Huixquilucan). After the glue was dry, squares with attached eggs were placed individually on foliage. In soil the number of eggs varied from 85 to 580, these were placed in Petri dishes at a distance approximately 5 cm from the broccoli stem. The tests on foliage and soil were performed as listed in table 1. The sentinel eggs were exposed 7 to 8 days in field, subsequently incubated at a temperature of 26-28 °C and a relative humidity of 60% in laboratory until the emergence of parasitoids. Sentinel eggs were exposed every month (if eggs were available) from November 2017 to Octuber2018.

Morphological identification

Individuals obtained from *Gryon myrmecophilum* and *Telenomus podisi* species were identified using the keys of Masner (1980) and Johnson (1984). Individuals of the *Trissolcus basalis* species were identified using the key of Talamas et al. (2015). Individuals of the Encyrtidae family (*Ooencyrtus californicus*) were identified using the key Noyes, 1985, Trjapitzin, et al. 2008 and Noyes (2010).

Due to the size of the wasps (less to 1 mm.), groups of photographs were taken at different depth and field with a Macropod image system, later these image piles were processed using HeliconFocus until obtaining a completely sharp image. Individuals of the Scelionid species were deposited in the Arthropod Collection of the State of Florida (FSCA), Gainesville, Florida, and individuals of the Scelionidae, Encytidae and Eulophidae families were deposited in the Collection of Entomology, Universidad Autonoma Agraria Antonio Narro, Saltillo, Mexico.

DNA analysis.

The insects were softened in 70% ethanol for two hours, the DNA was extracted using a DNeasy tissue and blood kit (Qiagen). The quantification of the DNA extracts was performed using a NanoDrop 2000 spectrophotometer (Thermo Scientific). 20 ng of genomic DNA was used for the PCR test. For PCR amplification, the methodology of Folmer et al. (1994). Each region of the 5'-CO1 code was amplified using the primers LCO1490 and HCO2198, the PCR was performed in 25 µl volumes using the HiStart HotStart DNA polymerase (Kapa Biosystems). The conditions of the PCR thermocycle were carried out in 5 phases: 1) denaturation of initial protein at 95 ° C for two minutes followed by 32 cycles of steps 2-4, 2) 98 ° C for 30 seconds, 3) 50 ° C during 30 seconds, 4) 72 ° C for 40 seconds and 5) final extension at 72 ° C for 7:00 minutes. The

PCR was verified by gel electrophoresis, sequencing was carried out with the QIAquick gel extraction kits (Qiagen). The purified products obtained by PCR were sequenced by Sanger in both directions using BigDye Chemical Terminator v3.1 (Applied Biosystems) on a SeqStudio gene analyzer (Applied Biosystems). The readings of the sequences were cut out and subsequently assembled the sequence in the sequencer 5.4.6 (Gene Codes Corporation). Finally, the CO1 bar codes obtained were deposited in GenBank.

Results and Discussion

Detection of insect parasitoids painted through sentinel eggs placed in foliage and soil

Parasitoids were collected in 2017 (November) and 2018 (April-August). From the tests carried out on soil and foliage in November 2017, the highest parasitism was observed in soil with 12.4% (29 wasps) respect to foliage with 4% (6 wasps), this parasitism corresponds only to *Trissolcus basalis* (Table 1.). In November 2017, 35 parasitoids were obtained, with a male: female ratio of 1:1. Therefore, in the following tests carried out in 2018, it was decided to carry out in soil. Table 1. Shows the parasitism of sentinel eggs of *Bagrada hilaris*: the site of sentinel eggs placement on the brassica plot (ground or foliage), the date eggs were placed in the field, the number of eggs exposed, the retrieval date of eggs from the field and total days exposed, the days of exposure and incubation in the laboratory until wasp emergence, the number of wasps that emerged from eggs, and percent parasitism.

In 2018 (April-August), 4 species were observed parasitizing *Bagrada hilaris* (*Aprostocetu*s sp. *Gryon myrmecophilum*, *Telenomus podisi* and *Ooencirtus californicus*) Figure 1-3. The parasitism fluctuated between 2.6% (May) and 49.6% (August) of exposed eggs. In the tests carried out in April, all the parasitism corresponded to the family Encyrtidae, genus *Ooencyrtus californicus* parasite, 7.1% and to the genus *Encyrtus* parasite, only 4.2% of the eggs exposed in the field. In the month of May, two species of parasitoids emerged, *Ooencyrtus californicus* parasite 2.6% of the exposed eggs, the emergence of a species of the family Eulophidae, subfamily Tetrastichinae, possibly the genus *Aprostocetus*, was recorded, the parasitism of this species corresponds to 0.52%.
From June to July the emergence of two parasitoids (*T. podisi* and *Gryon myrmecophilum*) was observed, in the two tests, *T. podisi* was more abundant compared to *Gryon myrmecophilum*. In August, two parasitoids (*Ooencyrtus californicus* and *T. basalis*) were observed, in this evaluation *Gryon myrmecophilum* was more abundant with respect to *Ooencyrtus sp.* with 34.2 and 15.3% respectively.

In overall, although in the monthly tests from November 2017 to December 2018, 6 species of the family Scelionidae, Encirtidae and Eulophidae emerged (246 wasps), according to the number of parasitoids obtained from field *Gryon myrmecophilum* (115 wasps) is the most abundant with 46.74%, followed by *Ooencyrtus californicus* (54 wasps) with 21.95 %, *T. podisi* and *Trissolcus basalis* (35 wasps) with 23.1%, the lowest number of wasps was obtained in the genera *Ooencyrtus* sp and *Aprostocetus* so with 2.47 and 0.40% respectively.

The first report on egg parasitism of Bagrada (*Bagrada cruciferarum*) was in India in 1959, where the Scelionidae *Hadrophanurus* sp, parasitized 85.1% of sentinel eggs exposed during the evaluation, (Narayanan et al., 1959). Later, in the years of 1961 and 1982, the species *Gryon karnalense* and *Telenomus samueli* are mentioned as parasitoids of *Bagrada cruciferarum* (Chacko & Katiyar 1961; Mani & Sharma 1982).

Mahmood et al. (2015) describe sentinel egg observations in Pakistan. After 4 days in the field, painted bug parasitoids (*Trissolcus* sp, *Gryon* sp. and *Ooencyrtus* sp.), emerged after 15-18 days of laboratory incubation. In our case, parasitoid emergence after laboratory incubation in November 2017 was somewhat delayed compared to these data: 18-23 days (foliage); 26 to 35 days (soil). In contrast, days to emergence of parasitoids in April and October 2018 were similar to those reported by Mahmood et al. (2015).

Ganjisaffar et al. (2018) reported parasitism (*Trissolcus basalis* and *T. hyalinipennis*) of painted bug eggs in the state of California (United States), however the percentage of parasitism is low (7%), compared to our observations in 2018 (49.6%). In other work Gözüaçık, (2018), mentions that *Bagrada abeillei* Puton, 1881 and *Bagrada amoenula*, are parasitized by *Trissolcus semistriatus* Nees., T. Grandis Thomson and T. vassilievi Mayr (Scelionidae). In similar tests, Pezzini et al. (2018) observed that sentinel egg predation was less than 3.7%. In our test on foliage, predation was low, below 3%; Orius (Hemiptera: *Anthocoridae*) bugs were observed feeding on eggs. Regarding soil tests, in June 14% of eggs were carried away by ants (*Pheidole* sp.: Hymenoptera: Formicidae) (40 eggs of 270). Our results may indicate that the wasp in the field is adapting rapidly in an exotic host, so that they can be used in the future as an effective biological control agent against the painted bug.

Pentatomid bugs like *Bagrada abeillei* (Puton, 1881), *B. amoenula* (Walker, 1870), *Halyomorpha halys* (Stål, 1855), and *Nezara viridula* (Linnaeus, 1758) are attacked, among others, by egg parasitoids of the genus *Trissolcus*, like *T. basalis* (Wollaston, 1858), *T. grandis* (Thomson, 1860), *T. semistriatus* (Nees, 1834) and *T. vassilievi* (Mayr, 1879) (Hymenoptera: Platygastridae (=Scelionidae, in part) (Colazza et al. 1999; Yang et al. 2009; Gözüaçık 2018).

Morphological identification

Specimens of the Scelionidae family collected were identified with the key of Masner (1980), Johnson (1984) and Talamas et al. (2015). *Trissolcus* was compared with the description of Ganjisaffar et al. (2018). Species of the Encyrtidae family were identified with the keys of Noyes, 1985, Trjapitzin, et al. 2008 and Noyes, 2010.

Subsequently, groups of males and females between 5 and 11 are deposited in the Arthropod Collection of the State of Florida (FSCA), Gainesville, Florida and in the Collection of Entomology, Universidad Autónoma Agraria Antonio Narro, Saltillo, México. (Table 2.)

Table 1. Percent parasitism of Bagrada hilaris sentinel eggs at Saltillo, Mexico, 2017-2018.

Sentinel eggs Placement	Date eggs placed in the field	# Eggs exposed	Retrieval date (days exposed in the field)	Time of incubation to wasp emergence	Wasps emerged	% parasitism
Eggs on foliage	20 November 2017	150	Nov 28 (8)	18 to 23	6 (*T. basalis*)	4
Eggs on soil	25 November 2017	234	Dec 3 (7)	27 to 35	29 (*T. basalis*)	12.4
	December	0*	-	-	-	-
	13 January 2018	450	Jan 21 (8)	-	0	0
	16 February 2018	580	Feb 23 (7)	-	0	0
	March	0*	-	-	-	-
	26 April 2018	140	May 3 (7)	14 to 20	10 (*O. californicus*)	7.1
					6 (*Ooencyrtus* sp.)	4.2
	17-may-18	189	May 24 (7)	15 to 18	5 (*O. californicus*)	2.6
					1 (*Aprostocetus sp*)	0.52
	19 June 2018	270	June 26 (7)	16 to 20	25 *(T. podisi)*	9.2
					8 (G. *myrmecophilum*)	2.9
	10 July 2018	262	July 17 (7)	14 to 17	26 (*T. podisi)*	9.9
					20 (*G. myrmecophilum*)	7.6
	12 August 2018	254	August 19 (7)	12 to 17	39 (*O. californicus*)	15.3
					87 (*G. myrmecophilum*)	34.2
	15 September 2018	112	September 22 (7)	-	0	0

17 October 2018	85	0ctober 24 (7)	-	0	0
November	0*	-	-	-	-
December	0*	-	-	-	-

* In December (2017-2018) March and November (2018) no sentinel eggs were placed, since the population of painted bug was very low or absent; adults could not be found to obtain eggs for tests.

DNA analysis.

The COI sequences obtained from each specimen from Saltillo, Coahuila Mexico, have been loaded in Genbank with accession numbers. *Trissolcus basalis* (MK720829), *Telenomus podisi* (MK720830), *Gryon myrmecophilum* (MK720831), *Gryon myrmecophilum* (MK720832), *Gryon myrmecophilum*- Rutgers, NJ, USA (MK937524). *Trissolcus basalis* (specimen FSCA 00090267) and *Telenomus podisi* (specimen FSCA 00090266) in BLAST comparison of the CO1 sequence from retrieved a 100% match to other sequences in Genbank. *Gryon myrmecophilum* in BLAST comparison of the CO1 sequence from specimen FSCA 00090442 when compared to specimen of *G. myrmecophilum* from New Jersey (FSCA 00090445) the yielded 88% sequence identity, indicating that *G. myrmecophilum* exhibits a high degree of variability in this gene region, or it is possibly a cryptic species complex. The comparison of the BLAST CO1 sequence of our specimens confirms the morphological identification made with the keys Masner (1980), Johnson (1984) (*Gryon myrmecophilum*- *Telenomus podisi*), Talamas et al. (2015), (*Trissolcus basalis*) Noyes, (1985), Trjapitzin, et al. (2008) and Noyes (2010) of for each of the identified species.

Table 2. Species, Collection Unit Identifier of eggs parasitoids of Bagrada hilaris in Saltillo, Coahuila, Mexico.

Species	Locality	Collection (UAAAN) Unit Identifier	Collection Unit Identifier
T. basalis	Saltillo, Mexico	MFV 001-007.	FSCA 00090267
			FSCA 00090476
			FSCA 00090479
Te. podisi	Saltillo, Mexico	MFV 008-0013.	FSCA 00090266
			FSCA 00033275
			FSCA 0003317-18
			FSCA 00090480-4
G. myrmecophilum	Saltillo, Mexico	MFV 014-019.	FSCA 00090442
			FSCA 00090443
			FSCA 00090446-7
Oo californicus.	Saltillo, Mexico	MFV014-021	

FSCA: Florida State Collection of Arthropods, UAAAN: Universidad Autónoma Agraria Antonio Narro; MFV: Moisés Felipe Victoriano (Manifold)

In this work, we observed greater parasitism levels in sentinel eggs placed in soil near plant stems compared to those placed on foliage. This parasitoid has the ability of searching for eggs on soil; this indicates that it might be adapted to the unusual trait of laying eggs in the soil or in soil cracks. However, testing of parasitism of eggs actually laying on the ground must be evaluated to determine the actual searching capacity of this wasp.

To our knowledge, this is the first report of significant field parasitism of painted bug eggs in North America. We are currently evaluating these parasitism rates and we have detected even higher percentages of attacked eggs in the summer (in preparation).

In the future, research should be carried out on the species reported in this work to determine their potential as biological control agents and the feasibility of augmentative releases of these wasps for the integrated pest management of the insect painted in Mexico.

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CONCLUSIONES GENERALES

Artículo 1.

En el futuro, se deben realizar investigaciones sobre las especies de Scelionidos (*Gryon myrmecophilum*, *Trissolcus basalis* y *Telenomus podisi*) colectados de huevos centinela en el noroeste de México para determinar su potencial como agentes de control biológico y la factibilidad de liberaciones aumentativas o inoculadoras para el manejo integrado de la chinche Bagrada en México.

Artículo 2.

En este trabajo, observamos mayores niveles de parasitismo en huevos centinela colocados en el suelo cerca de los tallos de las plantas en comparación con los colocados en el follaje, por ello en estudios futuros las investigaciones deben ser enfocados en la capacidad de búsqueda real de huevos sobre el suelo o grietas, debido a que la chinche Bagrada comparado con otras chinches tiene la particularidad de poner sus huevecillos en grieta sobre el suelo.

En el futuro, se deben realizar investigaciones sobre las especies de Encyrtidae (*Ooencyrtus* sp, *Encyrtus* sp.), Scelionidae, (*Gryon myrmecophilum*, *Trissolcus basalis* y *Telenomus podisi*) y Pteromalidae colectados de huevos centinela en el noroeste de México para determinar su potencial como agentes de control biológico y la factibilidad de liberaciones aumentativas o inoculadoras para el manejo integrado de la chinche Bagrada en México.

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