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Universidad de Guadalajara

Centro Universitario de Ciencias Biológicas y Agropecuarias

DOCTORADO EN CIENCIAS EN ECOFISIOLOGÍA Y RECURSOS GENÉTICOS

La familia Coccinellidae (Coleoptera: Cucujoidea) y su potencial como agentes de control biológico de *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae)

Tesis

Que para obtener el grado de

Doctor en Ciencias en Ecofisiología y Recursos Genéticos.

Presenta

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La Venta del Astillero, Zapopan, Jalisco 13 de Marzo de 2020

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Por

José Manuel Rodríguez Vélez

Tesis presentada como requisito parcial para obtener el grado de

Doctor en Ciencias en Ecofisiología y Recursos Genéticos

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DEDICATORIA

Dedico esta tesis a las primeras personas en amarme, mis padres María y Miguel. A mis hermanas y hermanos; Beatriz, Rosa Alicia, Miguel Ángel, Alberto y Luis David. A mis sobrinas y sobrinos; Luisa, Andrea, Sophia, Mariano, Leonardo y Daniel. Al amor de mi vida, mi esposa Adela Yarazed y especialmente a mi nuevo amor y mayor fuerza, mi hijo Dariel**.**

No hay nada que temer en la vida, únicamente se debe entender. Ahora es tiempo de entender más, para temer menos.

Marie Curie

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CONTENIDO

RESUMEN

En el presente trabajo se aportó una lista de doce especies de coccinélidos depredadores de *Melanaphis sacchari* (Zehntner) en México, enriquecida con comentarios taxonómicos, ilustraciones de referencia taxonómica e información molecular; además se realizaron avances en el entendimiento de las poblaciones de coccinélidos depredadores asociados a *M. sacchari*, mediante un análisis de componentes principales, considerando las variables ambientales y la fluctuación poblacional de las especies de coccinélidos y áfidos presentes, durante muestreos llevados a cabo durante tres periodos, correspondientes a los años 2016, 2017 y 2018. Se demostró que las poblaciones de *M. sacchari* disminuyeron de forma abrupta a través de los tres años muestreados, así como la formación de un grupo de depredadores de *M. sacchari* compuesto por 25 especies; de las cuales, destacan por su relación depredador-presa con *M. sacchari* los coccinélidos *Scymnus* (*Pullus*) *dozieri* Gordon, *Hippodamia convergens* Guérin-Méneville, *Cycloneda sanguinea sanguinea* (L.) y *Coleomegilla maculata lengi* Timberlake. Por último, se realizó una revisión de los estudios que han evaluado la depredación sobre *M. sacchari* por coccinélidos nativos de México. Se evidencia el potencial de las especies nativas de coccinélidos como agentes de control biológico de la especie plaga del sorgo, *M. sacchari*.

ABSTRACT

A list of twelve species of predatory cocinellids of *Melanaphis sacchari* (Zehntner) in Mexico, enriched with taxonomic comments, taxonomic reference illustrations and molecular information is provided. In addition, advances were made in the understanding of the populations of predatory coccinellids associated with *M. sacchari*, through an analysis of main components, considering the environmental variables and the population fluctuation of the coccinellid species and aphids present during the sampling period. Sampling was carried out during three periods; those corresponded to the years 2016, 2017 and 2018. *Melanaphis sacchari* population decreased abruptly throughout the three sampled years, as well as the formation of a group of predators of *M. sacchari* composed of 25 species; of which the coccinellids *Scymnus* (*Pullus*) *dozieri* Gordon, *Hippodamia convergens* Guérin-Méneville, *Cycloneda sanguinea sanguinea* (L.) and

Coleomegilla maculata lengi Timberlake stand out for their predatory-prey relationship with *M. sacchari*. Finally, a bibliographic review of the studies that have evaluated predation on *M. sacchari* by native coccinellids in Mexico was carried out. The potential of native coccinellid species as biological control agents of the sorghum pest species, *M. sacchari*, is evidenced.

INTRODUCCIÓN

El consumo de tejido fotosintético vivo por animales constituye la definición más general de herbivoría y se califica como herbívoros a todos aquellos organismos adaptados a vivir únicamente consumiendo a los productores primarios (Begon *et al*. 2006). Es probable que en la agricultura el herbivorismo se conozca desde el nacimiento de la misma (Painter 1951).

La agricultura moderna comprende la práctica del monocultivo, que elimina la diversidad vegetal, reduce las fuentes de alimento y de refugio de los organismos fitófagos y de los enemigos naturales, y por añadidura un aumento en los daños por insectos plaga (Holt-Giménez & Altieri 2013), entendiendo como plaga en el sentido antropocéntrico, cualquier especie, raza o biotipo vegetal o animal, o agente patógeno dañino para las plantas o productos vegetales de interés para el humano (FAO 2016).

Los monocultivos manejados con el amplio arsenal químico disponible actualmente, asegurando al menos en el corto plazo, el aumento de las cosechas, en contraste estos provocan contaminación ambiental, disminución de organismos benéficos y especies silvestres, intoxicaciones, efectos negativos sobre aplicadores y personas relacionadas con su manejo, y el desarrollo de resistencia de las plagas (García-Gutiérrez & Rodríguez-Meza 2012).

En contraparte al manejo con agroquímicos, se encuentra el relativamente nuevo concepto y vieja práctica milenaria del manejo sustentable de plagas, el cual se basa en la combinación de diferentes estrategias de forma armónica para el control de plagas agrícolas (Flint & Van den Bosch 1981). Hasta principios del siglo XX estas prácticas enfocadas en estrategias de control biológico, cultural, químico y mixto habían considerado el conocimiento de la interacción entre plantas y animales, con una tendencia a la sustentabilidad dichas prácticas eran indispensables debido a la escasez de productos químicos (Pichardo-González 2006). Entre las estrategias del manejo sustentable de plagas agrícolas se encuentra el control biológico el cual involucra la acción de organismos benéficos sobre organismos plaga (Van Driesche *et al*. 2007).

El control biológico es el uso de enemigos naturales, para prevenir, reducir o retrasar el desarrollo de poblaciones elevadas de las plagas (DeBach 1974). El control biológico puede emplearse con enemigos naturales nativos o exóticos. Cundo se emplean organismos exóticos se le denomina control biológico clásico, el cual nació a finales del siglo XIX a raíz de la introducción del coccinélido *Rodolia cardinalis* (Mulsant, 1850) para el control de la cochinilla acanalada *Icerya purchasi* (Maskell, 1878) (Caltagirone & Doutt 1989), plaga de los cítricos, originaria de Australia que aquejaba los cultivos de California en Estados Unidos.

El éxito de esta alternativa de manejo de plagas depende de los enemigos naturales usados, ya que estos constituyen el recurso fundamental. De lo anterior se origina la importancia de conocer la taxonomía, biología, ecología y el comportamiento del agente de control de interés (Nicholls y Altieri 2008). Los enemigos naturales pueden emplearse como control biológico por inoculación, por inundación y por conservación.

El control biológico por inoculación es la liberación intencional de pocos individuos de una o varias especies de enemigos naturales, con la expectativa de que se multipliquen, se establezcan y controlen la plaga durante un período extendido; el control biológico por inundación es el uso de números elevados de enemigos naturales, nativos o exóticos para controlar las plagas rápidamente (Eilenberg *et al*. 200l).

El control biológico por conservación consiste en preservar los insectos benéficos de la región para que ellos controlen a la plaga de forma paulatina y sin intervención humana. Para que el control biológico por conservación sea exitoso se recomienda reducir los factores que interfieren en la acción natural de los depredadores y parasitoides (insecticidas no selectivos), y la implementación de agroecosistemas de policultivos que brindarán la disponibilidad de presas alternativas, fuentes de néctar y microhábitats apropiados (Altieri 1994).

Los coleópteros de la familia Coccinellidae han sido históricamente empleados en programas de control biológico, siendo hasta el momento los depredadores más recurrentes en estos programas (Romoser 1981).

Un adecuado sistema de estudio para la implementación de programas sustentables de control biológico es el cultivo del sorgo. El cereal de sorgo es uno de los monocultivos más

importantes a nivel mundial (Espinosa-Ramírez & Serna-Saldívar 2016). En nuestro país para el ciclo otoño-invierno del año 2016 se le destinaron 1 706 601.91 hectáreas, con un valor de producción de 17 216 768.73 miles de pesos (SIAP 2019).

No obstante el rendimiento del cultivo de sorgo en México, se ve afectado por diversas plagas entre las cuales se encuentran los insectos suctores de la familia Aphididae como *Rhopalosiphum maidis* (Fitch, 1856), *Schizaphis graminum* (Rondani, 1852) y *Melanaphis sacchari* (Zehntner, 1897) (Michels & Matis 2008). *Melanaphis sacchari* es la especie que ocasiona mayores pérdidas, las cuales pueden variar en función al estrés hídrico de la planta, la etapa de crecimiento de la planta, el momento en que se produce la infestación, la intensidad y la duración de la misma, entre otros factores (Singh *et al*. 2004). Si la infestación ocurre en condiciones de sequía, ambos factores pueden tener una interacción sinérgica que intensifica la reducción del rendimiento (Singh *et al*. 2004).

El control de las poblaciones de *M. sacchari* se basa en la aplicación de insecticidas sintéticos, actualmente no existe un listado de insecticidas recomendados, no obstante no se recomienda el uso de insecticidas organofosforados ya que se ha demostrado la sobreproducción de enzimas β-esterasas y α-esterasas en *M. sacchari* como mecanismo detoxificativo a los pesticidas oganofosforados y por consecuencia el desarrollo de resistencia a los mismos (Hernández-Bautista *et al*. 2016).

En el norte del continente americano se ha reportado una gran actividad depredadora de especies de Coccinellidae depredando a *M. sacchari*. En los Estados Unidos existen reportes de *Diomus terminatus* (Say, 1835), (Hall 1987), *Coleomegilla maculata* (Degeer, 1775), *Hippodamia convergens* Guérin-Méneville 1842, *Cycloneda sanguinea* (L. 1763) y *Olla-v nigrum* (Mulsant, 1866) (Hall 1988) alimentansose de *M. sacchari*.

En el estado de Kansas en Estados Unidos, *H. convergens* es la especie más importante que controla naturalmente los áfidos en los cultivos de granos (Rice & Wilde 1988; Nechols & Harvey 1998; Michels *et al*. 2001).

En México, Rodríguez-Vélez *et al*. 2016, reportan 11 especies de coccinélidos en coexistencia con *M. sacchari* para el estado de Tamaulipas: *Coccinella septempuctata* L. (1758),

C. maculata, *Cycloneda sanguinea sanguinea*, (L. 1763) *H. convergens*, *Olla v-nigrum*, (Mulsant, 1866) *Brachiacantha decora* Casey, 1899, *Hyperaspis wickhami* Casey, 1899, *Diomus roseicollis* (Mulsant, 1853), *D. terminatus*, *Sycmnus* (*Pullus*) *loewii* Mulsant, 1850 *y Scymnus* (*Pullus*) sp. Por su parte Rodríguez-Palomera *et al.* (2016) reportan cinco especies de Coccinellidae asociadas a *M. sacchari* en el estado de Nayarit, México, entre las cuales se encuentra *Harmonia axyridis* (Pallas, 1773), la cual no se reporta para Tamaulipas en el trabajo de Rodríguez-Vélez *et al*. (2016). *Scymnus (Pullus) dozieri* Gordon, 2000 fue reportado como depredador de *M. sacchari* en el estado de Guanajuato (Marín-Jarillo 2017).

Vázquez-Navarro *et al*. (2016) mencionan al coccinélido *H. convergens* como la especie dominante en los muestreos enfocados en un estudio poblacional en un cultivar de sorgo forrajero infestado con *M. sacchari* en la comarca lagunera.

Marín-Jarillo (2017), en el estado de Guanajuato realizó muestreos para determinar la fluctuacion poblacional de *M. sacchari* y de sus enemigos naturales en plantas de sorgo, identificando las especies de Coccinellidae; *H. convergens*, *H. axyridis*, *C. sanguinea*, *S.* (*P.*) *loewii*, *Scymnus* spp., *O. v-nigrum*, *C. maculata*. Además de los Syrphidae; *Toxomerus politus (*Say, 1823), *Allograpta obliqua* (Say, 1823) y *Allograpta exotica* (Wiedemann, 1830), también se identificó al depredador, *Hemerobius* sp. (Hemerobidae), *Chrysoperla carnea s. l.* (Stephens, 1836) y *Chrysoperla comanche* (Banks, 1938) (Chrysopidae).

Por su parte Rodríguez-del-Bosque *et al*. (2018) presentaron un listado faunístico de enemigos naturales de *M. saccahri* en Tamaulipas en donde mencionaron las mismas especies de coccinélidos que ya se habían reportado con anterioridad por Rodríguez-Vélez *et al.* (2016).

Pese a que existen varios reportes documentados de depredación y asociación de coccinélidos con *M. sacchari* en México, no existe un documento que conjunte toda esa información. Por lo anterior, disponer de un documento que facilite la identificación de las especies de Coccinellidae que depredan a *M. sacchari*, basada tanto en información morfológica como molecular, consiste en el primer paso en el desarrollo de un programa de control biológico; tal como DeBach (1968) plantea, “la adecuada identificación de una plaga y sus enemigos

naturales es fundamental, dado que sin un nombre científico no podrían ser adecuadamente examinadas referencias previas de las especies”.

Al igual que conocer a las especies de Coccinellidae que depredan a *M. sacchari* como base de un programa de control de plagas, es necesario conocer las preferencias y capacidad de depredación de los enemigos naturales así como realizar estudios de ecología poblacional de estos depredadores, con la intención de tener una aproximación al entendimiento de las variables que coinciden con la oscilación poblacional de los mismos, entendiendo como variables, los parámetros ambientales y las fluctuaciones poblacionales de sus presas.

OBJETIVOS Y PREDICCIÓN

Objetivo general

Analizar el potencial de las especies nativas de Coccinellidae para el control biológico de *M. sacchari.*

Objetivos particulares:

- Conocer las especies de Coccinellidae depredadoras de *M. sacchari* en México.
- Determinar la relación entre las poblaciones de coccinélidos nativos con las poblaciones de *M. sacchari* y las condiciones ambientales en un cultivo de sorgo.
- Determinar la pertinencia como agentes de control biológico, de las especies de Coccinellidae nativas en México y depredadoras de *M. sacchari* a través de investigaciones documentadas.

Predicción

- Algunas especies nativas de coccinélidos depredadores mostrarán potencial para ser consideradas como controladores biológicos de *M. sacchari*.

El potencial de los coccinélidos como depredadores de plagas se basa, en que presentan gran actividad de búsqueda ya sean larvas o adultos, ocupando todos los ambientes de sus presas, especialmente escamas, áfidos, mosquitas blancas, ácaros fitófagos, y otros artrópodos de cuerpo blando (Hodek 1973). Asimismo, la mayor parte de los depredadores afidófagos nativos están preadaptados a depredar áfidos aun exóticos como *M. sacchari* adoptándolos rápidamente en su dieta (Colares *et al.* 2015a), lo que explicaría por qué el control biológico natural de los áfidos evoluciona en el tiempo. Por tanto, algunas de las especies de coccinellidae nativas de México que coexistan con *M. sacchari*, podrian regular poblaciones de manera que puedan ser utilizadas como agentes de control biológico.

SISTEMA DE ESTUDIO

Sorgum bicolor (L.) Moench

El sorgo es una gramínea proveniente del noreste de África que llegó a América en el siglo XVIII por medio de los españoles (Pérez *et al*. 2010), comúnmente cultivado anualmente, sin embargo, en los trópicos puede cosecharse varias veces al año (FAO 1995). Actualmente los principales países productores son Estados Unidos, Nigeria, México e India (SAGARPA 2018).

Históricamente el sorgo ha sido clasificado de varias formas, de acuerdo a Snowden (1936), fue el romano Plinio el primer autor que hizo una descripción de la planta en "*Historiae Naturalis"*. Linnaeus en 1753 en su obra "*Species Plantarium*" describe dos especies de sorgo silvestre; *Holcus glumis glabris* L. y *Holcus glumis villosis* L., y tres especies de sorgo cultivado: *Holcus sorghum* L., *H. saccaratus* L., y *H. bicolor* L. Persoon, en 1805 creó el nombre *Sorghum vulgare* para *Holcus sorghum* y *Holcus dora* (House 1982). Los conceptos actuales sobre género y especies de *Sorghum,* están de acuerdo con las definiciones de Moench, y todos los nombres específicos descritos anteriormente se consideran sinónimos de *Sorgum bicolor* (L.) Moench (House 1982).

El sorgo es utilizado principalmente como materia prima para la industria de alimentos balanceados para animales, la cual, a su vez, permite que el mercado alimentario humano disponga de proteínas de origen animal (Pérez *et al*. 2010).

Melanaphis sacchari (Zehntner, 1897)

Melanaphis sacchari, originalmente fue descrito como *Aphis sacchari* en cultivos de caña de azúcar en Java, Indonesia, comúnmente conocido como pulgón amarillo o pulgón amarillo del sorgo "PAS", es un insecto cosmopolita y su distribución geográfica asociado a los cultivos de caña y sorgo en todo el mundo (Eastop 1955, 1965; Mead 1978).

Melanaphis sacchari es una especie exótica en México, cuya presencia se detectó en cultivos de sorgo del estado de Tamaulipas en otoño del año 2013 (INIFAP 2014; Rodríguez-del-Bosque & Terán 2015).

Melanaphis sacchari actualmente se extiende por 26 estados de nuestro país, que incluyen a Campeche, Chiapas, Chihuahua, Coahuila, Colima, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, Quintana Roo, San Luis Potosí, Sinaloa, Sonora, Tabasco, Tamaulipas, Veracruz y Yucatán (Peña-Martínez *et al*. 2015).

El período de desarrollo de *M. sacchari*, desde su nacimiento hasta llegar al estado adulto, es de alrededor de nueve días tanto en el sorgo como en caña de azúcar, pero el período reproductivo es más largo en el sorgo que en la caña de azúcar, con un mayor número de ninfas producidas. Las hembras producen un promedio de 17 y 26 ninfas en 11 y siete días, con una longevidad de 17 y 20 días en sorgo y caña de azúcar respectivamente, con reproducción exclusivamente partenogenética (Nibouche *et al.* 2014; Brown *et al.* 2015). Las ninfas y los adultos suelen ser ápteros, el desarrollo de adultos alados se puede estimular bajo condiciones estresantes, como el deterioro de la planta, exceso de individuos en la colonia o condiciones ambientales adversas (Brown *et al*. 2015).

La colonización del PAS suele ocurrir cuando las plantas tienen 2 o 3 semanas de edad, los aumentos notables en el número de áfidos se originan después del desarrollo de la panícula (van Rensburg 1973). El daño directo es la succión de savia en hojas tiernas, pedúnculos de flores y granos, ocasionando deformación y lesiones de color marrón, mientras que el daño indirecto es la transmisión de enfermedades virales (Mazzuferi *et al.* 2011), así como la melaza que es producida por el pulgón donde pueden crecer hongos saprófitos, los cuales generan la patología llamada “fumagina”, la cual afecta la capacidad fotosintética de la planta (SENASICA 2014). Las infestaciones de *M. sacchari* alcanzan su máximo crecimiento cuando existen condiciones entre 11.4 a 30 ˚C y de 43 a 94% de HR (Waghmare *et al*. 1995), en contraste las poblaciones empiezan a morir a partir de los 35 ˚C (Behura & Bohidar 1983).

Enemigos naturales

A pesar de los múltiples daños que origina *M. sacchari* en el sorgo, existen registros de varios enemigos naturales que se alimentan de él en diferentes países. Singh *et al*. 2004, mencionan a seis especies de insectos parasitoides, una especie de hongo entomopatógeno y 39 especies de insectos depredadores, observándose con mayor frecuencia a los sírfidos, crisópidos y coccinélidos los que probablemente contribuyen con la mayor mortalidad de *M. sacchari*.

Parasitoides de las familias Aphidiidae, Aphelinidae, Elasmidae y Braconidae se han registrado atacando a *M. sacchari*, pero en general, a menores niveles comparados con los insectos depredadores (Singh *et al*. 2004). Además, el PAS se puede beneficiar de una asociación simbiótica con la bacteria *Hamiltonella defensa* debido a que este endosimbionte protege a los áfidos del parasitismo, por tanto, esta asociación podría ser un impedimento para el control biológico con avispas parasitoides, principalmente bracónidos (Moran *et al*. 2005, Vorburger *et al*. 2009).

Coccinellidae (Coleoptera: Cucujoidea)

Las especies de la familia Coccinellidae presentan forma generalmente ovalada a redonda y convexa, a veces alargada y débilmente convexa. Antenas generalmente de 11 artejos, a menudo reducidas a 10, 9, 8 o 7 segmentos. Segmento apical de palpo maxilar triangular (securiforme), o de lados paralelos, o cónico. Élitros nunca truncados, ni estriados. Prosterno que separa claramente las coxas frontales. Mesepimeron cercano a la cavidad coxal media. Abdomen con 5 o 6 esternitos visibles, séptimo rara vez visible. Primer esternito con línea postcoxal (a excepción de los géneros *Naemia, Paranemia* y *Coleomegilla*). Tarsos cryptotetrameros, a menudo trimeros, rara vez verdaderamente tetrameros. Espuelas tibiales presentes o ausentes. Garra tarsal simple o dentada. Genitales masculinos con sifo esclerotizado, falobase trilobulada. Los tarsos cryptotetrámeros y la presencia de líneas postcoxales en el primer segmento abdominal generalmente permitirán reconocer un Coccinellidae de otras familias de coleópteros.

La forma de la línea postcoxal del primer segmento abdomial es un carácter determinante para discernir entre subfamilias, tribus y géneros; a grandes rasgos existen cuatro formas de líneas postcoxales: tipo *Pullus*, tipo *Scymnus*, tipo *Diomus y* tipo *Nephus*. Línea tipo *Pullus* es

completa, recurvada apicalmente, alcanzando la base del primer esternito abdominal; línea tipo *Scymnus* es incompleta con extremo apical recurvado, dirigido hacia la base del primer esternito, pero sin alcanzarlo. Tipo *Diomus*, línea incompleta, que se extiende hacia abajo desde la base, uniéndose al margen apical del esternito; y de tipo *Nephus*, línea incompleta, que se extiende desde la base casi hasta el margen apical del esternito, extendiéndose paralelamente al margen apical hacia el margen lateral (Gordon 1985).

La familia Coccinellidae está conformada aproximadamente por 6,000 especies, distribuidas en 42 tribus y 360 géneros (Nedvěd & Kovář 2012). Para los Estados Unidos de América se han registrado 57 géneros y 475 especies descritas (Gordon 1985). En México, la Colección Entomológica del Instituto de Biología de la Universidad Nacional Autónoma de México (UNAM) cuenta con un registro de 23 géneros y 49 especies que fueron colectadas en diversas regiones del país (Juárez 1986). El registro oficial más reciente de la diversidad de la familia Coccinellidae en México, en un catálogo que incluye 65 especies (CONABIO 2008). A la fecha, en México se han registrado 47 géneros y 169 especies de Coccinellidae, las cuales 134 son nativas y 35 introducidas (Anexo 1).

CAPÍTULO I

Identification of Twelve Coccinellidae (Coleoptera) Species Predatory on *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) in Mexico, and Submission of Reference COI Sequences

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Resumen

Un extenso estudio de coccinélidos depredadores de *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) se llevó a cabo en cultivos de sorgo en siete estados de México. Se recolectó un total de 495 coccinélidos que pertenecen a 12 especies, 10 géneros, seis tribus y tres subfamilias: *Brachiacantha decora* Casey, *Coccinella septempunctata* (L.), *Coleomegilla maculata lengi* Timberlake, *Cycloneda sanguinea sanguinea* (L.), *Diomus roseicollis* (Mulsant), *Diomus terminatus* (Say), *Exochomus childreni guexi* LeConte, *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, *Olla v-nigrum* (Mulsant), *Scymnus* (*Pullus*) *dozieri* Gordon, y *Scymnus* (*Pullus*) *loewii* Mulsant. *Exochomus childreni guexi* es reportado por primera vez como depredador of *M. sacchari*. Se incluye una breve diagnosis y fotografías de referencia de las doce especies, además del depósito de secuencias COI en BOLD y GenBank de nueve de las especies.

Palabras clave: áfidos, sorgo, control biológico, DNA Barcoding

Abstract

An extensive survey of coccinellid beetles predatory on *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) was conducted in sorghum crop areas within seven states in Mexico. In total 495 coccinellid specimens were collected, belonging to 12 species, 10 genera, six tribes, and three subfamilies: *Brachiacantha decora* Casey, *Coccinella septempunctata* (L.), *Coleomegilla maculata lengi* Timberlake, *Cycloneda sanguinea sanguinea* (L.), *Diomus roseicollis* (Mulsant), *Diomus terminatus* (Say), *Exochomus childreni guexi* LeConte, *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, *Olla v-nigrum* (Mulsant), *Scymnus* (*Pullus*) *dozieri* Gordon, and *Scymnus* (*Pullus*) *loewii* Mulsant. The subspecies *E. childreni guexi* is recorded for the first time as a predator of *M. sacchari*. A brief diagnosis and photographs are provided for all twelve species, additionaly COI sequences were submitted to BOLD and GenBank for nine species.

Key Words: aphid, sorghum, biological control, DNA Barcoding

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is a cosmopolitan insect whose geographical distribution follows sugarcane and sorghum crops worldwide (Eastop 1954, 1965). In Mexico, *M. sacchari* was first reported during 2013 (Rodríguez-del-Bosque and Terán 2015), causing losses of up to 100% in sorghum crops (Maya-Hernández and Rodríguez-del-Bosque 2014).

Several species of predators that consume *M. sacchari* have been reported around the world, particularly species of the ladybird beetle family Coccinellidae. In Mexico, 11 species of Coccinellidae were recorded in coexistence with *M. sacchari* in the state of Tamaulipas: *Brachiacantha decora* Casey, *Coccinella septempuctata* (L.), *Coleomegilla maculata* (Degeer), *Cycloneda sanguinea sanguinea* (L.), *Diomus roseicollis* (Mulsant), *Diomus terminatus* (Say), *Hippodamia convergens* Guérin-Méneville, *Hyperaspis wickhami* Casey, *Olla v-nigrum* (Mulsant), *Scymnus* (*Pullus*) *loewii* Mulsant, and *Scymnus* sp. Kugelann (Rodríguez-Vélez *et al*. 2016; Rodríguez-del-Bosque *et al.* 2018). In Nayarit, five species of Coccinellidae associated with *M. sacchari* have been reported (Rodríguez-Palomera *et al*. 2016). In the state of Durango, Vázquez-Navarro *et al.* (2016) carried out a study on predators associated with *M. sacchari*, in which six species of Coccinellidae were documented, among them was *Cycloneda munda* (Say), recorded for the first time in coexistence with *M. sacchari* in Mexico; however predatory activity of *C. munda* on *M. sacchari* was not confirmed. *Scymnus* (*Pullus*) *dozieri* Gordon has been recorded as a predator of *M. sacchari* in the state of Guanajuato (Marín-Jarillo 2017), while Salas-Marina *et al*. (2017) recorded *H. convergens* and *Cycloneda sanguinea* (L.), feeding on *M. sacchari* in Chiapas.

Most studies reporting natural enemies of *M. sacchari* have been limited to species lists, and generally lack diagnoses or illustrations that would assist in identification of the predators. Few investigations have implemented molecular tools to assist in identification of predators of *M. sacchari.* One exception is Salas-Marina *et al*. (2017), who identified the green lacewing *Chrysoperla externa* (Hagen) by comparison of cytochrome c oxidase I (COI) against the GenBank database. Unfortunately, the sequences they obtained were not deposited in GenBank or BOLD (Barcode of Life Data Systems), and no species of Coccinellidae were reported using molecular tools.

This work offers an annotated and illustrated list of coccinellid species that feed on *M. sacchari* in Mexico. Each species entry provides reference to taxonomic sources and a brief diagnosis. A general discussion on molecular identification is included for all species. Therefore, this study contributes to a better knowledge of predatory coccinellid species that may be potential biological control agents of *M. sacchari* in Mexico.

Material and Methods

Specimen collecting. Survey work for predatory coccinellids was carried out in sorghum crop areas of the states of Colima, Guanajuato, Michoacán, Morelos, Nuevo León, Sinaloa, and Tamaulipas (Appendix 1). Collecting methods included sweep net, aspirator, and hand picking of coccinellids that were seen feeding on *M. sacchari*, documenting a direct association of predation. Specimens were preserved in 70% ethanol, dried at room temperature, mounted on paper triangles, and labeled with locality data, date, type of sampling, and name of collector.

Specimen collection was opportunistic and most of the localities were sampled occasionally, with the exception of Tecuanillo, Colima, where a greater number of collection events were conducted. Since collection was not quantitative, abundance or diversity at and across sites cannot be determined.

Morphological identification. Coccinellids were determined based on specialized literature: Chapin and Brou (1991), Gordon (1976, 1985, 2000), Lablokoff-Khnzorian (1982), Romero-Nápoles (1988), and Vandenberg (1990, 1992). Genitalia morphology is occasionally necessary to determine differences between species (Gordon 1974); dissections were performed following González (2006).

Molecular identification. In total, 17 specimens of ladybird beetles were used for molecular identification (Table 1). DNA extraction was carried out with a DNeasy® Blood & Tissue Kit (Qiagen, Valencia, USA). First, the whole insect for samples CIE-COL-001 to CIE-COL-003 (replacement vouchers from the same locality were retained) or abdominal tissue for samples CIE-COL-004 to CIE-COL-013 and CIE-COL-022 to CIE-COL-025, were placed in a 2 ml Eppendorf tube with a 3 mm diameter tungsten-carbide bead (Qiagen, Valencia, USA) for overnight incubation at -80 °C, then they were homogenized using TissueLyser II™ (Qiagen,

Valencia, USA) for 3 min at 20 Hz. Further steps of DNA extraction were performed according to manufacturer's instructions.

The cytochrome c oxidase I (COI) region of mitochondrial DNA was amplified by PCR using universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Amplification reactions were performed in the C1000 Touch™ thermal cycler (BioRad Laboratories Inc., Hercules, USA) in 25 µl reaction volumes. Each reaction contained 1X Phire Reaction Buffer (1.5 mM of $MgCl_2$ at final reaction concentration), 0.2 mM of each dNTP (Promega, Madison, USA), 0.4 µM of each primer, 0.4 µL of Phire Hot Start II DNA Polymerase (Thermo Scientific Inc., Pittsburgh, USA), and 2 µl of DNA template. Cycling conditions were: initial denaturation at 98°C for 5 min; 40 cycles of denaturation at 98°C for 5 s, annealing at 51°C for 5 s, and extension at 72°C for 20 s; and a final extension at 72°C for 5 min. PCR products were visualized using agarose gel (1.5%) in 1X TBE stained with 3X GelRed™ (Biotium Inc., USA), and prepared for sequencing by C

Purified products were sequenced in both directions at Macrogen Inc. (Seoul, South Korea). The chromatograms were assembled and edited using BioEdit sequencer software (Hall 1999). Sequences were compared in the BOLD platform (Ratnasingham and Hebert 2007) (www.boldsystems.org) and GenBank public collection of the National Center for Biotechnology Information (NCBI) to test morphological identification. Collection data, voucher information, taxonomy, sequences, electropherograms, and primer details for each specimen are available in BOLD, and all sequences have been deposited in GenBank (Table 1).

Illustrations. Reference images were captured with an AxionCam HRc camera connected to a Carl Zeiss Discovery V20 stereoscope. Images are product of the splicing of several layers integrated at the end on an image with depth of field. Splicing was performed with the Combine ZP software (Hadley 2010), and then images were edited with Photoshop, Adobe 2012.

Voucher specimens. Specimens were deposited at the facilities of Colección de Insectos Entomófagos del Centro Nacional de Referencia de Control Biológico (CIE-CNRCB), located in Tecomán, Colima, Mexico.

Results and Discussion

The present work includes a list of coccinellids predatory on *M. sacchari* collected on sorghum in the states of Colima, Guanajuato, Michoacán, Morelos, Nuevo León, Sinaloa, and Tamaulipas, Mexico; in total, 495 specimens were collected, representing 12 species within 10 genera, six tribes, and three subfamilies (Appendix 1).

Species diagnoses. The 12 species are highlighted below, unless stated, diagnoses are based on keys in Gordon (1985).

Brachiacantha decora Casey (Fig. 1A). *Brachiacantha decora* is similar to *Brachiacantha dentipes* (F.) and *Brachiacantha blaisdelli* Nunenmacher by coloration; however, the rounded to oval body form is useful in distinguishing *B. decora* from *B. dentipes* and *B. blaisdelli*, which are oblong. *Brachiacatha decora* was collected preying on *M. sacchari* in the states of Morelos and Tamaulipas (Appendix 1), in the latter state the species was previously recorded feeding on *M. sacchari* (Rodríguez-Vélez *et al.* 2016; Rodríguez-del-Bosque *et al.* 2018).

Coccinella septempunctata (L). (Fig. 1B). The species is similar to *Coccinella difficilis* Crotch, 1874, since both species have seven spots on the elytra, however *C. septempunctata* presents white triangular spots close to the anterolateral angle on the pronotum; *C. septempunctata* is 6.5–7.8 mm in length, while *C. difficilis* is 5.0–6.0 mm (Gordon 1985); and the two species do not occur in the same geographical region. *Coccinella septempunctata* was collected preying on *M. sacchari* in Nuevo León and Tamaulipas (Appendix 1), in the latter state the species was previously recorded feeding on *M. sacchari* (Rodríguez-Vélez *et al*. 2016; Rodríguez-del-Bosque *et al*. 2018).

Coleomegilla maculata lengi Timberlake (Fig. 1C). The subspecies is similar to *Coleomegilla maculata fuscilabris* (Mulsant) and *Coleomegilla maculata strenua* (Casey) in shape and coloration. *Coleomegilla m. lengi* may be distinguished from *Coleomegilla m. fuscilabris* by: spots on the pronotum that are large and triangular, the median elytral spot is large and oval, and the spot on the apical declivity is touching sutural margin; while *C. m. fuscilabris* has pronotal spots that are small, oval or curvilinear, the median elytral spot is

reduced or divided into a small lateral spot and a large median spot, and the spot at the apical declivity is not touching sutural margin (Gordon 1985).

Coleomegilla m. lengi and *C. m. strenua* are similar in coloration and shape of the spots; to distinguish them it is necessary to check characters of male genitalia; *C. m. lengi* presents the basal lobe of male genitalia with apex feebly notched, while *C. m. strenua* has the basal lobe of male genitalia with apex distinctly notched (Gordon 1985). *Coleomegilla m. lengi* was collected preying on *M. sacchari* in Colima, Sinaloa, and Tamaulipas (Appendix 1). There are records of *Coleomegilla maculata* (Degeer) without subspecies identification for the states of Durango (Vázquez-Navarro *et al.* 2016), Nayarit (Rodríguez-Palomera *et al.* 2016), and Tamaulipas (Rodríguez-Vélez *et al.* 2016; Rodríguez-del-Bosque *et al.* 2018).

Cycloneda sanguinea sanguinea (L.) (Fig. 1D). The pronotal color pattern will distinguish *C. s. sanguinea* from the other North American species in almost all instances (Gordon 1985). It is similar to *Cycloneda polita* Casey and *Cycloneda munda* (Say) by the shape of their body and elytron color. However, these species can be differentiated in most cases by the color pattern of the pronotum, which in *C. s. sanguinea* presents a pale lateral spot enclosed by black coloration, while in *C. polita* and *C. munda* it presents a pale lateral spot not entirely enclosed, or with isolated lateral black spot (Gordon 1985). *Cycloneda s. sanguinea* shares many characters with *Cycloneda sanguinea limbifer* Casey. The subspecies can be differentiated by the coloration of the edge of the elytra, which in *C. s. sanguinea* is slightly paler than the rest of the elytron, whereas in *C. s. limbifer* it is black. The most compelling character to differentiate *C. s. sanguinea* from similar subspecies is the male genitalia (Gordon 1985). *Cycloneda s. sanguinea* was collected preying on *M. sacchari* in Colima, Michoacán, Morelos, Nuevo León, Sinaloa, and Tamaulipas (Appendix 1). There are records of *C. sanguinea* without subspecies identification preying on *M. sacchari* for the states of Chiapas (Salas-Marina *et al*. 2017), Guanajuato (Marín-Jarillo 2017), Nayarit (Rodríguez-Palomera *et al.* 2016), and Tamaulipas (Rodríguez-Vélez *et al.* 2016; Rodríguez-del-Bosque *et al.* 2018).

Diomus roseicollis (Mulsant) (Fig. 1E). *Diomus roseicollis* resembles *Diomus dichorus* Mulsant and *Diomus bigemmeus* (Horn); however, the latter two do not have the distinct posterior spot on the elytron (Gordon 1976). *Diomus roseicollis* was collected preying on *M.*

sacchari in Colima and Tamaulipas (Appendix 1); it also has been recorded in Nayarit by Rodríguez-Palomera *et al.* (2016) and Tamaulipas (Rodríguez-Vélez *et al.* 2016; Rodríguez-del-Bosque *et al.* 2018).

Diomus terminatus (Say) (Fig. 1F). *Diomus terminatus* shares similar external characteristics with *Diomus xanthaspis* (Mulsant); however, a yellow pronotum; male genitalia with basal lobe strongly asymmetrical, much shorter than paramere; and the siphonal apex with small spherical protrusions are traits that clearly distinguish *D. terminatus* from other species of *Diomus* (Gordon 1976). *Diomus terminatus* was collected preying on *M. sacchari* in Tamaulipas (Appendix 1) where it had been previously recorded (Rodríguez-Vélez *et al.* 2016; Rodríguez-del-Bosque *et al.* 2018).

Exochomus childreni guexi LeConte (Fig. 1G). *Exochomus childreni guexi* bears a striking resemblance to the color and form of *Exochomus childreni childreni* Mulsant; however, *E. c. childreni* is not sexually dimorphic, whereas *E. c. guexi* is. *Exochomus marginipenis* (LeConte) shows the same color pattern and sexual dimorphism as *E. childreni guexi*, and there are no notable differences in the genitalia structures (Gordon 1985). However, the species can be separated by the almost round shape of *E. c. guexi* that differs from the oval shape of the body of *E. marginipennis* (Gordon 1985). *Exochomus childreni guexi* was collected preying on *M. sacchari* in Colima and Morelos (Appendix 1). The subspecies *E. c. guexi* is recorded for the first time as a predator of *M. sacchari*. There are records of *E. childreni* without subspecies identification in Texas (Villanueva and Sekula 2014); also *Exochomus concavus* (Furch) has been recorded preying on *M. sacchari* in South Africa (van Rensburg 1973).

Harmonia axyridis (Pallas) (Fig. 1H). A diagnosis of this species is presented by Chapin and Brou (1991). *Harmonia axyridis* can be confused with *Harmonia dimidiata* (F.) and *Harmonia quadripunctata* (Pontopiddian). Also, they may share the same geographical distribution. *Harmonia axyridis* can be distinguished from *H. quadripunctata*: the latter is more elongate in shape with 11 punctiform black spots on the pronotum with one or two pairs sometimes faint or absent; the elytron is immaculate or has a pair of elongate black marks at the lateral margin on either side of the mid-line (Vandenberg 1990); and the prosternal carinae, present in the other two species, are absent (Lablokoff-Khnzorian 1982). *Harmonia dimidiata* is

round and very convex with consistent markings composed of a bilobed black spot at the base of the pronotum and seven black spots arranged in a 1,3,2,1 pattern on each elytron (Chapin and Brou 1991). However, *H. axyridis* can present more than 100 color patterns (Korschefsky 1932), therefore genitalia or molecular identification should be used confirm the identity. *Harmonia axyridis* was collected preying on *M. sacchari* in the states of Michoacán and Morelos (Appendix 1); it had been previously recorded for Durango (Vázquez-Navarro *et al.* 2016), Guanajuato (Marín-Jarillo 2017), Nayarit (Rodríguez-Palomera *et al.* 2016), and Tamaulipas (Rodríguez-del-Bosque *et al.* 2018).

Hippodamia convergens Guérin-Méneville (Fig. 1I). *Hippodamia convergens* is similar to *Hippodamia koebelei* Timberlake; these species can be differentiated either by genitalia, or by the anterior tarsi, which are more robust in *H. koebelei* (Romero-Nápoles 1988). *Hippodamia convergens* preying on *M. sacchari* were collected in Colima, Guanajuato, Michoacán, Nuevo León, and Tamaulipas (Appendix 1). In the latter state it had been previously recorded (Rodríguez-Vélez *et al*. 2016; Rodríguez-del-Bosque *et al*. 2018), also it had been recorded for Chiapas (Salas-Marina *et al.* 2017), Guanajuato (Marín-Jarillo 2017), Durango (Vázquez-Navarro *et al*. 2016), and Nayarit (Rodríguez-Palomera *et al*. 2016).

Olla v-nigrum (Mulsant) (Fig. 1J). Diagnoses of this species are provided by Gordon (1985) and Vandenberg (1992). *Olla v-nigrum* is very similar to *Olla roatanensis* Vandenberg, *Olla hageni* Vandenberg, and *Olla timberlakei* Vandenberg. *Olla roatanensis* is differentiated by the male genitalia, where the basal lobe is short and wide; while in *O. v-nigrum*, it is long and slender; in addition, this species is only known in the islands of the Bay of Honduras. *Olla hageni* differs from *O. v-nigrum* also by the male genitalia; the basal lobe is elongated and narrow, and has a slightly longer abdomen than *O. v-nigrum*. *Olla timberlakei* differs from *O. v-nigrum* by the siphonal apex of male genitalia terminating in a corkscrew-shaped projection, distinctly longer than the subapical projection, while in *O. v-nigrum* the siphonal apex of male genitalia ends in a weakly sinuous projection subequal in length to the subapical projection (Vandenberg 1992). *Olla v-nigrum* was collected preying on *M. sacchari* in Colima, Nuevo León, Sinaloa, and Tamaulipas (Appendix 1). It has also been collected preying on *M. sacchari* in Durango (Vázquez- Navarro *et al*. 2016) and Guanajuato (Marín-Jarillo 2017).

Scymnus (*Pullus*) *dozieri* Gordon (Fig. 1K). The species was described by Gordon (2000). *Scymnus* (*Pullus*) *dozieri* shares many morphological and coloring characteristics with other species of the same genus, especially with *Scymnus* (*Pullus*) *simillimus* Gordon and *Scymnus* (*Pullus*) *calabozoensis* Gordon, all of which have the same broad-shouldered body tapered posteriorly (Gordon 2000). Positive identification can only be achieved by comparison of male (Gordon 2000). *Scymnus* (*Pullus*) *dozieri* was collected preying on *M. sacchari* in Colima (Appendix 1); it had been previously mentioned as predator of *M. sacchari* for the state of Guanajuato (Marín- Jarillo 2017).

Scymnus (*Pullus*) *loewii* Mulsant (Fig. 1L). A comprehensive description of this species is provided by Gordon (2000). *Scymnus* (*Pullus*) *loewii* differs from the other species of *Scymnus* by the coloration of the dorsal surface, with a dark middle V-shaped spot, which appears in the elytra and part of the pronotum, however specimens with reduced coloration will require examination of genitalia for positive identification (Gordon 2000). *Scymnus* (*Pullus*) *loewii* was collected preying on *M. sacchari* in Colima and Tamaulipas (Appendix 1). It was previously recorded from Tamaulipas (Rodríguez-Vélez *et al*. 2016; Rodríguez-del-Bosque *et al*. 2018), and Guanajuato (Marín-Jarillo 2017).

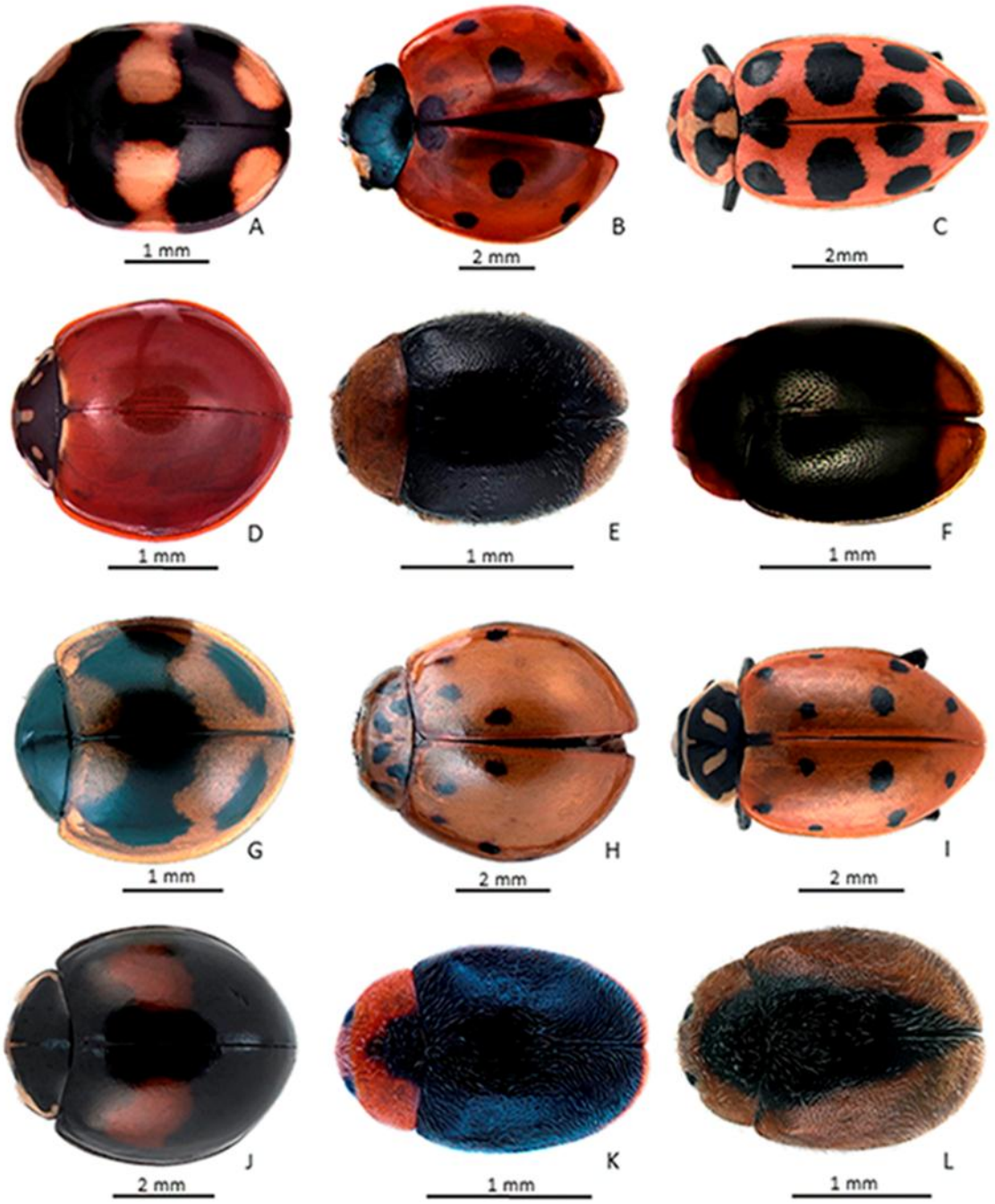

Fig. 1. Coccinellid predators of *Melanaphis sacchari* in Mexico. A) *Brachiacantha decora*, B) *Coccinella septempunctata*, C) *Coleomegilla maculata lengi*, D) *Cycloneda sanguinea sanguinea*, E) *Diomus roseicollis*, F) *Diomus terminatus*, G) *Exochomus childreni guexi*, H) *Harmonia axyridis*, I) *Hippodamia convergens*, J) *Olla v-nigrum*, K) *Scymnus* (*Pullus*) *dozieri*, and L) *Scymnus* (*Pullus*) *loewii*.

Molecular identification. Barcode sequences were obtained from 13 of the 17 samples (Table 1) (76%) and all met the quality criteria [i.e., more than 500 bp and less than 1% uncertain base calls (Ns)] required to allow their identification and incorporation in the BOLD system. Analyzed fragment lengths of samples CIE-COL-001 to CIE-COL-013 ranged from a minimum of 597 bp to the full fragment size of 658 bp, whereas PCR amplifications failed for samples CIE-COL-022 to CIE-COL-025.

The BOLD v4 database (as of 4th May 2018) was used to determine the availability of DNA barcodes (i.e., species level) for all samples. The 1% divergence threshold criterion was applied (Ratnasingham and Hebert 2007). For the 13 generated COI barcodes, seven matched to reference sequences in BOLD and confirmed the morphological identification of five species. Those samples included *C. maculata lengi* (CIE-COL-007), *H. axyridis* (CIE-COL-010), *H. convergens* (CIE-COL-001 and CIE-COL-006), *O. v-nigrum* (CIE-COL-005), and *S.* (*P.*) *loewii* (CIE-COL-003 and CIE-COL-004) (Table 1).

The other six samples were identified only at genus or family level because (1) no reference sequence existed in BOLD within 1% divergence of the query sample and/or (2) the reference sequence that matched with our query was only identified to family. Identifications were confirmed by BLAST searches in GenBank. Therefore, morphological identification confirmed that the six inconclusive sequences represented novel DNA barcodes from four species not previously represented in BOLD, i.e., *C. sanguinea sanguinea* (CIE-COL-002 and CIE-COL-11), *D. roseicollis* (CIE-COL-009), *E. childreni guexi* (CIE-COL-008), and *S.* (*P.*) *dozieri* (CIE-COL-012 and CIE-COL-013) (Table 1).

Table 1. Samples used for the DNA barcoding. NA= Not available

Acronym	Species	State	Region	Locality	BOLD	GenBank
CIE-COL-001	*Hippodamia convergens*	Colima	Tecomán	Tecuanillo	AGAAB001-18	MH308199
CIE-COL-002	*Cycloneda sanguinea*	Tamaulipas	Ciudad Mante	El Limón	AGAAB002-18	MH308194
CIE-COL-	*Sanguinea Scymnus* (*Pullus*)	Tamaulipas	Altamira	Villa	AGAAB003-	MH308205

003	*loewii*			Cuauhtémoc	18	
CIE-COL-004	*S.* (*P*) *loewii*	Colima	Tecomán	Tecuanillo	AGAAB004-18	MH308204
CIE-COL-005	*Olla v-nigrum*	Colima	Tecomán	Tecuanillo	AGAAB005-18	MH308201
CIE-COL-006	*H. convergens*	Colima	Tecomán	Tecuanillo	AGAAB006-18	MH308200
CIE-COL-007	*Coleomegilla maculata*	Colima	Tecomán	Tecuanillo	AGAAB007-18	MH308193
CIE-COL-008	*Lengi Exochomus childreni*	Colima	Tecomán	Tecuanillo	AGAAB008-18	MH308197
CIE-COL-009	*Guexi Diomus roseicollis*	Colima	Tecomán	Tecuanillo	AGAAB009-18	MH308196
CIE-COL-010	*Harmonia axyridis*	Morelos	Ayala	Xalostoc	AGAAB010-18	MH308198
CIE-COL-011	*C. sanguinea sanguinea*	Colima	Tecomán	Tecuanillo	AGAAB011-18	MH308195
CIE-COL-012	*Scymnus* (*Pullus*) *dozieri*	Colima	Tecomán	Tecuanillo	AGAAB012-18	MH308202
CIE-COL-013	*S.* (*P*) *dozieri*	Colima	Tecomán	Tecuanillo	AGAAB013-18	MH308203
CIE-COL-022	*Brachiacantha decora*	Tamaulipas	Altamira	Villa Cuauhtémoc	NA	NA
CIE-COL-023	*Coccinella septempunctata*	Tamaulipas	Altamira	Villa Cuauhtémoc	NA	NA
CIE-COL-024	*Diomus terminatus*	Tamaulipas	Ciudad Mante	El Limón	NA	NA
CIE-COL-025	*D. terminatus*	Tamaulipas	Altamira	Buenos Aires	NA	NA

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Appendix 1. Species of coccinellids predatory on *Melanaphis sacchari* collected on sorghum.

Species	State	Municipality	Locality	Georeferences	No. of specimens	Collecting date
Brachiacantha decora	Morelos	Tlaquiltenango	Campo Palapa	18° 33' N, 99° 07' W	7	17/ix/2016
	Tamaulipas	Altamira	Villa Cuauhtémoc	22° 31' N, 98° 09' W	2	31/iii/2014
			Cervantes	22° 26' N, 98° 03' W	1	31/iii/2014
Coccinella septempunctata	Nuevo León	Marín	Universidad Autónoma de Nuevo León	25° 52' N, 100° 02' W	1	27/x/2017
	Tamaulipas	Altamira	Villa Cuauhtémoc	22° 31' N, 98° 09' W	1	31/iii/2014
Coleomegilla maculata lengi	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 5' W	7	22/iv/2016
	Sinaloa	Culiacán		24° 43' N, 107° 27' W	3	12/x/2014
	Tamaulipas	Ciudad Mante	El Limón	22° 51' N, 99° 05' W	2	01/iv/2014
Cycloneda sanguinea sanguinea	Colima	Tecomán	CNRCB	18° 55' N, 103° 53' W	3	09/v/2014
			Tecuanillo	18° 51' N, 103° 54' W	78	04/iii/2014
		Coquimatlán	Pueblo Juárez	19° 09' N, 103° 52' W	1	01/ix/2016
	Michoacán de Ocampo	Álvaro Obregón		19° 49' N, 101° 03' W	1	27/vii/2017

	Morelos	Tepalcingo		18° 37' N, 98° 53' W	6	17/ix/2016
		Xalostoc	Ayala	18° 49' N, 98° 54' W	7	14/ix/2016
	Nuevo León	Marín	Universidad Autónoma de Nuevo León	25° 52' N, 100° 02' W	7	27/x/2017
	Sinaloa	Culiacán		24° 43' N, 107° 27' W	2	12/vii/2014
	Tamaulipas	Ciudad Mante	El Limón	22° 51' N, 99° 05' W	10	01/iv/2014
		Altamira	Cervantes	22° 26' N, 98° 02' W	1	31/i/2014
		Altamira	Buenos Aires	22° 26' N, 98° 01' W	2	31/iii/2014
		Altamira	Villa Cuauhtémoc	22° 31' N, 98° 09' W	4	31/iii/2014
Diomus roseicollis	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	103	11/v/2016
	Tamaulipas	Altamira	Buenos Aires	22° 26' N, 98° 01' W	3	31/iii/2014
Diomus terminatus	Tamaulipas	Altamira	Cervantes	22° 26' N, 98° 02' W	7	31/iii/2014
		Ciudad Mante	El Limón	22° 51' N, 99° 05' W	7	01/iv/2014
		Altamira	Buenos Aires	22° 26' N, 98° 01' W	2	31/iii/2014
Exochomus childreni guexi	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	4	01/vi/2016
Harmonia axyridis	Morelos	Tlaquiltenango	Campo Palapa	18° 33' N, 99° 07' W	6	17/ix/2016
	Michoacán de Ocampo	Álvaro Obregón		19° 49' N, 101° 03' W	2	28/vii/2017
	Morelos	Xalostoc	Ayala	18° 49' N, 98° 54' W	6	01/viii/2016
		Tepalcingo		18° 37' N, 98° 53' W	2	19/xii/2016
	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	8	01/vi/2016
Hippodamia convergens		Coquimatlán	Pueblo Juárez	19° 09' N, 103° 52' W	4	01/ix/2016
	Guanajuato	Santa Cruz de Juventino Rosas	Galera de la Esperanza	20.62194' N, 101.01729' W	33	23/v/2018
		Santa Cruz de Juventino Rosas	Santa María de Guadalupe	20.61042' N, 101.3281' W	20	23/v/2018
	Michoacán de Ocampo	Álvaro Obregón		19° 49' N, 101° 03' W	2	27/vii/2027

	Nuevo León	Marín	Facultad de Agronomía. Universidad Autónoma de Nuevo León	25° 52' N, 100° 02' W	11	27/x/2017
		Altamira	Villa Cuauhtémoc.	22° 31' N, 98° 09' W	4	31/iii/2014
	Tamaulipas	Ciudad Mante	El Limón	22° 51' N, 99° 05' W	7	01/iv/2014
		Ciudad Mante	El Limón	22° 50' N, 99° 06' W	9	31/iii/2014
		Altamira	Buenos Aires	22° 26' N, 98° 01' W	1	31/iv/2014
	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	1	01/vi/2016
		Tecomán	CNRCB	18° 55' N, 103° 53' W	1	09/v/2014
Olla v-nigrum	Nuevo León	Marín	Universidad Autónoma de Nuevo León	25° 52' N, 100° 02' W	2	27/x/2017
	Sinaloa	Culiacán		24° 43' N, 107° 27' W	1	12/x/2014
	Tamaulipas	Ciudad Mante	El Limón	22° 51' N, 99° 05' W	1	31/iii/2014
Scymnus* (*Pullus*) *dozieri	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	95	01/vi/2016
Scymnus* (*Pullus*) *loewii	Colima	Tecomán	Tecuanillo	18° 51' N, 103° 52' W	3	08/vi/2016
	Tamaulipas	Altamira	Villa Cuahutémoc	22° 31' N, 98° 09' W	4	01/iv/2014

CAPÍTULO II

Relationships among aphids (Hemiptera: Aphididae), their predator insect communities and climatic conditions in sorghum crops in Colima, Mexico

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Resumen

1. Se realizó un análisis de componentes principales (PCA por sus siglas en ingles) de las comunidades de insectos depredadores de (Coccinellidae, Syrphidae y Chrysopidae), de los insectos suctores (Aphididae) y de las condiciones ambientales en cultivos de sorgo en el municipio de Tecomán, Colima, México.
2. Se colectó durante tres periodos de muestreos correspondientes a los años 2016, 2017 y 2018 respectivamente. Se colectaron tres especies de afidos: *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch), y *Melanaphis sacchari* (Zehntner), siendo esta última especie la más abúndate. Por su parte se colectaron 12 especies de Coccinellidae depredadores de afidos, dos especies de Chrysopidae y 13 de Syrphidae.
3. Un abrupto descenso en las poblaciones de *M. sacchari* a través de los años fue evidente.
4. El PCA reveló mayor relación entre las comunidades de especies depredadores y *M. sacchari* que, con las condiciones climáticas, las cuales no tuvieron una influencia significativa.
5. Las especies *Hippodamia convergens* Guérin-Méneville, *Scymnus (Pullus) dozieri* Gordon, *Cycloneda sanguinea sanguinea* L., y *M. sacchari* presentaron una relación significativa entre sí.

Abstract

1. A principal component analysis (PCA) was conducted on the communities of predators (Coccinellidae, Syrphidae and Chrysopidae families), sucking insects (Aphididae family) and environmental conditions in the sorghum crop of the municipality of Tecomán, Colima, Mexico.
2. Sampling was undertaken in three periods, 2016, 2017 and 2018. In total, three aphid species were collected *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch), and *Melanaphis sacchari* (Zehntner) with the third found to be the most abundant. Twelve species of aphid predators were identified from the Coccinellidae family, two from the Chrysopidae family and 13 from the Syrphidae family.
3. The abrupt decrease of the abundance of *M. sacchari* over the years was evident.

4. The PCA revealed a stronger relationship between the predator species and *M. sacchari* than that found with the climatic conditions, which do not seem to have a significant influence on the predator populations.

5. A significant relationship was observed between *Hippodamia convergens* Guérin-Méneville, *Scymnus (Pullus) dozieri* Gordon, *Cycloneda sanguinea sanguinea* L., and *M. sacchari* in the graphical representation of the PCA.

Introduction

Since the arrival of the sugarcane aphid *Melanaphis sacchari* (Zehntner) to Mexico in 2013 (INIFAP, 2015; Rodríguez-del-Bosque & Terán, 2015), various studies have been undertaken with the objective of contributing information and tools for controlling the species. However, few publications have dealt with the relationship among the predator insect populations, *M. sacchari* (or other aphid species) populations and the climatic variables in sorghum crops. In this regard, Vázquez-Navarro *et al*. (2016) undertook a population study on a fodder sorghum crop infested with this pest, identifying the coccinellid *Hippodamia convergens* Guérin-Méneville as the dominant predator species. In the United States of America, Colares *et al*. (2015a) evaluated the capacity for using native predators against both the invasive species *M. sacchari* and *Schizaphis graminum* (Rondani), an established insect pest species. They conclude that the majority of native aphidophagous predators in cereal crops are pre-adapted to respond and feed on *M. sacchari* in the same way as on *S. graminum*, for that reason, they suggest that the capacity of native aphidophagous guilds to control invasive aphid species may often be underestimated. This would explain why the effective biological control of aphids typically evolves over time, even in circumstances where classical programs are unable to achieve the same level of control.

Melanaphis sacchari is capable of rapid population growth in sorghum (Colares *et al*., 2015b). For example, an initial population of 50 individuals can grow to more than 500 in a week, while unmanaged populations can reach densities of more than 10,000 aphids per leaf (Bowling *et al*., 2016). A severe infestation is devastating and substantially reduces sorghum yield and grain quality, causing chlorosis in the leaves, necrosis and even plant death (Bayoumy *et al*., 2015; Bowling *et al*., 2016). Moreover, fields untreated with insecticides can undergo

100% losses when infested during the early development of the plant. While insecticides are currently, the only effective measure for suppressing aphid populations in sorghum, the application of insecticides is costly (Elliott *et al*., 2017) and it has severe environmental implications.

Given the foregoing, there is an urgent need to explore and apply additional non-chemical management strategies, which would reduce both management and cost challenges while limiting resistance to insecticides. The incorporation of natural enemies into integrated pest management (IPM) has the potential to reduce both the costs of suppressing aphid populations and the use of insecticides (Zhang & Swinton, 2012). The effect of biological control in *M. sacchari* populations in sorghum fields has not been quantified and may have been underestimated (Colares *et al*., 2015b). Singh *et al*. (2004) report 47 species of *M. sacchari* pathogens, parasitoids and predators in different countries. Among these natural enemies, the coccinellids, chrysopids, and syrphids are observed with the greatest frequency, which probably contributes to the higher mortality of *M. sacchari* (Singh *et al*., 2004).

The objective of the present study was to analyse the combination of factors related to the presence of aphid predator species from the Coccinellidae, Chrysopidae and Syrphidae families in sorghum crops, in order to move closer to an understanding of the structure and operation of the community of predators surrounding aphids. Moreover, it sought to lay the foundations for research into the probable implementation of biological control programs using these predator species.

Methods

Study areas

The present study was developed in two locations found in the municipality of Tecomán, in the state of Colima, Mexico. No type of insecticide was applied in order not to interfere with the presence of the insects. The locations are described below:

Location 1. The town of Tecuanillo, found at 18°51'31" N, 103°52'55" W, with an elevation of 17 m a.s.l. It covers an area of four hectares cultivated with *Sorghum bicolor* (L.)

Moench (SG PICHON 70 forage variety) manually sown at a depth of 1.5 cm, with a 20 cm space between plants and 90 cm between furrows.

Location 2. The Faculty of Biological and Agricultural Sciences' Experimental Field, at the University of Colima, Tecomán campus, located at the 40 km of the Colima-Manzanillo highway, at 18° 57' N, 103° 53' W and an elevation of 50 m a.s.l. The experimental area covers 3,052.12 m² of an *S. bicolor* forage variety SG PICHON 70 crop manually sown at a depth of 1.5 cm, with a space of 20 cm between plants and 90 cm between furrows.

Collections

Three sampling periods were undertaken, each corresponding to a complete sorghum crop cycle. In each period, weekly collections were undertaken with a sweep net, covering an approximate distance of 180 linear meters per collection.

The first sampling period was applied at Location 1 from 18th February 2016 to 6th July the same year, comprising 21 collections. The second period was applied at Location 2, from 28th September 2017 to 4th January 2018, comprising 15 collections. The third period was applied at Location 2, from 15th March to 13th July 2018, comprising 17 collections.

Preservation and morphological identification

Collected insects were sacrificed via immersion in 70% alcohol, after which the Coccinellidae, Syrphidae and Chrysopidae individuals were mounted on entomological pins and labeled with the location, collection date and the name of the collector. The Aphididae examples were stored in 70% alcohol in 10 ml glass containers labeled with the corresponding data.

The identification of the coccinellids was undertaken based on the specialized literature produced by Gordon (1985; 2000), Romero-Nápoles (1988), and Vandenberg (1992). The Chrysopidae specimens were identified based on both Brooks (1994) and De Freitas *et al*. (2009), while the Syrphidae species were identified using the keys described by Miranda *et al*. (2013). The Aphididae species were identified based on Remaudière & Remaudière (1997) and Peña-Martínez *et al*. (2016). All specimens were deposited as backup in the *Colección de*

Insectos Entomófagos del Centro Nacional de Referencia de Control Biológico (CIE-CNRCB, or the Entomophagous Insect Collection of the National Reference Center for Biological Control) of the Directorate General for Vegetable Health at the *Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria* (SENASICA, or National Service for Agrifood Health, Safety and Quality), located in Tecomán, Colima, Mexico.

Statistical analysis

With the objective of ascertaining the relationships between the most abundant predator species and the most abundant aphid species, a correlation analysis was undertaken, while a PCA was undertaken in order to elucidate the relationship among the climatological variables, predator populations, and aphid populations. The above described analysis was conducted using the PC-ORD 5.31 program (McCune & Mefford, 2006).

Two matrices were constructed for the PCA, the first of which being environmental, containing the aphid populations (sampled weekly) and the weekly average for the climatological conditions obtained from the *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias* (INIFAP, or the National Institute for Forestry, Agricultural and Livestock Research) (2016), while the second was sociological, containing the predator populations (sampled weekly). Both matrices comprised 51 samples applied to the 15 environmental and 27 sociological variables (Annex 1).

Results

A total of 30,526 specimens of the Aphididae family were collected, three species were identified, but *M. sacchari* was the most abundant (98% of individuals). In terms of the predator insects, 2,088 specimens were collected, of which 1,342 adults corresponded to Coccinellidae with 12 species, while 622 individuals belonging to Chrysopidae with two species, and 124 Syrphidae specimens were distributed across 13 species (Annex 2).

During the first sampling period, 26 predator insect species were identified from the 1,573 specimens collected, while 11 species were identified from the 212 specimens collected in the second period, and 11 species were identified from the 303 specimens collected in the third

period. The increased abundance observed in the third period, as compared to the second, is mainly due to the increase in the *Chrysoperla externa* (Hagen) species number of individuals. With regard to the aphids, in Period 1, 27 254 *M. sacchari* and 650 *S. graminum* individuals were collected, while, in Period 2, 2 122 specimens were collected, all of which corresponded to the species *M. sacchari*. In Period 3, 198 *M. sacchari* and 102 *R. maidis* specimens were collected (Annex 2).

The correlation coefficient analysis showed a significant relationship between *M. sacchari* and the predators, of which *Cycloneda sanguinea sanguinea* L., *H. convergens*, *Coleomegilla maculata lengi* Timberlake and *Sycmnus* (*Pullus*) *dozieri* Gordon (Table 1) are highlighted. Similarly, the predator species correlate to each other, with the strong relationships between the following highlighted: *C. sanguínea sanguinea* and *H. convergens*; *S.* (*P.*) *dozieri*, *Ceraeochrysa valida* (Banks), and *Allograpta exotica* (Wiedemann); in similar way, *H. convergens* and *S.* (*P.*) *dozieri*; *Diomus roseicollis* (Mulsant), *S.* (*P.*) *dozieri* and *A. exotica*; *S.* (*P.*) *dozieri* and *Sthetorus* sp; *Sthetorus* sp. and *C. valida*; and finally, *C. valida* and *C. externa* (Table 1).

Table 1. Correlation coefficient values between the most abundant predator species and *M. sacchari*

	Cysan	Hipcon	Colmac	Dioroc	Scydoc	Stesp	Cerval	Chrext	Allexo	Melsac
Cycsan	1.000									
Hipcon	**0.868**	1.000								
Colmac	**0.429**	**0.417**	1.000							
Dioroc	**0.437**	0.219	**0.527**	1.000						
Scydoc	**0.745**	**0.906**	0.331	**0.592**	1.000					
Stesp	0.339	0.141	0.168	**0.469**	**0.884**	1.000				
Cerval	**0.645**	**0.450**	0.158	**0.663**	0.301	**0.545**	1.000			
Chrext	**0.432**	0.316	0.326	0.372	0.222	**0.412**	**0.542**	1.000		
Allexo	**0.609**	**0.430**	0.245	**0.684**	0.293	0.392	**0.715**	**0.549**	1.000	
Melsac	**0.871**	**0.790**	**0.450**	0.150	**0.719**	0.144	**0.386**	**0.348**	**0.353**	1.000

The first axis of the PCA explained 25.83% of the total variation, while the second explained 12.83% and the third 8.53%. The total accumulated variation explained by these three axes was 46.97% (Table 2).

Among the species that most strongly and positively correlated with the first principal component are: *C. sanguinea sanguinea*, *H. convergens*, *D. roseicollis*, *Exochomus childreni guexi* LeConte, *S.* (*P.*) *dozieri*, *Stethorus* sp., *Hyperaspis* sp., *Scymnus* (*Pullus*) *loewii* Mulsant, *C. valida*, *C. externa*, *A. exotica*, *Allograpta obliqua* (Say), *Ocyptamus antiphates* (Walker), *Ocyptamus dimidiatus* (F.) *Pseudodorus clavatus* (F.), and *Toxomerus dispar* (F.). With respect to the second component, *D. roseicollis*, *C. valida* and *A. obliqua* are highlighted, and with negative correlation: *H. convergens*, *E. childreni guexi*, *S.* (*P.*) *dozieri*, *Ocyptamus gastrostactus* (Wiedemann), and *P. clavatus*. Finally, *Coleomegilla maculata lengi* Timberlake, *Olla v-nigrum* (Mulsant), *Hyperaspis* sp., and *O. dimidiatus* are correlated to the third component, with the last species with negative correlation (Table 2).

Table 2. Principal components. Percentage of accumulated explained variance and correlation of the species with the components.

	COMPONENT 1	**COMPONENT 2**	**COMPONENT 3**
% Variance	25.83	12.60	8.53
% Accumulated var.	25.83	38.43	46.97
SPECIES	**Correlation**	**Correlation**	**Correlation**
Cycsan	**0.893**	-0.131	0.161
Cheext	**0.611**	0.266	-0.058
Scyloe	**0.587**	-0.305	0.210
Stesp	**0.451**	0.285	0.087
Hipcon	**0.825**	**-0.447**	0.007
Ocygas	**0.373**	**-0.511**	-0.159
Toxdis	**0.547**	**0.447**	-0.230
Psecla	**0.424**	**-0.565**	-0.134
Dioroc	**0.557**	**0.557**	-0.083
Cerval	**0.791**	**0.454**	0.045
Allexo	**0.788**	**0.377**	-0.295
Allobl	**0.421**	**0.514**	0.265
Exochi	**0.560**	**-0.586**	0.332
Scydoc	**0.709**	**-0.630**	0.028
Colmac	**0.370**	-0.162	**0.496**
Hypsp	**0.448**	0.337	**0.668**
Ocyant	**0.630**	0.043	**-0.391**
Ocydim	**0.575**	-0.113	**-0.482**
Ollvni	0.333	**0.346**	**0.592**

Figure 1 shows the dispersion of the sociological variables and the relationship between the environmental variables and ranked axes 1 and 2 from the PCA. Global radiation, reference evapotranspiration and *M. sacchari* were the environmental variables with the highest weighting on the positive side of Axis 1, while *M. sacchari* was the variable that contributed the most. All predator species, except *Nephus* sp.1 and *Toxomerus politus* (Say), were located in the positive portion of Axis 1, while, in contrast, *H. convergens*, *C. sanguinea sanguinea* and *S.* (*P.*) *dozieri* were the predator species with the strongest relationship with high values for *M. sacchari*, as shown by their close proximity (Fig. 1). Precipitation and average temperature were the environmental variables that correlated with the negative portion of Axis 1.

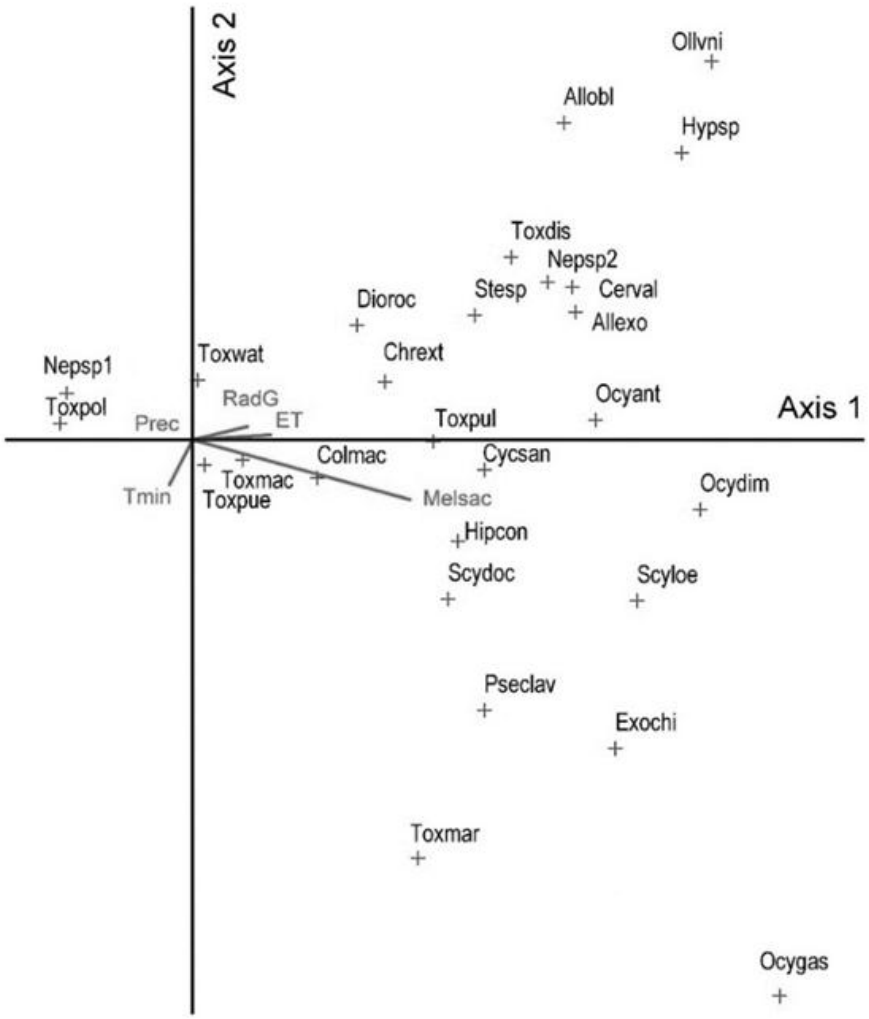

Figure 1. PCA diagram with predator species (+) and environmental variables (-). Annex 1. Variables

Discussion

The abundance of *M. sacchari* in Period 3 was lower than 1% with respect to Period 1 (Annex 2), which coincides with the observations of Colares *et al.* (2015b), who describe how exotic aphid infestations go through three stages: 1) Epidemic stage: in which large aphid populations have a devastating impact on a wide geographical region of crop production; 2) Attenuation stage: in which infestations gradually decrease to more limited periodic and intermittent outbreaks where the geographical range may contract; and 3) Endemic stage: in which the biological control exercised by a complex of resident natural enemies is widely

established, and the pest insect infestations that cause economic losses become rare and, generally, are limited to situations where the populations of natural enemies are interrupted by climatic or cultural impacts.

The time required for an invasive aphid species to reach an endemic level may vary from a few years to decades (Colares *et al*., 2015b). In accordance with the population decrease observed over the three sampling periods, it can be inferred that, currently, the invasion of *M. sacchari* in the state of Colima is around the endemic stage or is, at least, in the attenuation stage.

The *M. sacchari* infestations achieve maximum growth under conditions of 11.4 to 30 ˚C and 43 to 94% relative humidity (RH) (Waghmare *et al*., 1995), while such populations begin to die from 35 ˚C onwards (Behura & Bohidar, 1983). The average temperature during the sampling periods was 26 ˚C, with a maximum temperature of 35.22 ˚C and a minimum of 14.8 ˚C, while the average RH was 77.94%, with a maximum of 92.22% and a minimum of 67.15%. For this reason, the environmental factors do not significantly influence the predator populations, because such conditions do not pass the tolerance threshold for these species.

The predation capacity of some coccinellid species has already been evaluated in laboratory conditions, as in the research conducted by Delgado-Ramírez *et al*. (2019), who successfully evaluated the capacity of *H. convergens* larva and adults to consume *M. sacchari*. This supports the results obtained by the present research, which suggest that *H. convergens* is one of the dominant predator species associated with *M. sacchari*.

Rodríguez-Vélez *et al*. (2019) previously registered the species *C. sanguinea sanguinea*, *H. convergens, D. roseicollis, E. childreni guexi, S.* (*P*.) *dozieri, S.* (*P*.) *loewii, C. maculata lengi* and *O. v-nigrum* as *M. sacchari* predators in various states in Mexico. These species significantly correlate with PC1 (Table 2), and are, moreover, located on the positive portion of Axis 1 of the PCA, as is *M. saccahri* (Fig. 1), confirming the predator-prey association between these species in sorghum crops in Mexico.

Colares *et al.* (2015a) affirm that controlling *M. sacchari* populations is undertaken naturally by native predator guilds, with the research conducted in the United States of America suggesting the species *H. convergens*, *C. maculata* DeGeer, *Chrysoperla carnea* Stephens and

Orius Insidiosus Say as comprising a guild of predators genetically preadapted for the consumption of *M. sacchari* or other - aphids. The present study demonstrates the relationship between various predator species via correlation analysis (Table 1) and that the majority of the variation is explained in the first principal component of the PCA (Table 2), in which the majority of the species, as well as *M. sacchari*, are positively correlated. This reinforces the concept of a guild of natural enemies capable of controlling the presence of *M. sacchari* in the medium term.

Spite of the decrease, in recent years, of *M. sacchari* populations (as shown in the present study), biological control via conservation should be considered a priority action for the control of this sorghum insect pest in our country. Similarly, the species *C. sanguinea sanguinea*, *H. convergens*, and *S.* (*P.*) *dozieri* are organisms to be considered for future research seeking to identify agents of biological control against *M. sacchari*, based on the correlation analysis and the close relationship with this insect pest shown by the PCA conducted here.

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Annex 1. Variables

Environmental variables		Sociological variables	
Acronym	**Variable**	**Acronym**	**Variable**
Melsac	*Melanaphis sacchari*	**Cycsan**	*Cycloneda sanguinea sanguinea*
Shigra	*Schizaphis graminum*	**Hipcon**	*Hippodamia convergens*
Rhomai	*Rhopalosiphum maidis*	**Colmac**	*Coleomegilla maculata lengi*
Prec	Precipitation (mm)	**Ollvni**	*Olla v-nigrum*
MaxT	Maximum temperature (°C)	**Dioroc**	*Diomus roseicollis*
MinT	Minimum temperature (°C)	**Exochi**	*Exochomus childreni guexi*
AvT	Average temperature (°C)	**Scydoz**	*Scymnus (Pullus) dozieri*
MaxWS	Maximum wind speed (km/h)	**Nepsp1**	*Nephus* sp. 1
WS	Wind speed (km/h)	**Nepsp2**	*Nephus* sp. 2
GRad	Global Radiation (w/m2)	**Stesp**	*Sthetorus* sp.
RH	Relative humidity (%)	**Hypsp**	*Hyperaspis* sp.
ET	Reference evapotranspiration (mm)	**Scyloe**	*Scymnus (Pullus) loewii*
PE	Potential evaporation (mm)	**Cerval**	*Ceraeochrysa valida*
		Chrext	*Chrysoperla externa*
		Allexo	*Allograpta exotica*
		Allobl	*Allograpta obliqua*
		Ocyant	*Ocyptamus antiphates*
		Ocydim	*Ocyptamus dimidiatus*
		Ocygas	*Ocyptamus gastrostactus*
		Psecla	*Pseudodorus clavatus*
		Toxdis	*Toxomerus dispar*
		Toxmac	*Toxomerus maculatus* Macquart
		Toxmar	*Toxomerus marginatus* (Say)
		Toxpol	*Toxomerus politus*
		Toxpue	*Toxomerus puellus* (Hull)
		Toxpul	*Toxomerus pulchellus* (Macquart)
		Toxwat	*Toxomerus watsoni* (Curran)

Annex 2. Abundance of the species. Period (P).

Species	Order and Family	P1	P2	P3
Melanaphis sacchari	Hemiptera: Aphididae	27454	2122	198
Rhopalosiphum maidis	Hemiptera: Aphididae	0	0	102
Schizaphis graminum	Hemiptera: Aphididae	650	0	0
Coleomegilla maculata lengi	Coleoptera: Coccinellidae	41	41	44
Cycloneda sanguinea sanguinea	Coleoptera: Coccinellidae	342	52	4
Diomus roseicollis	Coleoptera: Coccinellidae	132	39	27
Exochomus childreni guexi	Coleoptera: Coccinellidae	4	0	0
***Hyperaspis* sp.**	Coleoptera: Coccinellidae	2	0	0
Hippodamia convergens	Coleoptera: Coccinellidae	159	22	11
***Nephus* sp. 1**	Coleoptera: Coccinellidae	1	0	0
***Nephus* sp. 2**	Coleoptera: Coccinellidae	1	0	0
Olla v-nigrum	Coleoptera: Coccinellidae	2	0	0
Scymnus* (*Pullus*) *dozieri	Coleoptera: Coccinellidae	353	28	8
Scymnus* (*Pullus*) *loewii	Coleoptera: Coccinellidae	4	0	0
***Stethorus* sp.**	Coleoptera: Coccinellidae	17	7	1
Ceraeochrysa valida	Neuroptera: Chrysopidae	80	0	0
Chrysoperla externa	Neuroptera: Chrysopidae	338	12	192
Allograpta exotica	Diptera: Syrphidae	51	0	1
Allograpta obliqua	Diptera: Syrphidae	3	0	0
Ocyptamus antiphates	Diptera: Syrphidae	1	0	0
Ocyptamus dimidiatus	Diptera: Syrphidae	3	0	0
Ocyptamus gastrostactus	Diptera: Syrphidae	1	0	0
Pseudodorus clavatus	Diptera: Syrphidae	9	0	0
Toxomerus dispar	Diptera: Syrphidae	18	0	6
Toxomerus maculatus	Diptera: Syrphidae	3	1	0
Toxomerus marginatus	Diptera: Syrphidae	1	0	0
Toxomerus politus	Diptera: Syrphidae	0	6	8
Toxomerus puellus	Diptera: Syrphidae	1	0	0
Toxomerus pulchellus	Diptera: Syrphidae	4	1	0
Toxomerus watsoni	Diptera: Syrphidae	2	3	1

CAPÍTULO III

Native predatory coccinellids (Coleoptera: Coccinellidae) of Mexico as regulators of populations of *Melanaphis sacchari*

Resumen

Se presenta un análisis de investigaciónes publicadas acerca del potencial de depredación sobre *Melanaphis sacchari* (Zehntner) por especies de Coccinellidae presentes en México. Las especies estudiadas fueron: *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* Pallas, *Diomus terminatus* (Say) y *Coleomegilla maculata* Degeer. Se comprobó que las hembras de *H. convergens* consumen más pulgones que los machos y las larvas de este coccinélido comen casi el doble de *M. sacchari* que los adultos. Individuos de *Harmonia axyridis* presentaron respuesta funcional tipo II a diferentes densidades de presas de *M. sacchari* lo que indica aumento desacelerado de la capacidad de depredación a medida que aumentan las presas consumidas. Larvas de *Diomus terminatus* consumieron un total de 30 ± 1.8 ninfas de pulgón, con una tasa de consumo diario de 4,7 ± 0,4; mientras que en adultos se mostró que *D. terminatus* puede consumir hasta 19 ± 9 pulgones por día. *Hippodamia convergens* y *Coleomegilla maculata* demostraron estar pre adaptados para explotar *M. sacchari* a pesar de ser una especie de reciente ingreso, con la capacidad de funcionar como un consorcio de excelentes depredadores capaces de proporcionar un control biológico sostenible de esta plaga. Se considera que las especies de Coccinellidae nativas en cultivos de sorgo junto con otros depredadores nativos pueden formar un consorcio de depredadores capaz de controlar a las poblaciones de *M. sacchari* a mediano plazo.

The yellow aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) (Fig. 1) is a cosmopolitan insect and its geographical distribution follows sugar cane and sorghum crops throughout the world (Mead 1978). *Melanaphis sacchari* causes direct damage inherent to feeding on the sap of the plant and indirect as the growth of molasses on the plant and the transmission of viruses, causing losses in the yield of crops up to 25% (Akbar et al. 2010).

Melanaphis sacchari was detected for the first time in Mexico in 2013, in the state of Tamaulipas (Rodríguez-del-Bosque and Terán 2015), currently, it is present in 26 states, and although the economic losses in Mexico have not been quantified precisely, the estimates assign values of billions of pesos (Peña-Martínez et al. 2015).

Different strategies have been used for the control of *M. sacchari*, among which is the chemical management, mainly the treatment of sorghum seed before sowing, which can be

crucial to prevent early infestation. Some systemic insecticides have been shown to be effective (Singh et al. 2004), but applications are expensive (Elliott et al. 2017), it is difficult for them to penetrate the foliage of sorghum (Armstrong et al. 2016), and their use threaten the sustainability and quality of crops as well as human health and natural systems (Meehan et al. 2011).

Figure 1. Individual of *Melanaphis sacchari* feeding on the sap of a sorghum leaf.

One of the recurrent measures to control aphids is based on the use of plant varieties resistant or tolerant to attack, however, most are effective towards a single species of aphid or, only towards a biotype (Dogimont et al. 2010). There is also the possibility of the use of entomopathogenic fungi; they have peculiar mechanisms during infection and colonization of the host, so they can be used to control sucking insects (Charnley 1992). Results showed that the fungus *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae), managed to decrease the presence of *M. sacchari* below the record of the control plots, however, the relative humidity and temperature are limiting factors in the development of *B. bassiana*, its biological activity is higher when the relative humidity is in a range of 81-92% (Ortíz-Catón et al. 2011).

The strategy of biological control using insects may be the most appropriate in the regulation of long-term populations of *M. sacchari* in Mexico, mainly with native predators; since there is little evidence to suggest that exotic biological control agents introduced against invading aphids have ever made measurable long-term contributions (Colares et al. 2015).

In Mexico there are several species of natural enemies of *M. sacchari*, particularly of the Coccinellidae family, 12 species have been registered for different regions of the country (Rodríguez-Vélez et al. 2019), fortunately some studies have already been done related to the predation capacity of some species of coccinellids present in Mexico on *M. sacchari*, that corroborate the importance of these species as agents of biological control.

Among the research studies relevant to pest predation, those related to functional response tests stand out since they used to consider a basic component to initiate a biological control study.

The functional response concept is defined as the relationship between the number of prey consumed by the predator and the density of the prey (Solomon 1949), expressing the influence of the behavior of natural enemies as individuals on the population dynamics of the prey (Fernández-Arhex and Corley 2004). Holling (1959) described three types of functional response, and in 1984, a fourth type called Holling type IV or non-monotonous functional response was described (Taylor 1984).

Type I functional response, there is a linear increase in the attack rate of the predator with respect to the density of the prey, until reaching a point from which the maximum attack rate becomes constant (Fernández-Arhex and Corley 2004).

In the type II functional response of, the manipulation time is considered, in addition to the search efficiency. This type of response results in a decelerated increase as the prey consumed increases, until arriving at an asymptote in which the maximum attack rate is reached. Consequently, long handling times lead to low attack rates and vice versa, which implies that preys at high densities, will have a lower probability of being attacked than when they are at low densities (Hassell 2000).

In type III functional response, the number of prey consumed per unit of time is accelerated by increasing the density of prey, until the time of manipulation begins to limit its consumption. This type of response produces, at low densities of the prey, direct dense-dependent mortality. It is for this reason that only type III response is potentially stabilizing prey-predator dynamics and it is important to note that this happens at low prey densities (Hassell et al. 1977, Hassell 1978, and Bernstein 2000). Once the inflection point has been passed, the curve resembles the one described for the type II response.

Type IV functional response is used to model antipredatory behaviors, such as the formation of defense groups and aggregation phenomena, which are used by dams to avoid predation (Taylor 1984), the first part of the curve can correspond to any of the previous types of functional responses followed by an abrupt decrease in the amount of prey consumed that is sometimes observed at high prey densities.

The following section is a bibliographic review of research related to the predation of *M. saccari* by native predatory coccinellid species in Mexico.

Hippodamia convergens Guerin-Meneville (Fig. 2A) is a species native to America, which was described based on a Mexican specimen, it is the most abundant species of its genus in the north of the continent (Gordon 1985), in Mexico it is recorded in 608 localities distributed in most of the territory (NaturaLista 2019a).

Delgado-Ramírez et al. (2016) evaluated the consumption capacity of larvae and adults of *H. convergens* on *M. sacchari*, under conditions of 25 ± 3 °C, 60-65% RH and photoperiod of 16 h light by eight of darkness, the time of the dam's exposure was 24 h. They did not find significant differences in the percentage of consumption with different densities of yellow aphid between males and females, except when this was of 64 individuals, with this density the females consumed 55 individuals, surpassing the males that ingested 43 in 30 minutes. With 100 aphids available, the larvae devoured 87 individuals on average and the adults 45 individuals, it was found that in the fourth larval stage they consumed more aphids, recording significant differences. They concluded that females of *H. convergens* consume more aphids than males and their larvae eat almost twice as many aphids as adults.

Harmonia axyridis Pallas (Fig. 2B), is a species native to Asia that has been introduced in North America, Europe and South America for the purpose of controlling aphid pests, its use in biological control programs in many countries is currently discussed, due to its voracity and success as a predator is such that it can displace native species of predatory insects (Roy et al. 2006). In Mexico it is recorded in 590 localities distributed mainly in the center of the country (NaturaLista 2019b).

Wu et al. (2018) determined the functional response of *H. axyridis* on *M. sacchari* under laboratory conditions; they used individuals from the four larval stages and female and male adults of *H. axyridis* on different densities of 12-h-old adults of *M. sacchari*. Each treatment had ten repetitions. The results showed that all stages of *H. axyridis* displayed a type II functional response to *M. sacchari*, where the the fourth larval instar and female adults were the most effective stages of *H. axyridis* in the context of biological control but suitable predator densities need to be considered before natural enemy release.

Diomus Mulsant, is a genus of Coccinellidae distributed worldwide with most of its species in the Neotropical region (Gordon 1999). *Diomus terminatus* (Say) (Fig. 1C) is the most widely collected species in the United States, (Gordon 1976). In Mexico, it is recorded predating *M. sacchari* in the state of Tamaulipas (Rodríguez-Vélez et al. 2016, Rodríguez-Vélez et al. 2019).

Akbar et al. (2009) documented the life cycle of *D. terminatus* and evaluated the potential as a biological control agent on *M. sacchari*. *Diomus terminatus* individuals were collected in the field and reared successfully until the adult stage under controlled conditions at 26 °C, using *M. sacchari* as food. On the other hand, the colonies of *M. sacchari* were kept of small pieces of sugarcane leaves. The eggs of *D. terminatus* placed on leaves of sugarcane hatched at 4.5 ± 0.1 days. The larval and pupal stages lasted an average of 6.8 ± 0.6 and 4.9 ± 0.2 days, respectively. *Diomus terminatus* required 12.1 ± 0.6 days, from egg hatching to adult emergence. The voracity test showed that the larvae consumed a total of 30 ± 1.8 aphid nymphs, with a daily consumption rate of 4.7 ± 0.4. While in adults it was shown that *D. terminatus* consume up to 19 ± 9 aphids per day.

Colares et al. (2015), compared the predation of the coccinellids *H. convergens*, Guérin-Méneville and *Coleomegilla maculata* Degeer, the latter species is considered native to North America and is recorded in Mexico in 95 localities mainly near the coast of the Pacific Ocean (NaturaLista 2019c). *Coleomegilla maculata lengi* Timberlake (Fig. 2D), is reported as a predator of *M. sacchari* in the states of Tamaulipas, Sinaloa, and Colima, (Rodríguez-Vélez et al. 2019). In addition to the previously mentioned species, *Chrysoperla carnea* (Stephens) (Neuroptera:Chrysopidae), and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) were also evaluated on *Shyzaphis graminum* (Rondani) which had already been part of the diet of the studied predators for years, and *M. sacchari* was .considered a pest of newly introduced, and on eggs *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

The survival from immature stage to adulthood was high for all predators, regardless of the prey, except for the nymphs of *O. insidiosus* that had greater survival feeding on *E. kuehniella*. The development times differed slightly according to the dams, with *S. graminum* that produced the fastest development of *H. convergens* and with *E. kuehniella* eggs a more accelerated development of *C. carnea* and *O. insidiosus* was originated. The diet with *S. graminum* also produced larger adults of *H. convergens*, while the larger adults of other predatory species were obtained with *E. kuehniella*.

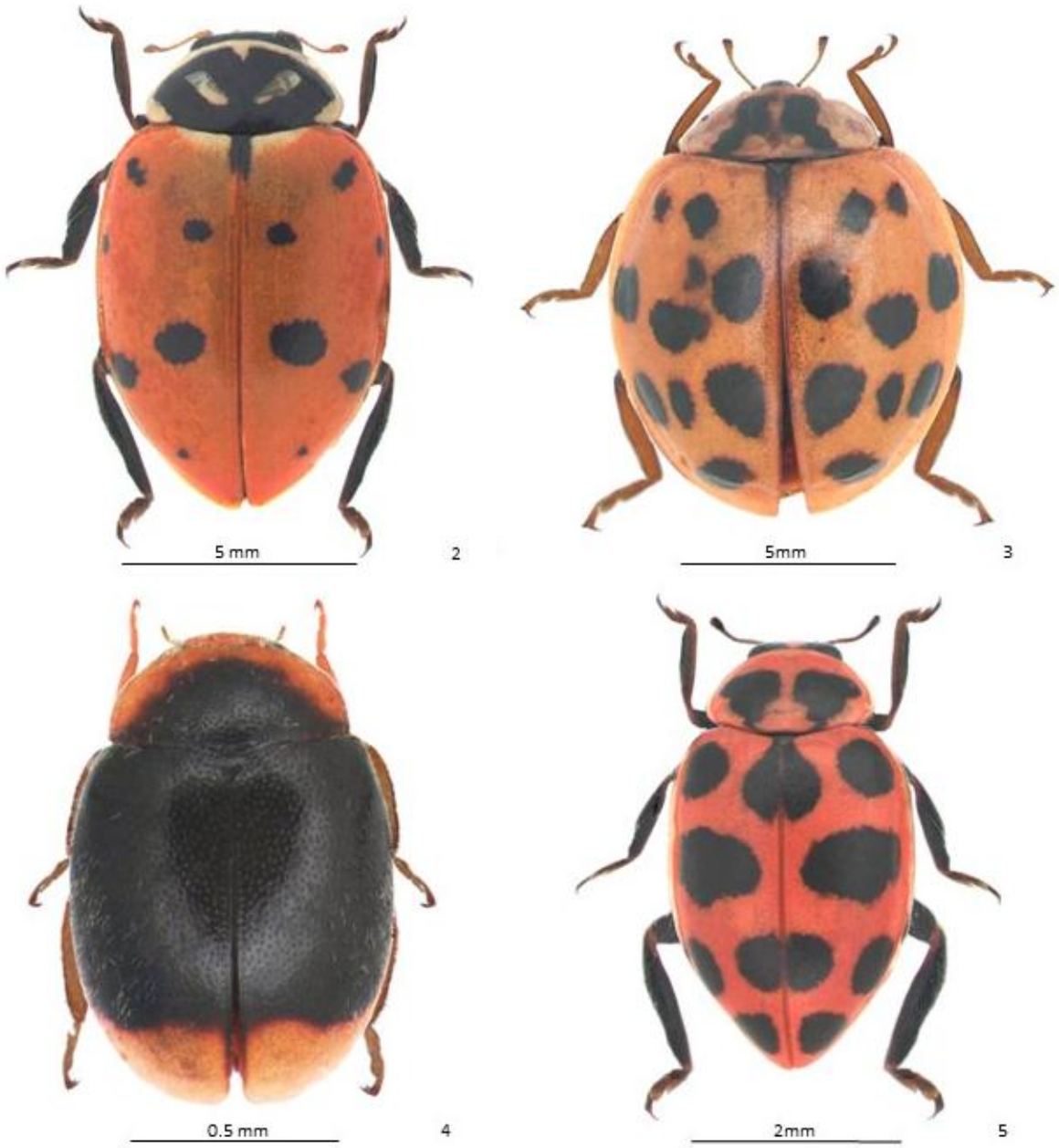

Figure. 2. Some predator coccinellid species native from Mexico: A) *Hippodamia convergens*; B) *Harmonia axyridis*; C) *Diomus terminatus*; D) *Coleomegilla maculata lengi*.

Based on the research reviewed, adult females of *H. convergens* are more voracious than adult males of the same species; in addition, larvae of *H. convergens* and *D. terminatus* are more

voracious than adults, when they feed on *M. sacchari*. *Hippodamia convergens* presents greater voracity than *D. terminatus* (Delgado-Ramírez et al. 2016), and wider distribution in Mexico.

Harmonia axyridis presented a type II functional response, which confirms its availability to feed on *M. sacchari*, however only a functional type III response can be considered as potentially stabilizing prey-predator dynamic. Wu et al. (2018) concluded that in the context of biological control both the fourth-stage larvae and the adult females of *H. axyridis* are efficient predators, but adequate densities of predators are needed before considering their release. Notwithstanding the foregoing, *H. axyridis* is considered the most invasive species of Coccinellidae on the planet (Roy et al. 2006), and a threat to biodiversity (Roy and Wajnberg 2008), so it is preferable to dispense with its use in augmentative or even inoculative biological control programs.

Colares et al. (2015), concluded that there is no evidence that *M. sacchari* even though an exotic just-arrived species, not represent an empty niche. They also conclude that the predators *H. convergens*, *C. maculata*, *C. carnea* and *O. insidiosus* are pre-adapted to exploit *M. sacchari* as prey, and can complete their life cycle by feeding on it, in addition, can work in the field as a consortium of excellent predators capable of providing sustainable biological control of this pest.

In conclusion, the potential of the species *H. convergens*, *D. terminatus*, *H. axyridis*, and *C. maculata* as predators of the exotic species *M. sacchari*, is ratified. Whereas the conservation of natural bioregulatory species is the most likely strategy in the management of pests in sustainable agriculture; likewise, since *M. sacchari* is a prey widely adapted for predators in general (Singh et al. 2004, Colares et al. 2015).

We estimated that the introduction of natural enemies is not necessary, but rather an integrated pest management program that considers the conservation biological control by (1) limited use of aphidicides, and if a chemical application is necessary, that they have a high degree of specificity on the pest; consider the stage of crop development, its effect on natural enemies, the type of spraying, and economic competitiveness (Cortez-Mondaca et al. 2018). (2) Having sufficient irrigation, since it has been shown that drought increases the susceptibility of plants to be attacked by pests (Suarez et al. 2004). Furthermore, if the infestation of *M. sacchari*

occurs under drought conditions, both factors can have a synergistic interaction that intensifies the reduction of the yield of the plant (Singh et al. 2004). (3) Planting or conservation of herbaceous vegetation, since this technique, has given good results with species such as *Fagopyrum esculentum*, which attracts parasitoids and coccinellids to cabbage and soybean fields (Lee and Heimpel 2005, Woltz et al. 2012).

Native Coccinellidae species together with other native predators can form a consortium of *M. sacchari* predators (Colares et al. 2015); In addition, the fact that the consortium species perform the same function in the agroecosystem potentiates the phenomenon of functional redundancy (Walker 1992) with the capacity to control the populations of *M. sacchari* in the medium term.

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DISCUSIÓN GENERAL

Las especies de Coccinellidae reportadas en este trabajo ya han sido registradas como depredadores de *M. sacchari* por otros autores en México (Rodríguez-Vélez *et al*. 2016; Rodíguez-Palomera *et al*. 2016; Salas-Marina *et al*. 2017; Rodríguez-del-Bosque *et al*. 2018) con excepción de *E. childreni guexi* que es registrada como depredador de *M. sacchari* por primera vez a nivel mundial.

La especie *C. sanguinea* ha sido reportada como depredador de *M. sacchari* en México por Rodríguez-Palomera *et al.* (2016), Salas-Marina *et al.* (2017) y Rodríguez-del Bosque *et al.* 2018. Sin embargo, la especie *C. sanguinea* como tal, no ofrece certeza en su identidad ya que esta se divide en dos subespecies, *C. sanguinea sanguinea* y *C. sanguinea limbifer* Casey 1899 (Gordon, 1985). Un caso similar es el de *C. maculata*, la cual se ha reportado en trabajos anteriores solamente a nivel de especie, ignorando que existen tres subespecies de la misma: *Coleomegilla maculata lengi* Timberlake 1943, *Coleomegilla maculata strenua* (Casey, 1899) y *Coleomegilla maculata fuscilabris* (Mulsant, 1866) (Gordon, 1985). En el capítulo uno se identifica al nivel más fino posible a todas las especies; determinado que los ejemplares de *C. sanguinea* estudiados pertenecen a la subspecie *C. sanguinea sanguinea* y los de *C. maculata* corresponden a la subespecie *C. maculata lengi.*

En el capítulo I a diferencia de otras publicaciones, se mostró una breve diagnosis, fotografías y caracterización molecular de cada una de las especies, por lo anterior se ofrece certeza acerca de las identidades de los taxa y brinda al lector referencias para su identificación.

En el capítulo II se demostró que las poblaciones de *M. sacchari* disminuyeron de forma abrupta a través de los tres años muestreados; este fenómeno coincide con las observaciones de Colares *et al.* (2015b), en donde mencionan que las infestaciones por pulgones exóticos pasan por tres etapas: (1) etapa epidémica, donde las poblaciones grandes de pulgones tienen impacto devastador sobre una amplia región geográfica de producción de cultivos; (2) etapa de atenuación, en que las infestaciones disminuyen gradualmente a brotes periódicos e intermitentes que son más limitados y el rango geográfico puede contraerse; y (3) etapa endémica, en la que el

control biológico ejercido por un complejo de enemigos naturales residentes se establece ampliamente, las infestaciones de la plaga que provocan pérdidas económicas se vuelven raras y, por lo general, se limitan a situaciones donde las poblaciones de enemigos naturales se ven interrumpidas por impactos climáticos o culturales.

De acuerdo a la disminución poblacional observada durante los tres años podemos sugerir que la invasión de *M. sacchari* en el estado de Colima, se encuentra en la etapa endémica o por lo menos en atenuación, gracias a la intervención de los enemigos naturales de la región, sin embargo son necesarios más estudios para aseverar este punto.

Además, los datos del capítulo II sugieren la formación de un grupo de depredadores de *M. sacchari* compuesto por 25 especies; de las cuales, destacan por su relación depredador-presa con *M. sacchari* los coccinélidos *S.* (*P.*) *dozieri, H. convergens, C. sanguinea sanguinea* y *C. maculata lengi*. Los resultados anteriormente señalados corroboran la idea planteada por Colares *et al.* (2016b), quienes evaluaron la depredación de *H. convergens* y *C. maculata* sobre el ya establecido *S. graminum* y sobre *M. sacchari* una plaga exótica de recién ingreso. Concluyendo que los coccinélidos afidófagos estudiados estaban pre adaptados genéticamente para depredar y controlar las poblaciones de *M. sacchari* a mediano plazo.

De acuerdo con la revisión bibliográfica de investigaciones relacionadas con pruebas de depredación realizadas con coccinélidos nativos en México sobre *M. sacchari*; las evidencias confirmaron la efectividad depredadora de *C. maculata, D. terminatus, H. axyridis* e *H. convergens*. Las hembras adultas de *H. convergens* son más voraces que los machos adultos; las larvas de *H. convergens* y *D. terminatus* son más voraces que los adultos de su especie, cuando se alimentan de *M. sacchari*. No obstante *H. convergens* presentó una mayor voracidad que *D. terminatus* (Delgado-Ramírez *et al*., 2016), además que despliega una mayor distribución en México. Lo anterior prueba el potencial como depredador de *H. convergens* sobre *M. sacchari* planteado por Delgado-Ramírez *et al*. (2016); así mismo se corrobora también la importancia de las especies *H. convergens* y *C. maculata lengi* como parte de un consorcio de depredadores sobre *M. sacchari*, de acuerdo a Colares *et al*. (2015b).

No se han realizado investigaciones pertinentes al potencial de depredación y respuesta funcional de *S.* (*P.*) *dozieri* sobre *M. sacchari*, por lo cual se sugiere lo anterior, basándonos en el grado de correlacion de esta especie en el primer comoponente principal y en su cercanía con *M. saccahri* en la representación gráfica del PCA, en el capitulo 2.

Cabe destacar que *H. axyridis* presentó una respuesta funcional tipo II, lo cual ratifica su disponibilidad para alimentarse de *M. sacchari*, sin embargo, solo una respuesta funcional tipo III puede considerarse potencialmente estabilizadora de la dinámica presa– depredador. Wu *et al*. (2018), concluyen que en el contexto del control biológico tanto las larvas de cuarto estadio como las hembras adultas de *H. axyridis* son eficientes depredadores, pero se necesitan densidades adecuadas de depredadores antes de considerar la liberación de los mismos. Sin embargo, a pesar de la eficiencia como depredador de *H. axyridis*, esta especie ha sido considerada como el coccinélido más invasivo en el planeta (Roy *et al*., 2006), y una amenaza inaceptable a la biodiversidad (Roy & Wajnberg, 2008), por lo cual es preferible prescindir de su uso en programas de control biológico aumentativo e inclusive inoculativo.

Se confirma la predicción de este trabajo, los coccinélidos nativos en el área de estudio tienen potencial de controlar a las poblaciones de *M. sacchari*. Los resultados evidenciaron que los depredadores han efectuado un control biológico por conservación en el significativo declive de las poblaciones de *M. sacchari* en los últimos años en el estado de Colima. Por lo planteado, se sugiere que el control biológico clásico no es una alternativa ya que se dispone con un arsenal vasto de enemigos naturales y el costo de un programa de este tipo es muy alto. El control biológico por aumento, con los enemigos naturales comercialmente disponibles hoy en día, no parece ser una alternativa técnica y económicamente viable ya que las liberaciones de huevos de *C. carnea* no ha resultado ser efectiva en la supresión de poblaciones del PAS en los trabajos efectuados en Guanajuato (Lomeli-Flores *et al*., 2016). El uso de los coccinélidos es muy costoso y difícilmente se podrá liberar poblaciones mayores a las que naturalmente existen (Lomelí-Flores *et al*., 2016). Sin embargo, no se debería descartar la inoculación de los mismos en el caso de que no se encotraran naturalmente. En contraste, el control biológico natural con coccinélidos y otros depredadores parece estar funcionando como lo sugiere la evidencia mostrada en la presente investigación y en los trabajos de Colares *et al*. (2015a & 2015 b).

CONCLUSIONES

1. Se identificaron mediante métodos morfológicos y moleculares 12 especies de coccinélidos que depredan a *Melanaphis sacchari* en México, entre las que destaca la especie *Exochomus childreni guexi*, la cual es reportada por primera vez como depredador of *M. sacchari* a nivel mundial.

2. Las fluctuaciones poblaciones de las especies *Coleomegilla maculata lengi*, *Cycloneda sanguinea sanguinea*, *Hippodamia convergens*, *Scymnus* (*Pullus*) *loewi*, *Scymnus* (*Pullus*) *dozieri*, *Diomus roseicollis* y *Exochomus childreni guexi* ocurren en función de las poblaciones de *M. sacchari* y no de las condiciones climáticas.

3. *Hippodamia convergens*, *Scymnus* (*Pullus*) *dozieri*, *Cycloneda sanguinea sanguinea* son especies con potencial como agentes de control biológico de *M. sacchari.*

4. Los depredadores nativos de la familia Coccinellidae, tienen el potencial de menguar y eventualmente controlar las poblaciones de *M. sacchari* de manera natural.

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Anexo 1. Especies de Coccinellidae registradas en México.

Especie	Referencia	Localidad de origen	Referencia de origen
Adalia bipunctata (L.)	CONABIO 2008, 2019	Asia y Europa	Gonzalez 2006
Anatis hydropica (Mulsant)	Blackwelder 1945; Gorham 1889	México	Blackwelder 1945; Trejo-Loyo *et al*. 2012
Anatis labiculata Say	CONABIO 2019	Norte América	ITIS 2019
Axion plagiatum (Olivier)	CONABIO 2019; Gorham 1889; Trejo 2001	EUA (Arizona y California), México	Blackwelder 1945; Leeper 2015
Azya luteipes Mulsant	Blackwelder 1945; Gorham 1889	Brasil, Colombia, Guatemala, Guayana Francesa, Honduras, Nicaragua, Costa Rica, México, Panamá, Venezuela	Blackwelder 1945
Azya orbiguera Mulsant	CONABIO 2008, 2019	México	Leeper 2015
Azya pontbrianti Mulsant	Blackwelder 1945	Brasil, Chile, EUA, Guayana Francesa, México, Uruguay,	Blackwelder 1945
Brachiacantha arizonica Schaeffer	Blackwelder 1945; ITIS 2019	México,	Blackwelder 1945; ITIS 2019
Brachiacantha aymardi Gorham	Gorham 1889	México	Néstor-Arriola & Toledo Hernández 2016
Brachiacantha barberi Gordon	CONABIO 2019	EUA	ITIS 2019
Brachiacantha bipartita Mulsant	Néstor-Arriola & Toledo Hérnandez 2016	México	Néstor-Arriola & Toledo Hérnandez 2016
Brachiacantha bistripustulata F.	Gorhan 1887-1889	Sudamérica	Gordon *et al*. 2014
Brachiacantha conjunta Mulsant	Gorham 1889; Néstor-Arriola & Toledo-Hérnandez 2016	México	Néstor-Arriola & Toledo Hérnandez 2016
Brachiacantha cryptocephalina Gorham	Blackwelder 1945	México	Blackwelder 1945

Brachiacantha decora Casey	CONABIO 2008, 2019	EUA	ITIS 2019
Brachiacantha dentipes F.	CONABIO 2019	Canadá y EUA	ITIS 2019
Brachiacantha erythrura Mulsant	Blackwelder 1945	México, Guatemala, Costa Rica, Venezuela	Blackwelder 1945
Brachiacantha fenestra Gorham	Blackwelder 1945	México, Costa Rica y Panamá	Blackwelder 1945
Brachiacantha lepida Mulsant	CONABIO 2019	México	ITIS 2019
Brachiacantha mexicana Leng	Blackwelder 1945	México	Blackwelder 1945
Brachiacantha octostigma Mulsant	Blackwelder 1945	México, Guatemala, Panamá	Blackwelder 1945
Brachiacantha pygidialis Mulsant	Gorham 1889	México	Leng 1911
Brachiacantha subfasciata Mulsant	Blackwelder 1945; ITIS 2019	México, EUA	Blackwelder 1945; ITIS 2019
Brachiacantha ursina (F.)	CONABIO 2019	Canadá y EUA	ITIS 2019
Brachiacantha westwoodii Mulsant	Néstor-Arriola & Toledo Hérnandez 2016	México	Néstor-Arriola & Toledo Hérnandez 2016
Brumoides septentrionis hogei (Gorham)	Blackwelder 1945; ITIS 2019	Estados Unidos, México	Blackwelder 1945; ITIS 2019
Calvia quatuordecimguttata (L.)	Blackwelder 1945; ITIS 2019	México, EUA, Alaska, Canadá	Blackwelder 1945; ITIS 2019
Chilocorus cacti (L.)	CONABIO 2008; Gorham 1889	México, Honduras, Guatemala, Nicaragua, Costa Rica, Sudamérica, Cuba	Blackwelder 1945; Leeper 2015
Chilocorus nigrita F.	CONABIO 2019	India	Leeper 2015
Chilocorus renipustulatus (Scriba)	CONABIO 2019	Europa	Laurent *et al*. 2002
Chilocorus stigma (Say)	CONABIO 2019	Canadá, EUA, México	Blackwelder 1945; ITIS 2019
Chnoodes sanguinipes (Crotgh)	Gorham 1889	México	Booth & Pope 1989
Chnoodes terminalis Mulsant	Blackwelder 1945	México	Blackwelder 1945
Coccinella califórnica Mannerheim	CONABIO 2019	EUA	Leeper 2015
Coccinella emarginata (Mulsant)	CONABIO 2008; Gorham 1889	México, Belice, Guatemala, Nicaragua	Gorham 1889
Coccinella maculata (De Geer)	Gorham 1889	Canadá, Estados Unidos, México	ITIS 2019
Coccinella montícola Mulsant	CONABIO 2019	Canadá y EUA	ITIS 2019

Coccinella novemnotata Herbs	Blackwelder 1945; Ková 2005	México, Guatemala, EUA	Blackwelder 1945; Ková 2005
Coccinella novemnotata franciscana Mulsant	Blackwelder 1945; Ková 2005	México, EUA	Blackwelder 1945; Ková 2005
Coccinella nugatoria sonorica Casey	Blackwelder 1945; Ková 2005	México	Blackwelder 1945; Ková 2005
Coccinella septempuntata L.	CONABIO 2019	Europa y Asia	Leeper 2015
Coccinella transverguttata Faldermann	Blackwelder 1945; Ková 2005	México, EUA, Viejo mundo	Blackwelder 1945; Ková 2005
Coleomegilla maculata (De Geer)	CONABIO 2008, 2019	Canadá, EUA y México	ITIS 2019
Cryptognatha auriculata Mulsant	Blackwelder 1945	México, Guatemala, Panamá, Colombia, Guayana Francesa	Blackwelder 1945
Cryptognatha flaviceps Crotch	Blackwelder 1945	México, Honduras, Panamá, Brasil	Blackwelder 1945
Cryptolaemus montrouzieri Mulsant	CONABIO 2019	Australia	Leeper 2015
Curinus coeruleus (Mulsant)	CONABIO 2019; Gorham 1899; Trejo-Loyo *et al*. 2012	Desde México hasta Argentina	González 2008
Cycloneda emarginata (Mulsant)	CONABIO 2019; Gorham 1889	Antillas, Argentina, Bolivia, Ecuador, México, Paraguay, Venezuela	
Cycloneda munda Say	CONABIO 2019	Canadá y México	
Cycloneda polita Casey	CONABIO 2019	Canadá y EUA	ITIS 2019
Cycloneda retrospiciens Crotch	Blackwelder 1945	México, Honduras, Guatemala	Blackwelder 1945
Cycloneda sanguinea (L.)	Blackwelder 1945; CONABIO 2008, 2019	América	Backwelder 1945; Hogue 1993
Delphastus diversipes Champion	Blackwelder 1945	México, Honduras, Guatemala	Blackwelder 1945
Delphastus pusillus (LeConte)	CONABIO 2008	EUA	
Diomus seminulus (Mulsant)	Blackwelder 1945; Gonzalez, 2016	México	Blackwelder 1945
Diomus thoracicus (F.)	Blackwelder 1945; Gonzalez 2016	México	Blackwelder 1945
Diora sórdida Mulsant	Blackwelder 1945;	Centroamérica y México	Blackwelder 1945;

	González 2008		González 2008
Epilachna aubei Mulsant	Gorham 1889; Gordon 1975	México	Gordon 1975
Epilachna borealis (Fabricius)	Gorham 1889	Centro América, Colombia, Venezuela, Argentina, Brasil, Cuba, EUA, México	Blackwelder 1945; ITIS 2019; Gordon 1975
Epilachna discincta Weise	CONABIO 2019	Honduras	Gordon 1975
Epilachna mexicana (Guerin)	Blackwelder 1945; CONABIO 2019; Gordon 1975; Gorham 1889	México	Blackwelder 1945; Gordon 1975
Epilachna modesta Mulsant	Gorham 1889; Gordon 1975	México	Gordon 1975
Epilachna nigrocincta Mulsant	Blackwelder 1945; CONABIO 2019; Gordon 1975	México	Blackwelder 1945; Gordon 1975
Epilachna obscurella Mulsant	Blackwelder 1945; CONABIO 2019; Gordon 1975	México	Blackwelder 1945; Gordon 1975
Epilachna olivácea Mulsant	Blackwelder 1945; Gordon 1975	México	Blackwelder 1945; Gordon 1975
Epilachna patula Mulsant	Gordon 1975	México	Blachwelder 1945; Gordon 1975
Epilachna tredecimnotata (Latreille)	CONABIO 2019	EUA y México	ITIS 2019
Epilachna varivestis Mulsant	CONABIO 2019; Gordon 1975; Gorham 1889	Canadá, Costa Rica, Guatemala, EUA y México	ITIS 2019; Gordon 1975
Epilachna vincta Crotch	Blachwelder 1945; Gordon 1975	México, Honduras, Guatemala	Blachwelder 1945; Gordon 1975
Epilachna vulnerata Gorham	Blachwelder 1945; Gordon 1975	México	Blachwelder 1945; Gordon 1975
Exochomus bisbinotatus Gorham	Blackwelder 1945; Gorham 1899	México, Honduras, Guatemala	Blackwelder 1945; Gorham 1899
Exochomus californicus Casey	Gordon 1985	EUA; México	Gordon 1985
Exochomus childreni childreni Mulsant	CONABIO 2019, Gordon 1985	EUA, México	ITIS 2019; Gordon 1985

Exochomus childreni guexi LeConte	Gordon 1985	EUA, México	Gordon 1985
Exochomus contristatus Mulsant	Crotch 1874; Gorham 1899	México	Crotch 1874; Gorham 1899
Exochomus decemnotatus Mulsant	Gordon 1974	México	Gordon 1974
Exochomus fasciatus Casey	CONABIO 2019	EUA	ITIS 2019
Exochomus hogey Gorham	Gorham 1899	México	Gorham 1899
Exochomus insatiabilis Rodríguez-Vélez	Rodríguez- Vélez 2018	México	Rodríguez- Vélez 2018
Exochomus ixtlenis Casey	Blackwelder 1945, Casey 1924	México	Blackwelder 1945, Casey 1924
Exochomus marginipennis (LeConte)	Blackwelder 1945; Gordon 1985	México, EUA	Blackwelder 1945; Gordon 1985
Exochomus minutus Gordon	Gordon 1974	México	Gordon 1974
Exochomus sallaei Gorham	Blackwelder 1945; Gorham 1899	México	Blackwelder 1945; Gorham 1899
Exochomus townsendi Casey	Blackwelder 1945; Gordon 1985	EUA, México	Blackwelder 1945; Gordon 1985
Exoplectra stevensi Mulsant	Blackwelder 1945	México, Guatemala	Blackwelder 1945
Harmonia axyridis (Pallas)	CONABIO 2019	Originaria de Europa y Asia	Gonzalez 2006
Harmonia venusta ampla (Mulsant)	Blackwelder 1945; Coutanceau 2008	México	Blackwelder 1945; Coutanceau 2008
Hippodamia convergens Guerin	Bustillo 2011; CONABIO 2008, 2019; Gorham 1889; Kirkaldy 1909	Ampliamente distribuida en toda América, Europa y Asia	Gonzalez 2006
Hippodamia koebelei Timberlake	Blackwelder, 1945	México	Blackwelder 1945
Hippodamia parenthesis (Say)	CONABIO 2008, 2019	Canadá Estados Unidos	ITIS 2019
Hyperaspidius brevilinea Casey	Blackwelder 1945	México	Blackwelder 1945
Hyperaspidius mexicanus Nunenmacher	Blackwelder 1945	México	Blackwelder 1945
Hyperaspidius trimaculatus L.	Blackwelder 1945	México	Blackwelder 1945
Hyperaspidius vittiger (LeConte)	Blackwelder 1945; ITIS 2019	México, Canadá y EUA	Blackwelder 1945; ITIS 2019
Hyperaspis Adelaida (Gorham)	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis balsasia Casey	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis billoti Mulsant	Blackwelder 1945	México, Guatemala,	Blackwelder 1945

		Nicaragua, Costa Rica, Brasil, Argentina	
Hyperaspis centralis Mulsant	Gorham 1889	Estados Unidos y México	ITIS 2019
Hyperaspis compedita Mulsant	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis connectens(Thunberg)	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis deyrollei Crotch	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis durangoensis Casey	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis festiva Mulsant	González 2008	Desde México y Puerto Rico hasta Argentina	González 2008
Hyperaspis fimbriolata Melsheimer	Blackwelder 1945; ITIS, 2019	Canadá, EUA, México	Blackwelder 1945; ITIS 2019
Hyperaspis globula Casey	Blackwelder 1945; ITIS 2019	USA, México	Blackwelder 1945; ITIS 2019
Hyperaspis invertens Casey	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis jocosa Mulsant	Blackwelder 1945	México, Panamá, Colombia, Brasil, Peru	Blackwelder 1945
Hyperaspis lateralis Mulsant	CONABIO 2019	Canadá, EUA y México	ITIS 2019
Hyperaspis levrati (Mulsant)	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis lunulata Mulsant	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis silvestrii Weise	Blackwelder 1945; ITIS 2019	México	Blackwelder 1945; ITIS 2019
Hyperaspis subsignata Crotch	Blackwelder 1945	México	Blackwelder 1945
Hyperaspis trifurcata Shaeffer	CONABIO 2008, 2019	EUA	ITIS 2019
Hyperaspis undulata (Say)	Blackwelder 1945; ITIS 2019	México, EUA, Canadá	Blackwelder 1945; ITIS 2019
Mada aplexata (Mulsant)	Gordon 1975	México	Gordon 1975
Mada desarmata (Mulsant)	Blackwelder 1945; Gordon 1975	México, Panama, Brasil	Blackwelder 1945; Gordon 1975
Mada inepta (Gorham)	Gordon 1975	México	Gordon 1975
Mada miltis (Mulsant)	Blackwelder 1945; Gordon 1975	México	Blackwelder 1945; Gordon 1975
Mada polluta (Mulsant)	Gordon 1975	México	Gordon 1975
Malata delphinae (Gorham)	Blackwelder 1945; Gordon 1975; Gorham 1889	México	Blackwelder 1945
Microscymnus calvus Champion	Blackwelder 1945	México	Blackwelder 1945
Mulsantina concolor Crotch	Blackwelder 1945	México	Blackwelder 1945

Mulsantina curva Chapin	Chapin 1985	México, Guatemala	Chapin 1985
Mulsantina lynx (Mulsant)	Blackwelder 1945; Chapin 1985	México, Guatemala	Blackwelder 1945; Chapin 1985
Mulsantina mexicana Chapin	Chapin 1985	México	Chapin 1985
Mulsantina picta (Randall)	CONABIO 2008	Canadá, EUA y México	Blackwelder 1945
Mulsantina quinquelineata (Mulsant)	Blackwelder 1945; Chapin 1985	México, Guatemala	Blackwelder 1945; Chapin 1985
Naemia seriata (Melsheimer)	Blacwelder 1945; ITIS 2019	México, América	Blacwelder 1945; ITIS 2019
Neaporia argentifrons Gorhan	Gordon & Hanley 2017	México	Blackwelder 1945; Gorgon 2017
Neda marginalis Mulsant	Blackwelder 1945; CONABIO 2008, 2019; Gorham 1889; Kirkaldy 1909	México, Guatemala, Costa Rica	Blackwelder 1945
Neda ostrina Mulsant	CONABIO 2019	Perú	Gonzalez 2006
Neohalyzia perroudi Mulsant	CONABIO 2019	Colombia, Centroamérica y México	Gonzalez 2006
Olla v-nigrum (Mulsant)	Blackwelder 1945; CONABIO 2008, 2019; Gorham 1889; Vandenberg 1992	Canadá, EUA y México	Blackwelder 1945; ITIS 2019; Vandenberg 1992
Olla timberlake Vandenberg	Vandenberg 1992	México y Guatemala	Vandenberg 1992
Ortalistes pexa Gorham	Blackwelder 1945	México, Guatemala, Islas Perlas, Panamá	Blackwelder 1945
Paranaemia vittigera (Mannerheim)	CONABIO 2008, 2019; Gorham 1889	Canada y Estados Unidos	ITIS 2019
Paraneda pallidulla guticollis (Mulsant)	Blackwelder 1945; CONABIO 2019; Gorham 1889	México, Guatemala, Colombia, Venezuela, Guayana Francesa, Brasil	Blackwelder 1945
Pentilia nigella Weise	Blackwelder 1945	México	Blackwelder 1945
Prodilis cribrata (Gorham)	Blackwelder 1945; Gordon & Hanley 2017	México	Blackwelder 1945; Gordon & Hanley 2017
Prodilis guatemalana (Gorham)	Blackwelder 1945; Gordon & Hanley 2017	Guatemala, México	Blackwelder 1945; Gordon & Hanley 2017

Psyllobora confluens (F.)	Blackwelder 1945	México	Blackwelder 1945
Psyllobora decipiens Mulsant	Blackwelder 1945; Gorham 1889	México, Colombia, Brasil, Ecuador	Blackwelder 1945; Gonzalez 2006
Psyllobora luctuosa Mulsant	Blackwelder 1945	México, Guatemala, Colombia, Venezuela, Guayana Francesa, Brasil	Blackwelder 1945
Psyllobora parvinotata Casey	CONABIO 2019	Estados Unidos	ITIS 2019
Psyllobora renifer Casey	CONABIO 2008, 2019	EUA y México	ITIS 2019
Psyllobora roei Mulsant	Blackwelder 1945	México, Guatemala	Blackwelder 1945
Psyllobora vigintimaculata (Say)	CONABIO 2019	Alaska, Canadá y Estados Unidos	ITIS 2019
Rhyzobius lophanthae (Blaisdell)	CONABIO 2008	Australia	ITIS 2019
Rodolia cardinalis (Mulsant)	CONABIO 2019	Australia	CONABIO 2019
Scymnobius bilucernarius Mulsant	Blackwelder 1945; Rodríguez-Palomera 2016	México	Blackwelder 1945
Scymnus (Pullus) apicalis Mulsant	Blackwelder 1945	Brasil, Colombia, Guatemala, México	Blackwelder 1945
Scymnus (Pullus) dubius Weise	Blackwelder 1945; Maes & Duverger 2019	México	Bkackwelder 1945; Maes & Duverger 2019
Scymnus (Pullus) jansoni Gorham	Blackwelder 1945; Gonzalez 2006	México	Blackwelder1945; Gonzalez 2006
Scymnus (Pullus) loewi Mulsant	CONABIO 2008	EUA y México	González 2008
Scymnus aztecanus Casey	Blackwelder 1945	México	Blackwelder 1945
Scymnus coloratus Gorham	Blackwelder 1945	México	Blackwelder 1945
Scymnus cribripennis Champion	Blackwelder 1945	México	Blackwelder 1945
Scymnus duplicatus Casey	Blackwelder 1945	México	Blackwelder 1945
Scymnus högei Gorhan	Blackwelder 1945	México	Blackwelder 1945
Scymnus horni Gorham	Blackwelder 1945	Guatemala, México, Panamá, EUA	Blackwelder 1945
Scymnus huachuca Gordon	CONABIO 2008	Canadá y Estados Unidos	ITIS 2019
Scymnus margipallens (Mulsant)	Blackwelder 1945; ITIS 2019	México	Blackwelder 1945; ITIS 2019
Scymnus nugator Casey	CONABIO 2008	Estados Unidos	ITIS 2019
Scymnus uncinatus Sicard	Blackwelder 1945; ITIS 2019	México	Blackwelder 1945

Semra picta (Gorham)	Özdikmen 2007	México	Blackwelder 1945
Spiloneda gilardini Mulsant	Blackwelder 1945	México	Blackwelder 1945
Thalassa montezumae Mulsant	Blackwelder, 1945,; ITIS 2019	México, Guatemala, USA	Blackwelder 1945; ITIS 2019
Thalassa pentaspilota (Chevrolat)	Blackwelder 1945	México, Guatemala, Cuba	Blackwelder 1945
Zagloba beaumonti Casey	Gonzalez 2016	Honduras, Panamá, Colombia, Venezuela, Brasil, Paraguay	Gordon 1970