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**DETERMINACIÓN DE LA ACAROFAUNA ASOCIADA  
A ZARZAMORA (*Rubus ulmifolius*),  
CARACTERIZACIÓN Y BIOLOGÍA DE *Diptacus* sp.  
(ERIOPHYIOIDEA: DIPTILOMIOPIDAE)**

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T E S I S

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**DETERMINACIÓN DE LA ACAROFAUNA ASOCIADA A ZARZAMORA (*Rubus ulmifolius*), CARACTERIZACIÓN Y BIOLOGÍA DE *Diptacus* sp. (ERIOPHYIOIDEA: DIPTILOMIOPIDAE)**

Sandra Guadalupe González Domínguez, Dra.  
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**RESUMEN**

La zarzamora es una frutilla de importancia económica para nuestro país por la superficie sembrada y las divisas que genera. Sin embargo, al igual que otros monocultivos, se ve limitado por diferentes plagas y enfermedades, entre las cuales se mencionan los ácaros fitófagos que pueden causar daños importantes por alimentarse del contenido celular, lo que provoca alteraciones fisiológicas y pérdidas en el rendimiento. Es por ello, que el objetivo de este trabajo fue determinar la diversidad, distribución y fluctuación poblacional de ácaros asociados al cultivo de zarzamora en huertas con producción orgánica y convencional; así como caracterizar y determinar la biología de *Diptacus* sp., en *Rubus ulmifolius* var. Tupy, en el municipio de los Reyes de Salgado, Michoacán, México. La diversidad de especies de ácaros fue similar en los sistemas de producción orgánica y convencional. Las especies dominantes fueron *Tetranychus urticae* y *Diptacus* sp. El manejo agronómico de la producción de zarzamora no influye en la distribución y fluctuación de la acarofauna que se observó en este cultivo. Se identificó a *Diptacus rubus* n.sp. González-Domínguez & Santillán-Galicia, una nueva especie asociada a zarzamora en México, por lo que se describió su morfología y se determinó su biología.

**Palabras clave:** *Diversidad, Rubus sp., producción orgánica, producción convencional, acarofauna.*

**DETERMINATION OF THE ACAROFUNA ASSOCIATED WITH BLACKBERRY  
(*Rubus ulmifolius*), CHARACTERIZATION AND BIOLOGY OF *Diptacus* sp.  
(ERIOPHYIOIDEA: DIPTILOMIOPIDAE)**

Sandra Guadalupe González Domínguez, Dra.  
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**ABSTRACT**

The blackberry, *Rubus ulmifolius* Schott, is an important crop in Mexico for the large extension used for its production and the economic gain. However, similar to other crops, pest and diseases are limiting factors for its production. Among the most important pests are phytophagous mites, which can cause severe damages to the plant by their feeding activities. Therefore, the aim of our research was to determine the species diversity, distribution and population dynamics of mites in blackberry crops with an organic and conventional production. In addition, we described the taxonomy and biology of a species of the genus *Diptacus* inhabiting blackberry plants (var. Tupy). Species diversity, distribution and population dynamics of the mites studied were similar in blackberry crops regardless the type of production, organic or conventional. The main species were *Tetranychus urticae* and *Diptacus* sp. We identified the species of *Diptacus*, as new species of this genus, *Diptacus rubus* n.sp. González-Domínguez & Santillán-Galicia, in Mexican blackberry crops. We described its morphology and biology.

**Key words:** *Species diversity, Rubus, organic production, conventional production, phytophagous mites.*

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*Con amor*

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## CONTENIDO

RESUMEN .....	iii
ABSTRACT.....	iv
LISTA DE FIGURAS.....	viii
LISTA DE CUADROS.....	ix
INTRODUCCIÓN .....	1
REVISIÓN DE LITERATURA .....	2
<b>Literatura Citada</b> .....	12
CHAPTER I. MITES IN BLACKBERRY: DIVERSITY, POPULATION DYNAMICS AND SPATIAL DISTRIBUTION .....	17
<b>1.1. ABSTRACT</b> .....	17
<b>1.2. INTRODUCTION</b> .....	18
<b>1.3. MATERIAL AND METHODS</b> .....	19
<b>1.4. RESULTS</b> .....	22
<b>1.5. DISCUSSION</b> .....	29
<b>1.6. REFERENCES</b> .....	33
CHAPTER II. BIOLOGY AND DESCRIPTION OF A NEW SPECIES OF <i>Diptacus</i> (ACARI: ERIOPHYOIDEA: DIPTILOMIOPIDAE) ON BLACKBERRY ( <i>Rubus ulmifolius</i> SCHOTT) IN MEXICO.....	38
<b>2.1. ABSTRACT</b> .....	38
<b>2.2. INTRODUCTION</b> .....	39
<b>2.3. MATERIAL AND METHODS</b> .....	40
<b>2.4. RESULTS</b> .....	42
<b>2.5. DISCUSSION</b> .....	51
<b>2.6. REFERENCES</b> .....	53
CONCLUSIONES GENERALES.....	56

## LISTA DE FIGURAS

### CAPITULO II

Figure 1. Mean number of *Diptacus* sp. mites in orchards with organic (a) and conventional (b)  
-----26

Figure 2. Mean number of *Tetranychus urticae* mites in orchards with organic (a) and conventional  
(b)-----27

### CAPITULO III

Figure 1 *Diptacus rubus* n.sp. -----50



## LISTA DE CUADROS

Cuadro 1 Producción de zarzamora nacional----- 2

### Capítulo I

Table 1 Species, taxonomic families and number of specimens of mites mounted for the identification in two systems of production (organic and conventional). -----23

Table 2 Diversity indices estimated for each species in the blackberry orchards with organic production -----24

Table 3 Diversity indices estimated for each species in the orchards with conventional production -----24

Table 4 Diversity indices estimated for mite populations in organic and conventional production systems-----25

Table 5 SADIE index of aggregation (ia) and distribution for each group of mites -----28

### Capítulo II

Table 1 Measurements of *Diptacus rubus* n.sp. mites from *Rubus* sp. (l—length, w—width). --46

## INTRODUCCIÓN

En México hay más de 9 000 ha establecidas de zarzamora (*Rubus ulmifolius* Scoth) principalmente en los estados de Baja California, Jalisco, México y Michoacán (SIAP 2020), este último concentra más del 60 % de la producción (Strik *et al.* 2007). Este cultivo, junto con el de fresa, el arándano y la frambuesa, ocupan el segundo lugar en exportación de productos agrícolas después del aguacate (SIAP 2020).

El cultivo es afectado por diferentes plagas y enfermedades que conllevan importantes costos en el manejo (Segura *et al.* 2012; Trinidad *et al.* 2019). Los ácaros fitófagos causan daños ya que sus estiletes penetran al tejido foliar y succionan su contenido (Nyoike y Liburd 2013), lo que provoca alteraciones fisiológicas tales como, pérdida del vigor, disminución de la calidad y en la composición de nutrientes, lo que se refleja en pérdidas en el rendimiento (Archer *et al.* 2016).

Los ácaros fitófagos tienen una alta capacidad reproductiva, lo que les permite sobrevivir y establecerse con éxito en diferentes ambientes (Nyoike y Liburd 2013), incluso han seleccionado mecanismos de resistencia ante el combate químico de sus poblaciones (Van Leeuwen *et al.* 2015), aunque también existen especies depredadoras en el cultivo que son utilizadas para suprimir a las especies plagas (McMurtry *et al.* 2013).

En México, la principal plaga de ácaros en zarzamora, *Tetranychus urticae*, ocasiona daños en frambuesa y zarzamora (Márquez-Chávez *et al.* 2019); además, se han encontrado otros fitófagos de las familias Eriophyidae y Tarsonemidae (Ayala-Ortega *et al.* 2019) y algunos depredadores de la familia Phytoseiidae. Debido a la importancia económica de la zarzamora en nuestro país, es necesario conocer la diversidad de ácaros fitófagos y depredadores en la producción orgánica y convencional de este cultivo, lo cual permitirá proponer diferentes métodos de manejo, que minimicen los daños causados por los ácaros fitófagos.

## REVISIÓN DE LITERATURA

### *Rubus ulmifolius*

Las moras son frutas ricas en antioxidantes y nutrientes, por ello, su demanda aumentó considerablemente durante las últimas dos décadas (Strick *et al.* 2007; Stupková 2016). México es el principal exportador de zarzamoras *R. ulmifolius* en el mundo por volumen de venta y la mayor producción está concentrada geográficamente en el Valle de Los Reyes, Michoacán (Stupková 2016), aunque también se produce en otros estados (Cuadro 1).

Cuadro 1 Producción de zarzamora nacional

Estado	Superficie (ha)			Producción (ton)	Rendimiento (ton/ha)
	Sembrada	Cosechada	Siniestrada	Obtenida	Obtenido
Baja California	86				
Colima	152	152		1320	8683
Ciudad de México	5	5		17	3646
Guanajuato	27	26		260	9812
Hidalgo	1	1		2	2200
Jalisco	742	732		8032	10 979
México	17	16		111	6948
Michoacán	8 675	8 112		148 048	18 250
Morelos	13	11		33	3 000
Puebla	34	34		386	11 399
Querétaro	2	2		13	11 399
Tlaxcala	1	1		13	12 700
<b>Total</b>	<b>9 754</b>	<b>9 092</b>		<b>158 239</b>	<b>17 404</b>

La zarzamora es una baya del género *Rubus* L. subgénero *Rubus* Watson que tiene más de 400 cultivares denominados originarios que van desde selecciones silvestres hasta liberadas (Clark y Finn 2011). Existe una gran cantidad de variedades de *Rubus* debido al mejoramiento genético, necesario para obtener características deseables como, calidad de la fruta, rendimiento y resistencia a factores bióticos y abióticos. Es una planta perenne, con estructuras vegetativas bianuales, con dos tipos de estructuras frutíferas que determinan las fechas de cosecha, con hábitos de crecimiento

erectos y rastreros, cuyo fruto es una polidrupa, y se adapta a diversas condiciones climáticas (Clark y Finn 2011; Finn y Clark 2011).

Actualmente, este cultivo se produce en sistema convencional y orgánico; de este último sistema, en los últimos años se ha extendido la superficie sembrada. El cultivo de zarzamoras en el país es por medio de la producción forzada que consiste en la aplicación de reguladores de crecimiento como el tidiazurón (TDZ) (N-fenil-N-1,2,3-tidiazol5-ilurea), ácido giberelico (AG<sub>3</sub>) y el uso de prácticas agrícolas como la poda, con el objetivo de estimular la formación de flores y frutos de las principales estructuras fructíferas como las primocañas que propician la primera cosecha durante el otoño (Galindo-Reyes *et al.* 2004 y 2006), y las frutocañas que dan origen a las frutas de segundo corte (Clark y Perkins-Veazi 2011), lo cual permite el retraso o adelanto en la programación de las cosechas y el manejo del mercado (Vincent *et al.* 2010; Rebek 2017); además, también determinan la disponibilidad de follaje, lo que trae como consecuencia el establecimiento temporal de diversas especies de artrópodos (Kumral y Kovanci 2007). El éxito del establecimiento y desarrollo de las zarzamoras en México, está determinado por la capacidad adaptativa principalmente a temperaturas más elevadas y a la tolerancia a suelos más “pesados” (Finn y Clark 2011).

Los monocultivos permiten la estandarización de técnicas y la aplicación generalizada en grandes superficies como la utilización de variedades mejoradas, manejo de la inocuidad y producción forzada que determina las diferentes etapas fenológicas, porte de la planta y períodos de cosecha (Thrupp 2000; Segura *et al.* 2012; Pritts 2012; Kumral y Kovanci 2007).

## **Ácaros asociados a zarzamora**

### **Familia Tetranychidae**

La mayoría de las especies de esta familia son polífagas; pocas discriminan a sus hospederos (McGregor 1950). Esta familia es considerada de las más importantes entre los ácaros fitófagos, por las pérdidas en la agricultura y plantas ornamentales (Othman y Zhang 2003). Se alimentan del contenido celular del parénquima de sus hospedantes (Nyoike y Liburd 2013), lo que provoca decoloración, amarillamiento o desecación y un retraso del crecimiento (McGregor 1950; Nyoike y Liburd 2013). Su ciclo de vida corto y su alto potencial reproductivo, les ha permitido generar diversos mecanismos de resistencia, principalmente a plaguicidas sintéticos (Nyoike y Liburd 2013; Onstad y Knolhoff 2013).

En zarzamora *Tetranychus urticae* (Koch), o ácaro de dos manchas, es considerada la plaga más importante (Márquez- Chaves *et al.* 2019), ya que se alimenta del envés de las hojas, extrae la clorofila y reducen el rendimiento (Akyazi y Liburd 2019). También ocasiona pérdidas importantes en fresa y frambuesa por las altas poblaciones que presenta (Gong *et al.* 2018).

### **Familia Tarsonemidae**

El ácaro blanco o araña ciclamina *Polyphagotarsonemus latus* (Banks, 1904), se asocia a más de 55 especies de plantas (Gerson 1992). Debido a su alimentación causa un bronceado y distorsión de los márgenes de las hojas tiernas en fresa (Renkema *et al.* 2017). Está ampliamente distribuido en estados productores de zarzamora de EE. UU. y donde ocasiona detención del crecimiento y distorsión de hojas apicales jóvenes, principalmente de las primocañas (Vincent *et al.* 2010). En Portugal se reportó como una plaga de importancia en moras bajo invernadero (Ferreira 2016), y en Brasil se ha reportado en bajas poblaciones, en zarzamora var. Tupy

(Marchetti y Ferla 2011). Otras especies de la familia Tarsonemidae fueron encontrados con menor frecuencia como *Xanotarsonemus* sp., y *Tarsonemus* sp. en el mismo cultivar (Marchetti y Ferla 2011).

### **Superfamilia Eriophyiodea**

Estos ácaros tienen una amplia distribución y se asocian a plantas con flores, coníferas y helechos; son cosmopolitas y se han descrito más de 3000 especies en las familias Phytoptidae, Eriophyiidae y Diptilomiopidae (Oldfield 1996); tienen un alto nivel de especificidad e inyectan toxinas a sus hospederos que pueden provocar distorsiones (Oldfield 1996). Aun cuando estas sintomatologías se asocian a estos ácaros, también hay otros llamados “vagabundos” que han logrado un alto nivel adaptativo y aparentemente no ocasionan ningún tipo de daño (Manson y Oldfield 1996). Por su alimentación pueden ocasionar agallas en hojas, yemas y bronceados, además algunas plantas presentan alargamiento de los tallos florales, laterales y deformaciones de ramas completas, como consecuencia se detiene el crecimiento (Keifer 1982). En *Rubus* sp. se han descrito especies del género *Acalitus* de la familia Eriophyiidae (Keifer 1982), *Diptacus*, *Rhynacus* (Keifer) y *Apodiptacus* (Kuang) de la familia Diptilomiopidae (Xin & Dong 1983; Domes 2000; Wang *et al.* 2009).

En esta superfamilia se presentan dos tipos de ciclo de vida: el sencillo, que se caracteriza por tener sólo un tipo de hembra llamada protoginia y el ciclo complejo, que además de presentar una protoginia, tiene otra forma morfológica de hembra conocida como deutoginia (Oldfield 1996). Los eriofidos pasan por los estados de huevo, larva, ninfocrisálida, ninfa, imagocrisálida y el adulto (tanto la ninfocrisálida como la imagocrisálida son estados quiescentes) (Manson y Oldfield 1996).

El estudio de la biología de estos ácaros es limitado por la dificultad en su manejo debido a su tamaño diminuto (Olfield 1996).

### **Familia Tenuipalpidae**

Los ácaros de esta familia son conocidos como falsas arañas y se conocen más de 600 especies descritas, asociados a más de 900 especies vegetales (Childers *et al.* 2003). La relación con su hospedante es por su alimentación en células epidérmicas de tallos, frutos y hojas (Mesa *et al.* 2009) y son plagas importantes de algunos cultivos por ser transmisores de virus (Gerson 2008). En *Rubus* sp. se han asociado las especies de *Cenopalpus brachypalpus*, *C. naupakticus* y *C. taygeticus* en Grecia (Hatzinikolis *et al.* 1999). En Irán se describió *Cenopalpus irani* y *Brevipalpus obovatus* en *Rubus* sp. (Pejman 2013; Ferreira 2016); mientras que, en Brasil se reportó la presencia de *B. phoenecis* y *B. yothersi* (Marchetti y Ferla 2011; Trinidad *et al.* 2019).

### **Familia Tydeidae**

Estos ácaros son cosmopolitas y se asocian a varias especies vegetales, donde además de sus hábitos fitófagos, pueden ser micofágos y depredadores. *Brachytydeus* es el género con mayor número de especies, seguido de *Tydeus* (da Silva *et al.* 2013). En estudios faunísticos en Irán *Rubus* sp. reportan a *Tydeus* sp. y a *Brachytydeus* sp. (Pejman 2013), y en Suecia a *Orthotydeys kochi* (Momen y Lundqvist 1996). En Brasil, se encontró en diferentes variedades de zarzamora a *T. californicus* y a *Tydeus* sp. (Marchetti y Ferla 2011; Trinidad *et al.* 2019), este último también se ha reportado en México (Ayala-Ortega 2019).

## **Familia Acaridae**

Los acaridos son cosmopolitas y sus principales hábitos alimenticios son fungívoros en una amplia gama de materiales almacenados, como cereales, productos lácteos, frutos secos, paja y en animales domésticos. Entre las especies más importantes se encuentra *Tyrophagus putrescentiae*, este ácaro se alimenta principalmente de hongos saprófitos; sin embargo, algunas especies pueden causar daño económico en plantas ornamentales y hortalizas en invernadero por su alimentación de raíces y brotes tiernos (Kiriskik *et al.* 2018).

Existe evidencia de que *Acarus siro*, *Rhizoglyphus robini* y *Tyrophagus putrescentiae* son parte de la fauna en *Rubus* sp. (Pejman 2013), siendo esta última la que tiene mayor asociación con moras silvestres y cultivadas (Marchetti y Ferla 2011).

## **Familia Phytoseiidae**

Los ácaros de la familia Phytoseiidae son los agentes de control biológico de ácaros más importantes y usados en la agricultura (McMurtry y Croft 1997). Algunas especies se han estudiado y utilizado ampliamente en el combate biológico de ácaros y trips plaga (McMurtry y Croft 1997; De Moraes *et al.* 2004); actualmente hay más de 2250 especies descritas distribuidas en diferentes hábitats (De Moraes *et al.* 2004). Algunos se alimentan de otros ácaros y otros pueden sobrevivir con polen (Dhooia 2016). Hoy en día, son un componente importante en la supresión de plagas; sin embargo, sus poblaciones pueden ser susceptibles a los acaricidas de amplio espectro (Dhooia 2016; McMurtry 1997).

En zarzamora se han encontrado los depredadores de los géneros *Metaseiulus*, *Neoseiulus*, *Amblyseis*, *Euseius*, *Galendromus* y *Typhlodromus*, principalmente en brotes tiernos (McMurtry 2012). En Portugal, Brasil y México la mayoría de los depredadores de Phytoseiidae que se han



encontrado en moras silvestres son principalmente de los géneros *Typhlodromus* y *Amblyseius* (Ferreira 2016; Marchetti y Ferla 2011; Ayala-Ortega *et al.* 2019). Los ácaros más utilizados de forma artificial en cultivos con alto valor comercial incluyen a especies de *Phytoseiulus* que, están dirigidos al control de la familia de la Tetranychidae y *Typhlodromus* y *Amblyseius* que son más generalistas (McMurtry y Croft 1997).

### **Diversidad específica**

La diversidad biológica representa la variedad de vida de una población desde el genoma hasta los ecosistemas más complejos; para determinarla es necesario el análisis en poblaciones con individuos del mismo orden taxonómico pertenecientes a un sistema; además, la diversidad está estrechamente relacionada con la riqueza de especies y la uniformidad con la que los individuos se distribuyen en un área geográfica (Delang y Li 2013).

La riqueza es el número de especies presentes en una muestra, aunque, también se puede expresar en una unidad de área que va desde un sitio determinado y es conocida como diversidad alfa, hasta un área o región de estudio completa llamada gamma (Fedor y Zvaríková 2018). La dominancia es otra característica importante, ésta refleja la energía acumulada de las especies presentes en el sistema; es decir, si cada especie tiene la misma cantidad se puede considerar con la misma dominancia, por el contrario, si una de las especies tiene un número máximo de individuos se considera altamente dominante (Thukral *et al.* 2019).

Para cuantificar cada uno de estos elementos se han implementado cálculos o índices que permiten conocer las características ecológicas y biológicas, así como la estructura de las poblaciones (Thukral *et al.* 2019; Washington 1984). La mayoría de los índices de dominancia y diversidad consideran la probabilidad de ocurrencia de una especie en una comunidad o muestra

y el análisis de la organización de una comunidad permite conocer el número de especies, individuos, el promedio de individuos por especie y el número total de ello en una comunidad dispersa o densa (Thukral *et al.* 2019)

Los más utilizados en el estudio de comunidades son los de Shannon que permiten conocer la diversidad de especies y la dominancia se establece por medio del índice de Simpson's (Spellerberg y Fedor 2003; Thukral *et al.* 2019).

### **Dinámica y distribución espacial**

Es importante comprender las interacciones que tienen los organismos de un ecosistema con los factores bióticos y abióticos, para poder predecir la función que tienen en el sistema y su comportamiento.

La dinámica de una subpoblación es impulsada por varios procesos como la natalidad y mortalidad, o la densidad y movimiento de individuos que resultan en patrones de distribución dentro de un área determinada, además, los procesos ecológicos actúan sobre el ciclo de vida de los organismos e influyen en su distribución (Borregaard *et al.* 2008), y son afectados por factores naturales como la migración, dispersión y por el hombre (Gilad 2008; Kumral y Kovanci 2007).

Respecto a la distribución poblacional, existen tres tipos: el aleatorio, que se presenta cuando el entorno es muy homogéneo y no hay ventajas para la agregación en torno a un recurso; es decir, los individuos se van a distribuir al azar; la distribución uniforme es más regular; mientras que, la distribución agregada es la más común en la naturaleza; en este tipo de distribución los individuos están más cerca de lo que estarían si se distribuyen al azar o de manera uniforme, esto puede ser por la disponibilidad de alimento, agua, refugio u otros recursos o factores (Gilad 2008). Estos patrones espaciales son básicos para comprender los procesos y dinámicas ecológicas, así

como la competencia intra e interespecífica, los sistemas de depredación y diseminación de plagas (Borregaard *et al.* 2008).

En los monocultivos como la zarzamora, las complejas prácticas agrícolas pueden generar cambios importantes en las estructuras de poblaciones que integran un agroecosistema e incluso, pueden alterar la composición de especies presentes en la zona, afectando su biología, comportamiento y dinámica poblacional (Kumral y Kovanci 2007).

## OBJETIVOS E HIPÓTESIS

### Objetivo general

Determinar la diversidad específica, fluctuación poblacional y distribución espacial de las especies de ácaros asociadas al cultivo de zarzamora en huertas con producción orgánica y convencional.

### Objetivos específicos

1. Determinar la diversidad específica de ácaros en huertas con producción orgánica y convencional de zarzamora.
2. Determinar la fluctuación poblacional y distribución espacial de las principales familias de ácaros en huertas con producción orgánica y convencional de zarzamora.
3. Identificar y describir las especies nuevas de la familia Diptilomiopidae presentes en zarzamora
4. Determinar la biología de *Diptacus* sp. en zarzamora

### Hipótesis

La diversidad específica de ácaros es mayor en el cultivo de zarzamora con producción orgánica comparada con la de producción convencional. El tipo de producción influye en los cambios de su población y distribución espacial.

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# CHAPTER I. MITES IN BLACKBERRY: DIVERSITY, POPULATION DYNAMICS AND SPATIAL DISTRIBUTION

## 1.1.ABSTRACT

We studied the species diversity of mites in five blackberry orchards, three with organic and two with conventional production. The study was carried out in 2018 during eight months with samplings on a monthly interval. From each orchard, 40 samples containing leaves, flowers and fruits were collected every month. We collected 16 667 mites in total, and 1 391 specimens were mounted for identification. We analysed the population fluctuation of the most abundant species, and determined their spatial distribution using the Spatial Analysis by Distance Indices (SADIE). We found 11 species distributed in seven taxonomic families. The most abundant species throughout the study were *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Diptacus* sp. Keifer (Acari: Diptilomiopidae). Two predatory mite species were present in both production systems, *Amblyseius andersoni* Chant and *Neoseiullus fallacis* Garman (Acari: Phytoseiidae). The species diversity in both production systems were similar, but a greater number of mites were found in organic orchards in comparison to orchards with conventional production. The distribution of the two phytophagous species were mostly aggregated or uniform throughout the study. We suggest additional studies at a larger scale, including geographically distant blackberry production areas to confirm our results.

**Keywords:** SADIE analysis, organic production, conventional production, phytophagous mites.

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## 1.2.INTRODUCTION

In Mexico, the production of the blackberry, *Rubus ulmifolius* Schott, has greatly increased due to the high demand of this fruit in Asia, Europe and USA (Segura *et al.* 2012; Clark y Finn 2014). As a result, Mexico have become the main producer worldwide (Strik 2007; Stupková 2016). Currently, there are more than 13,000 ha in Mexico producing blackberry (SIAP 2020), and Michoacan is the main producer in Mexico with more than 60% of the total production (Strik *et al.* 2007; FIRA 2016).

Mites represent one of the main problems affecting the blackberry production (De Lillo and Duso 1996; Trinidad *et al.* 2019). Mite populations has normally been reduced using synthetic acaricides in different crops (Van Leeuwen *et al.* 2014), including blackberry (Gordon 1997). However, for any control strategy, the correct and accurate identification of all phytophagous mite species is very important in order to design and apply the appropriate control methods (Bajwa *et al.* 2013). Furthermore, the presence of natural enemies, such as predatory mites, needs to be determined to elucidate the potential role of these mites to reduce phytophagous mite populations (Cardinale *et al.* 2003). Regardless the control method adopted, knowing when to apply a control method is important, and such decision should be based on a periodic sampling to determine population dynamics, where knowledge of the spatial distribution becomes vital to develop accurate sampling methodologies (Kuno 1991).

Despite the importance of blackberry, studies reporting species diversity of mites or their spatial distribution in this crop are still very scarce. Recent studies from Mexico and Brazil done in blackberry, reported as the main mite species, *Tetranychus urticae* (Koch), *Diptacus rubuculosum* (Trinidad, Duarte & Navia, 2018), *Brevipalpus yothersi* (Baker) and *Polyphagotarsonemus latus* (Banks, 1904). In addition, different natural enemies from the genera

*Amblyseius*, *Galendromus*, *Euseius* and *Neoseiulus*, and the family Tydeidae have also been reported (Marchetti & Ferla 2011; Trinidad *et al.* 2019; Ayala-Ortega *et al.* 2019).

Species diversity of mites and their spatial distribution can be affected by abiotic and biotic factors, and such effects are important to understand, before any management program is proposed (Barbosa *et al.* 2003; McCoy 2009; Alves *et al.* 2010; Marchetti & Ferla 2012). An increasing demand for organic food (Jensen 2011), has also caused a greater demand for organic blackberry. Differences between organic and conventional methods of production may also affect the species diversity of mites, as the use of pesticides in the conventional regime may have an impact on all mites including phytophagous and predatory species (Barbar 2017). We believe that blackberry crops under organic production will have greater species diversity of mites, favouring also the presence of predatory mites. Therefore, our aim was to compare the species diversity, population dynamics and spatial distribution of mites in blackberry crops produced under organic and conventional methods.

### **1.3.MATERIAL AND METHODS**

#### **Collection of mites, mounting and identification**

The study was conducted in five orchards of blackberry (*R. ulmifolius*) var. Tupi. Two orchards using conventional production method (HPC2 and HPC3), and three using organic production method (HPO1, HPO4 and HPO6). All orchards were located in the region named “Los Reyes de Salgado”, in the state of Michoacan. In each orchard, samplings were carried out on a monthly basis from April to December 2018. In each orchard, 40 plants were systematically sampled, and each sample contained leaves from the lower, medium and upper strata of the plant.

In each site, samples containing leaves, flowers and fruits were collected. Samples were deposited inside plastic bags in a cool box, and transported to the laboratory. All samples were maintained at 7 °C until processed but never longer than seven days. Mites from samples were recovered by washing them with soapy water and passing the water through different sieves with openings of 200, 300 and 500 µM. Mites recovered from each sieve were preserved in 70% ethanol. We collected a total of 16,667 mites in total, and from these, 1,391 specimens were mounted for identification which represented 8% of the mites collected. For the identification, individual mites were mounted using a modified Keifer medium (De Lillo *et al.* 2010). Mountings were maintained at 40 °C for 15 days. Identification of mites were carried out using phase contrast microscope (Olympus BX41, Olympus Corporation of the Americas, PA, USA) using the 40X and 100X objectives. For the Eriophyiodea and Tarsonemidae genera, the taxonomic keys developed by Amrine *et al.* (2003) and Lindquist (1982) respectively were used. For the rest of the mites the following keys were used: Ewing (1939), Keifer (1952), Baker (1968), Gupta (1975), Papadoulis & Emmanouel (1991), Seeman & Beard (2011), da Silva *et al.* (2013), Darbemamieh *et al.* (2016) and Tixier *et al.* (2016). Climatological data were kindly provided by the company “GRUPO LOS CERRITOS Berries & Aguacate S.A. DE C.V.” from its meteorological station located at Los Reyes de Salgado (19° 35.93' N - 102° 29.44' W).

### **Diversity indices**

Diversity indices were estimated using the 1,391 individuals mounted and identified. We used the software ANAFAU (Moraes *et al.* 20003), which performs faunistic analyses that classifies each mite species found into categories within each index. The indices estimated were frequency (percentage of individuals of the same species in relation to the total individuals),

abundance (number of individuals of the same species per area), dominance (action exerted by some species over others causing the appearance or disappearance of other species) and constancy (percentage of species present throughout time) (Moraes *et al.*, 2003).

In addition, the following indices for each production system were estimated. The species diversity index of Shannon-Wiener ( $H'$ ) as described by Spellerberg and Fedor (2003):

$$H = - \sum_{i=1}^n p_i \ln p_i$$

the index of Pielou ( $J'$ ) to determine the evenness of the species diversity (Pielou 1966), where the closest the value to “1” the more even the number of individuals among the different species:

$$J' = \frac{H}{H'_{max}}$$

the Simpson’s dominance index ( $C'$ ) to determine the dominance of the species as described by (Thukral *et al.* 2019).

$$C' = \sum_{i=1}^k p_i^2$$

where the probability of occurrence of individuals of a species in a community ( $P_i$ ) is estimated as,

$$P_i = \frac{\text{Number of individuals from a given species in a community or area}}{\text{Total number of individuals of all species in the community or area}}$$

## Population dynamics

Studies describing the population dynamics and spatial distribution were done using all mites collected from the most abundant species (*T. urticae* and *Diptacus* sp). Data for each group of mites was analysed separately. For the population dynamics studies, prior the analyses, data were transformed using Box-Cox ( $\lambda=0.1$ ). Transformed data were analysed using linear mixed models that included a power variance model with production method (organic vs. conventional)

and time as main effects, and their interactions. Variance components were estimated using the restricted maximum likelihood method (REML) using the statistical software GenStat v. 8.0 (Payne *et al.* 2005).

### **Spatiotemporal distribution**

Distribution of the different group of mites were analysed using Spatial Analysis by Distances Indices (SADIE) (Perry 1996) using the software SADIEshell v. 1.22 (Conrad and Perry 2001). This analysis provided indices and tests of non-randomness for spatial patterns of counts using all the available spatial information (Perry 1997; 1998). The output of the analysis provided index of aggregation ( $Ia$ ) where  $Ia > 1$  indicates aggregation,  $Ia = 1$  indicates random distribution and  $Ia < 1$  indicates uniform distribution (Korie *et al.* 2000).

## **1.4.RESULTS**

In the blackberry eleven species of mites were identified distributed in seven taxonomic families comprising phytophagous, predators and mycophagous. Ten species were found in the organic crops and 10 in the conventional crops (Table 1).

The most abundant species were *Diptacus* sp. and *T. urticae*, followed by the predatory mite *Amblyseius andersoni*, with a greatest number in the organic compared to the conventional orchards. The remaining species represented approximately the 8% of the total specimens identified.

Table 1 Species, taxonomic families and number of specimens of mites mounted for the identification in two systems of production (organic and conventional).

Family	Species	Organic	Conventional	Habits
Tetranychidae	<i>Tetranychus urticae</i>	348	89	
Diptilomiopidae	<i>Diptacus</i> sp.	527	230	
	<i>Brevipalpus yothersi</i>	7	2	Phytophagous
Tenuipalpidae	<i>B. californicus</i>	1	1	
Tarsonemidae	<i>Polyphagotarsonemus latus</i>	2	0	
	<i>Tarsonemus</i> sp.	11	35	
Phytoseiidae	<i>Amblyseius andersoni</i>	56	28	Predator
	<i>Neoseiullus fallacis</i>	9	1	
Tydeidae	<i>Brachytydeus Mexicana</i>	4	7	Mycophagous
	<i>Tydeus kochi</i>	0	15	Predators
Acaridae	<i>Tyrophagus putrescentiae</i>	6	12	Mycophagous
<b>Total</b>		<b>971</b>	<b>420</b>	

### Diversity indices

The characteristics of each mite species found in both production systems are shown in Table 2 and 3, for the organic and conventional systems respectively. In both cases, *T. urticae* and *Diptacus* sp. are the most dominant, abundant and consistent species. The remaining species showed a range of indices indicating a not significant presence in both blackberry production systems. However, the two predatory mite species *A. andersoni* and *N. fallacis* showed a greater dominance, frequency and constancy in the organic system (Table 2) compared to the conventional production system (Table 3).



Table 2 Diversity indices estimated for each species in the blackberry orchards with organic production after analysis with ANAFAU.

Species	No. individuals	Dominance	Abundance	Frequency	Constance
<i>Tetranychus urticae</i>	348	SD	Sa	SF	W
<i>Diptacus sp.</i>	527	SD	Sa	SF	W
<i>Amblyseius andersoni</i>	56	SD	Sa	SF	W
<i>Neoseiullus fallacis.</i>	9	D	c	F	Y
<i>Brevipalpus yothersi</i>	7	D	c	F	Y
<i>B. californicus</i>	1	ND	r	IF	Y
<i>Polyphagotarsonemus latus</i>	2	ND	d	IF	Y
<i>Tarsonemus sp.</i>	11	ND	ma	VF	Y
<i>Tyrophagus putrescentiae</i>	6	ND	c	F	W

SD = super dominant, D = dominant, ND = not dominant, Sa = super abundant, c = common, r = rare, d = disperse, va = very abundant, SF= super frequent

Table 3 Diversity indices estimated for each species in the orchards with conventional production after analysis with ANAFAU.

Specie	No. Individuals	Dominance	Abundance	Frecuency	Constance
<i>Tetranychus urticae</i>	89	SD	sa	SF	Y
<i>Diptacus sp.</i>	230	SD	sa	SF	W
<i>Amblyseius andersoni</i>	28	D	ma	VF	W
<i>Neoseiullus fallacis</i>	1	ND	d	IF	Z
<i>Brevipalpus yothersi</i>	2	ND	d	IF	Y
<i>B. californicus</i>	1	D	d	IF	Z
<i>Tarsonemus sp.</i>	35	D	ma	VF	W
<i>Brachytydeus mexicana</i>	7	D	c	F	Y
<i>Tydeus kochi</i>	15	D	c	F	W
<i>Tyrophagus putrescentiae</i>	12	D	c	F	Y

SD = super dominant, D = dominant, ND = not dominant, Sa = super abundant, c = common, r = rare, d = disperse, va = very abundant, SF= super frequent

The greater diversity index ( $H'$ ) was found in orchards with a conventional production system (1.61) compared to the organic orchards (1.06). The evenness of species was grater in the

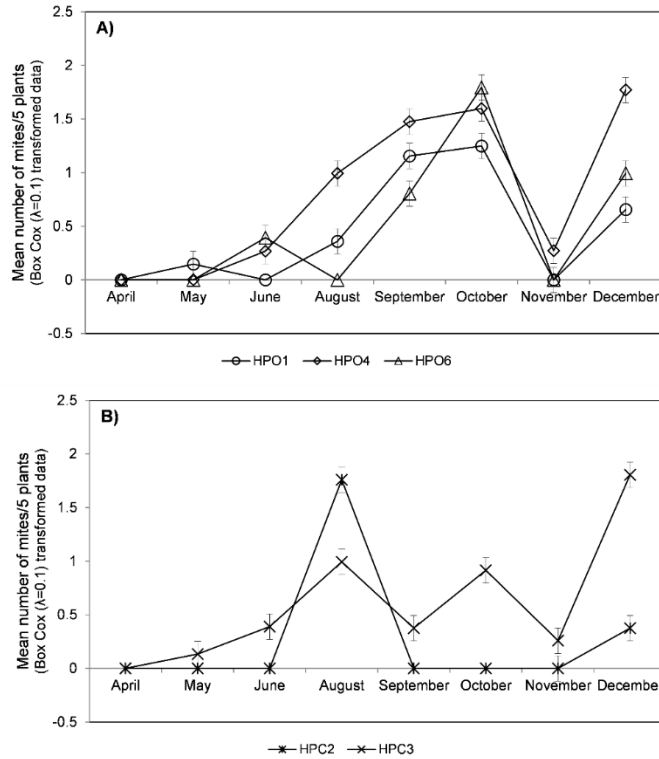
conventional system compared to the organic; however, the dominance of some species over the others was greater in the organic production system compared to the conventional system (Table 4).

Table 4 Diversity indices estimated for mite populations in organic and conventional production systems. N = number of individuals, S = number of species, H' = Shannon-Wiener index, J' = Pielou's index, D = Simpsons's index of dominance.

<b>Production system</b>	<b>N</b>	<b>S</b>	<b>(H')</b>	<b>(J')</b>	<b>D</b>
Organic	971	10	1.06	0.46	0.95
Conventional	420	10	1.61	0.69	0.64

### Population dynamics

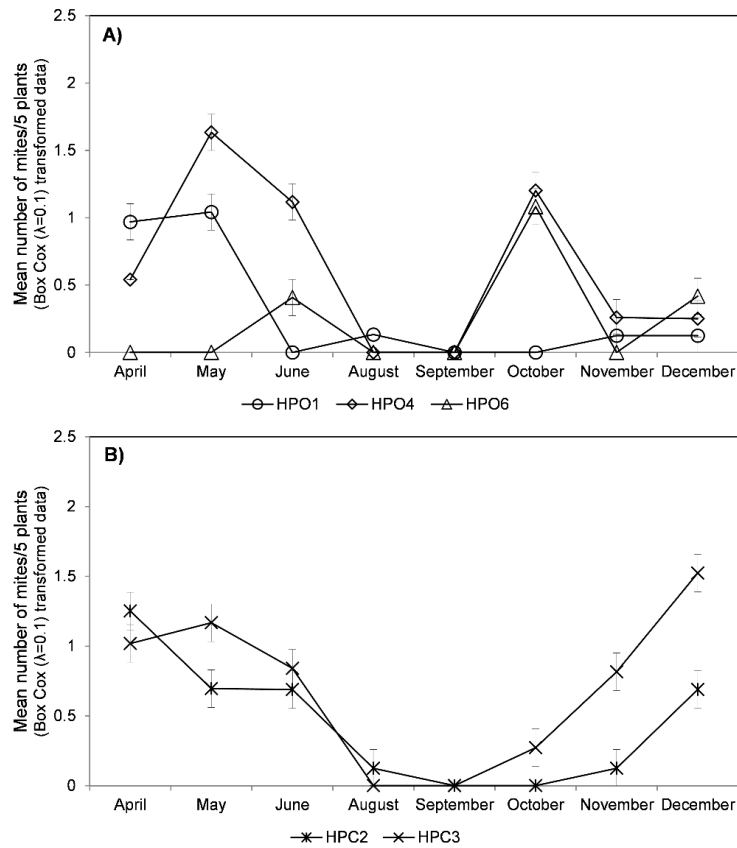
The population dynamics of *Diptacus* sp. was different between crops under organic (combining data from the three crops) and conventional (combining data from the two crops) production systems ( $\chi^2_1=24.26$ ,  $P<0.001$ ). When a comparison was made only among organic crops, significant differences were found ( $\chi^2_2=35.90$ ,  $P<0.001$ ), so as between the two conventional crops ( $\chi^2_1=21.64$ ,  $P<0.001$ ). The effect of time in the population dynamics of *Diptacus* sp. was significant among the organic ( $\chi^2_{11}=61.37$ ,  $P<0.001$ ) and conventional ( $\chi^2_7=113.63$ ,  $P<0.001$ ) crops. Overall, the *Diptacus* sp. population was greater in the HPO4 orchard compared to the other orchards; then, populations declined in all three orchards by November and again increased with the HPO4 orchard showing the greatest populations (Fig 1A). Populations of *Diptacus* sp, were generally smaller compared to the organic orchards (Fig 1). Overall, no individuals were detected until August in the orchard HPC2, and then again, another peak in December; however, this pattern was different for the orchard HPC3, with individuals detected throughout the study with three peaks in August, October and December (Fig 1B).



**Figure 1.** Mean number of *Diptacus* sp. mites per five plants collected in orchards with organic (A) and conventional (B) production systems. Error bars represents  $1 \pm \text{SEM}$  (Standard Error of the Mean).

When comparing the population dynamics of *T. urticae* between organic (combining the three orchards) and conventional (combining the two orchards) production systems, significant differences were found ( $\chi^2_{1}=6.48$ ,  $P<0.001$ ). Overall, greater populations were observed in the organic orchards (Fig 2). Significant differences were obtained when compared among the organic orchards ( $\chi^2_{3}=26.43$ ,  $P<0.001$ ) where populations of *T. urticae* were always greater in orchards HPO1 and HPO4 compared to HPO6 (Fig 2A). Similarly, significant differences were found between the two conventional orchards ( $\chi^2_{1}=14.96$ ,  $P<0.001$ ), which despite showing similar patterns, the greatest populations were normally found in the orchard HPC3 (Fig 2B). Time had a significant interaction with the population dynamics of *T. urticae* in the organic orchards ( $\chi^2_{11}=76.77$ ,  $P<0.001$ ). Populations were always lower in the orchard HPO6, with increases in June, October and December. The largest populations of *T. urticae* were found in the orchard

HPO4, especially during May, June and October (Fig 2A). Time had also a significant interaction with time in the *T. urticae* populations in the conventional orchards ( $\chi^2_7=28.96, P=0.001$ ). Overall, in the orchard HPC3 were normally greater compared to HPC2 in all sampling times, except in April (Fig 2B).



**Figure 2.** Mean number of *Tetranychus urticae* mites per five plants collected in orchards with organic (A) and conventional (B) production systems. Error bars represents  $1 \pm \text{SEM}$ .

### Spatiotemporal distribution

The distribution of this mite species was not consistent throughout the different sampling times, being mostly aggregated or uniform, and this was regardless the type of production system used (organic and conventional). However, it was evident that the presence of *Diptacus* sp. was more consistent in the organic orchards as more sampling dates can be found with mites, compared

to the orchards with conventional production (Table 5). The distribution of *T. urticae* was also mostly aggregated and uniform, with only one date reporting a random distribution (Table 5). Interestingly, the presence of this mite species was slightly more consistent in the conventional orchards compared to the organic.

Table 5 SADIE Index of Aggregation (Ia) and distribution for each group of mites during the eight months of the study. P values estimated after randomization tests.

Orchards	Month	<i>Diptacus sp.</i>			<i>Tetranychus urticae</i>		
		Ia	P	Distribution	Ia	P	Distribution
HPO1	April	-	-	-	1.612	0.203	Aggregated
	May	-	-	Aggregated	1.97	0.002	Aggregated
	June	-	-	-	-	-	-
	August	2.775	0.0002	Aggregated	-	-	-
	September	0.827	0.771	Uniform	-	-	-
	October	1.213	0.149	Aggregated	-	-	-
	November	-	-	Uniform	-	-	-
	December	0.981	0.44	Uniform	-	-	-
HPO4	April	-	-	-	1.217	0.167	Aggregated
	May	-	-	-	0.95	0.48	Uniform
	June	-	-	-	0.952	0.475	Uniform
	August	1.259	0.131	Aggregated	-	-	-
	September	1.553	0.036	Aggregated	-	-	-
	October	1.538	0.037	Aggregated	1.002	0.393	Random
	November	-	-	-	-	-	-
	December	1.1	0.242	Aggregated	-	-	-
HPO6	April	-	-	-	-	-	-
	May	-	-	-	-	-	-
	June	-	-	-	-	-	-
	August	-	-	-	-	-	-
	September	0.859	0.693	Uniform	-	-	-
	October	1.984	0.003	Aggregated	1.514	0.035	Aggregated
	November	-	-	-	-	-	Uniform
	December	1.045	0.31	Random	1.179	0.184	Aggregated
HPC2	April	-	-	-	1.263	0.139	Aggregated
	May	-	-	-	0.911	0.56	Uniform
	June	-	-	-	0.811	0.829	Uniform
	August	1	0.388	Random	-	-	-
	September	-	-	-	-	-	-
	October	-	-	-	-	-	-
	November	-	-	-	-	-	-
	December	-	-	-	1.164	0.208	Aggregated

**Table 1.** SADIE Index of Aggregation (Ia) and distribution for each group of mites during the eight months of the study. P values estimated after randomization tests.

	April	-	-	-	2.27	0.003	Aggregated
	May	-	-	-	0.976	0.042	Uniform
	June	-	-	-	1.065	0.31	Aggregated
HPC3	August	1.295	0.114	Aggregated	-	-	-
	September	-	-	-	-	-	-
	October	0.892	0.606	Uniform	1.161	0.205	Aggregated
	November	-	-	-	0.97	0.449	Uniform
	December	2.516	0.0002	Aggregated	0.905	0.577	Uniform

## 1.5.DISCUSSION

Our results represent one of the most complete reports of mite diversity in Mexican blackberry orchards. We compared organic and conventional production systems. The greater index diversity ( $H'$ ) was obtained in the conventional production system compared to the organic production (Table 4). Overall, populations of mites were always greater in the organic orchards (Fig 1), compared to conventional orchards (Fig 2). This is not surprising as the synthetic acaricides used in the conventional production systems, may have caused the reduction in the overall mite populations. We found *Diptacus* sp. as the most abundant species in both production systems, which is in line to other reports from Brazil where another species, *D. rubusculosum*, was reported as the most abundant in blackberry (Trinidad *et al.* 2019). However, our data on the morphological characteristics of the *Diptacus* specimens we found, suggest that is indeed a new species, the manuscript reporting this information has just been submitted. In Brazil individuals of the genus *Chakrabartiella* sp. which is in the family Diptilomipidae, as *Diptacus* has been reported as abundant in blackberry (Marchetti and Ferla 2011). In Mexico, the genus *Asetadiptacus* (Diptilomiopidae) has also been reported in blackberry (Ayala-Ortega *et al.* 2019). The abundance

of taxonomically related species on the same crop could be due to the close relationship between mites and the host plant as well as similarities in agronomical practices (Olfield 1996; Dhooria 2016). It is important to notice that the type of damage that *Diptacus* may cause to blackberries remains unclear. For example, no evident relationship has been reported between *D. rubusculosum* and damages to blackberry in Brazil (Trinidad *et al.*, 2019).

After *Diptacus* sp., *T. urticae* has been found as the second largest populations in blackberry, regardless the orchards were organic or conventional, and such results are in line to what was reported previously for Mexican blackberry orchards (Ayala-Ortega *et al.* 2019; Márquez-Chávez *et al.* 2019). This species has also been reported causing damages in strawberry orchards (Monteiro *et al.* 2014). Other studies with strawberry from Brazil, reported *Neotetranychus asper* and *Olygonychus yothersi* as the main spider mites (Tetranychidae) affecting blackberry (Marchetti and Ferla 2011; Trinidad *et al.* 2019). Differences in species diversity and abundance among orchards of the same host plant, in this case blackberry, may be the result of differences due to physiological alterations in orchards caused by the chemicals used to force flower production (Clarck and Finn 2014). This physiological alteration may cause production or reduction of leaves, which eventually will induce changes in species diversity or the spatial distribution of mites (Kumral and Kovanci 2005; Rijal *et al.* 2016).

The spatial distribution of *Diptacus* sp. and *T. urticae* were not consistent throughout the study (Table 5), and this was regardless the production system studied. It is likely that changes in the developmental stage of the blackberry plants, which are artificially induced using chemicals in both, organic and conventional, will produce changes in the distribution within the plants. This was observed in *Tetranychus* sp. populations in cotton crops, where the distribution of the mites changed according to the different developmental stages of the plant (Wilson *et al.* 1983).

Regarding the predatory mites, we found *Amblyseius andersoni* and *Neoseiullus fallacis* in both types of production systems with a greater number of individuals of *A. andersoni* compared to *N. fallacis*. Both mite species showed always a greater number of individuals in the organic orchards compared to the conventional (Cuadro 2). The mite *A. andersoni* has been previously reported in *Rubus* sp. (Grabovska and Kolodochka 2014; Stojnic *et al.* 2018), and as the most abundant predatory mite in strawberry and raspberry feeding on *T. urticae* (Ragusa and Ragusa 1997; Sikorska *et al.* 2019). However, in other studies in Michoacan Mexico, blackberry orchards, reported the presence of *Typhlodromalus peregrinus* and *Neoseiulus californicus* as the most abundant species (Ayala-Ortega *et al.* 2019). We believe our results differed from these other studies due to differences in agronomical practices including control strategies used of the production of blackberry.

We found *B. yothersi* and *B. californicus* in both production systems but with a greater abundance of the former species. *B. yothersi* has been previously reported in Mexican blackberry orchards (Ayala-Ortega *et al.* 2019); however, to the best of our knowledge this is the first report of *B. californicus* in blackberry from Mexico. *Brevipalpus phoenicis* s.l. has been previously reported in *Rubus* sp., from Brazil, apparently without causing evident damages (Marquetti and Ferla 2011).

*Tarsonemus* sp. was found in both production systems but in very low populations, and even fewer *P. latus* individuals, and only in organic orchards. These species were also reported previously in blackberry in Mexico (Ayala-Ortega *et al.* 2019) and Brazil (Marquetti and Ferla 2011). The few individuals of *Tarsonemus* sp. and *P. latus* we found here, were similar to what has been reported in Brazil (Marquetti and Ferla 2011; Trinidad *et al.* 2019a *et al.* 2019). However, *P. latus* has been reported as an emergent important pest in strawberry in Florida (LeFors *et al.*



2017), Arkansas, North and South Caroline (Johnson *et al.* 2016), which suggest this species needs to be consistently monitored to detect changes in its population density. This represent the first report of *T. putrescentiae* for blackberry in Mexico. Is not clear why we found this species in blackberry as *T. putrescentiae* has been previously reported in stored grains and as parasites of rodents in Mexico (Estebanes-Gonzalez and Rodriguez-Navarro 1991; Estébanes-González *et al.* 2011; Abundes-Arteaga *et al.* 2020). This species has been reported in soybean plants (de Oliveira *et al.* 2007), and the authors suggested that the presence of this mite in the field could be from using infested seeds, and we believe something similar happened in blackberry. We also found *B. mexicana* (Syn. *Paralorryia mexicana*) and *Tydeus kochi* (Tydeidae). Their role in blackberry orchards are unclear, as *B. mexicana* is considered as a generalist predator and *T. kochi* as a mycophagous. *B. mexicana* has been previously reported in citrus orchards (Baker 1968; Liberato *et al.* 2016) and *T. kochi* in *Capsicum annum* var. *glabriusculum* (Dunal) Heiser & Pickersgill, and none of them with a significant presence in these orchards.

Overall, the species diversity in both blackberry production systems we studied were similar. In both cases, the most important species was *T. urticae*, considered as the most damaging species, and *Diptacus* sp. as the most dominant species. We expected a greater diversity of mites including predatory species in the organic orchards compared to conventional orchards, mostly due to the differences in the synthetic acaricides application regimes; however, we only obtained differences in number of specimens of these species rather than diversity. It is likely that more diversity could be found on a larger scale, for example comparing blackberry orchards between states of Michoacan, Jalisco and Colima, Mexico, where the effect of management intensity on a landscape scale can be studied.

In conclusion, the production system (organic vs conventional) in blackberry orchards from Michoacan had no significant effect on the diversity of mite species, nor their spatial distribution. The main species of phytophagous mites were *T. urticae* and *Diptacus* sp. Our study was done only in orchards from the estate of Michoacan, we suggest that additional studies are required at a larger scale, which should include other geographically distant production areas, to confirm our results in blackberry.

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**CHAPTER II. BIOLOGY AND DESCRIPTION OF A NEW SPECIES OF *Diptacus*  
(ACARI: ERIOPHYOIDEA: DIPTILOMIOPIDAE) ON BLACKBERRY (*Rubus  
ulmifolius* SCHOTT) IN MEXICO**

**2.1. ABSTRACT**

Blackberry (*Rubus ulmifolius* Scott), is an economically important crop in Mexico. Eryophyoid mites are amongst the most important arthropod pests affecting production of this crop. Despite this, reports on species diversity of Eriophyidae on blackberry in Mexico are very scarce. We sampled six orchards from two municipalities in the state of Michoacan, the most important region for blackberry production. Sampling was done monthly between May and November 2018. Only one species of eryophyoid mite was found, *Diptacus rubus* **n.sp.** González-Domínguez & Santillán-Galicia, a new species associated with blackberry in Mexico. The biology and morphology of this new species are described.

**Keywords:** *Taxonomy, measurements, developmental stage.*

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## 2.2.INTRODUCTION

Blackberries (*Rubus ulmifolius* Scott) have become increasingly attractive to consumers due to their high levels of antioxidants (Schulz *et al.*, 2019). Currently, Mexico is the main producer of blackberries worldwide (Stupková 2016) and economically it is amongst the most important crops in Mexico; the state of Michoacan produces 60% of the Mexican blackberry crop (Strik *et al.* 2007; Servicio de Información Agroalimentaria y Pesquera [SIAP] 2020).

Mites from the family Tetranychidae and the superfamily Eriophyoidae are amongst the most important arthropod pests affecting the blackberry production (Marchetti & Ferla 2011). Typically, egg, larvae, nymphochrysalis, nymph, imagochrysalis and can be observed in the life cycle of eriophyoid mites (Sternlicht & Goldenberg 1971; Mason & Oldfield 1996). The nymphochrysalis and imagochrysalis are pre-ecdysial inactive stages between larva and nymph and between nymph and adult respectively (Sternlicht & Goldenberg 1971).

Despite the economic importance of this crop, there are few studies reporting species diversity and biology of eriophyoids (Eriophyidae, Phytoptidae and Diptilomiopidae) in Mexico (Marchetti & Ferla 2011; Trinidad *et al.* 2018). Currently, only *Acalitus essigi*, *A. orthomera* (Eriophyidae), *Chakrabartiella* sp. and *Diptacus rubusculosum* (Diptilomiopidae) have been reported on blackberry (Trinidad *et al.* 2018; Marchetti & Ferla 2011). Other mite species of the family Diptilomiopidae reported in *Rubus* spp. are *D. glaber* (Huang & Wang 2009), *D. gigantorubra* (Xin & Dong 1983), *D. caeseius* (Domes 2000), *D. chihouensis* (Wang *et al.* 2009), *D. rubi* (Kuang 2001), *Rhynacus abronius* (Keifer) (Cham. & Schltldl.) and *Apodiptacus rubi* (Kuang) (Domes 2000). To determine the species diversity of eriophyoid mites in Mexico, a field survey was done between April and November 2018, in six orchards from two municipalities in



the state of Michoacan. We also report on the morphology and biology of a new species from the genus *Diptacus*.

### 2.3.MATERIAL AND METHODS

#### Collection and identification of species

Monthly samples were taken between May and November 2018 from six blackberry (*R. ulmifolius* var. Tupi) orchards in the municipalities of Los Reyes de Salgado and Periban, Michoacan. In each orchard there were 40 sampling points that were randomly selected but covered the entire orchard. At each sampling point samples composed of the basal, intermediate and apical strata, buds, flowers, leaves and fruits infested with mites were collected in plastic bags and transported to the Acarology Laboratory, Colegio de Postgraduados, Mexico state in a cool box. Mites were washed off the plant samples and collected after filtration through 200, 300 and 500µm aperture sieves and were preserved in alcohol at 70%.

Identification of mites was done using phase contrast microscopy (PCM) and scanning electronic microscopy (SEM). For PCM evaluation, specimens were mounted in modified Keifer mounting liquid on glass slides (De Lillo *et al.* 2010). Slides were maintained at 40° C for 15 days before observations were made. All specimens were evaluated using an Olympus microscope (model BX41, Olympus Corporation of the Americas, PA, USA) with the 40X and 100X objectives. Photographs were taken using a digital camera (Olympus) attached to the microscope. For genus identification, we used the information reported by Amrine *et al.* (2003) and the genitalia anatomy reported by Lindquist (1996). We measured all the structures suggested for the identification of eriophyid mites (De Lillo *et al.* 2010). All measurements were made in microns (µM) using the software Image J v 1.5 (Abramoff *et al.* 2004) and are shown in Table 1. For the

description of the new species we used one female holotype and three male paratypes. Eight hundred permanent slides were made for species identification, all mounted specimens were deposited in the Acarology collection of the Crop Protection Department, Colegio de Postgraduados.

For SEM analysis, two females and one male, previously maintained in 70% ethanol, were used. The specimens were freeze dried, to ensure all ethanol was removed, and then coated with colloidal gold by ionization (1 mA) for 100 seconds in a sputter coater (Fine-Coat Ion Sputter JFC-1100, JEOL Ltd., Tokyo, Japan). They were then observed and photographed in the vacuum chamber of a SEM (JOEL JSM-6390, JEOL Ltd., Tokyo, Japan).

### **Biology and development**

For this experiment, adult mites of the new species identified were collected from blackberry plants at the same sampling sites as described previously and the development time of their progeny determined on leaf discs in Petri dish arenas. Blackberry leaf discs were cut from leaves harvested from four-month-old blackberry plants grown under glasshouse conditions in 20 L pots of compost (1:1:1 mix of peat moss: coconut fibre: vermiculite). Leaf discs (0.5 cm diameter) were cut from leaves avoiding leaf ribs, washed and rinsed with distilled water, and dried with a clean paper towel. Single leaf discs were placed in the base of 5 cm diameter Petri dishes containing damp sterile cotton wool. For ventilation the lid of the Petri dish had a 3 cm diameter hole covered with printing mesh. An individual adult female mite was placed, using a fine brush (size 15/0), into each arena (n=24) and incubated at 25 °C, 60% RH in a 12:12 light:dark regime for 10 hours during which time she laid eggs. The females and any additional eggs were then removed leaving only one egg in each arena. The cotton wool in each arena was moistened every 24 h with 3 mL of

sterile distilled water and leaf discs replaced every four days. Observations were made every 12 h using a stereomicroscope (Nikon SMZ 1500, Nikon Instruments, Inc. Melville, NY) to determine when the egg hatched and how many and how long each life stage was until the adult stage was reached. The duration of each developmental stage was estimated using the equations described by Perring *et al.* (1984). The duration of the egg stage was estimated using the following equation:

$$\frac{Te_1 + Te_2}{2} - T0 = TE$$

Where:  $Te_1$ = time before egg hatch,  $Te_2$  = time after egg hatch,  $T0$ = time when oviposition took place, and  $TE$ = time to complete egg stage. The larval stage was considered to last until it became inactive (nymphochrysalis). The total larval developmental time was estimated using the equation:

$$\frac{Tl_1 + Tl_2}{2} - TE = TL$$

Where,  $Tl_1$ = time before the larva became quiescence (nymphochrysalis),  $Tl_2$ = time spent as a nymphochrysalis,  $TE$ = time spent as an egg, and  $TL$ = time spent as a larva. All other developmental stages were estimated using the same equation.

## 2.4.RESULTS

All 800 specimens mounted were from the genus *Diptacus* and from the same species. According to our data, the morphological characteristics obtained did not match any of the six species currently described associated to *Rubus* sp. Therefore, we consider that the specimens collected belong to a new species of Diptilomiopidae, genus *Diptacus*, namely *Diptacus rubus* **sp. nov.** Gonzalez-Domínguez & Santillán-Galicia from blackberry plants (*R. ulmifolius* var. Tupy) in

Mexico. We consider that individuals of this species were vagrants on the leaf surface because no visible damage was observed on the plants.

## **Taxonomy**

Family Diptilomiopidae Keifer 1944

Subfamily Diptilomiopinae Keifer 1944

Genus *Diptacus* Keifer 1951

*Diptacus rubus* **sp. nov.** Gonzalez-Domínguez & Santillán-Galicia (**Figs. 1, Table 1**)

**Differential diagnosis.** The main differences to other species were the number of dorsal and ventral annuli, the length of the scapular setae (*sc*), the pattern present on the microtubercles of the genital shield, the shape of the spermathecal apparatus and the number of empodium rays.

## **Description**

All measurements are presented in microns ( $\mu\text{M}$ ) followed by the minimum and maximum values in parenthesis. The asterisk indicates no variation was found amongst the measurements made for that specific morphological attribute.

**FEMALE:** (n=16). Body fusiform, 231(225-237), 69 (69-83) wide, yellowish to brownish.

**Gnathosoma** 55 (48-60), projecting downwards; pedipalp coxal seta *ep* 2 (1-2), dorsal pedipalp genual seta *d* 10 (7-15); chelicerae 55 (52-60), auxiliary stylets 53 (46-60). **Prodorsal shield** 35 (33-37), 45 (39-54) wide, broad based and apically rounded frontal lobe 2 (1-2), 3 (3-4) wide; prodorsal shield ornamented, with a net of sinuous lines, incomplete median line connected to admedian and submedian lines by short transversal lines forming cell like structures; epicoxal area

with small sinuous lines and protruding structures aligned longitudinally; fused prosternal apodeme. Scapular tubercles on rear shield margin 2 (1-2) apart, scapular seta *sc* 2 (1-2) projecting posteriad. **Coxigenital region** with incomplete (3-4) and complete (6-7) annuli. Shield with incomplete lines and small granulations aligned transversally. Lateral seta on coxisternum I *lb* 14 (11-19), 11 (10-12) apart; proximal seta on coxisternum I *la* 11 (10-13), (10) apart; proximal seta on coxisternum II *2a* 35 (30-40), 30 (30-32) apart. **Legs** with all segments; seta *bv* absent on both legs. **Leg I** 42 (40-44), femur 13 (12-15); genu 6 (4-7), antaxial genual setae (*l''*) 36 (32-39); tibia 13 (11-15), paraxial tibial setae (*l'*) (11); tarsus 8 (6-9), paraxial, fastigial tarsal setae (*ft'*) 14 (11-23), antaxial, fastigial tarsal setae (*ft''*) 16 (13-22), paraxial, unguinal tarsal setae (*u'*) 5 (4-7), tarsal empodium (*em*) 7 (6-7), divided, 15-rayed, tarsal solenidion  $\omega$  7 (5-8), slightly curved and knobbed. **Leg II** 39 (36-43); femur 13 (12-14); genu 5 (4-7), antaxial genual seta (*l''*) 11 (10-13); tibia 12 (10-13); tarsus 8 (6-10), paraxial, fastigial tarsal setae (*ft'*) 16 (13-33), antaxial, fastigial tarsal setae (*ft''*) 20 (15-25), paraxial, unguinal tarsal setae (*u'*) 4 (4-7); tarsal empodium (*em*) 8 (6-7), divided, 15-rayed\*; tarsal solenidion ( $\omega$ ) 8 (6-10), slightly curved and knobbed. **Opisthosoma** with 74 (72-77) dorsal annuli, with minute microtubercles on rear margin of each annulus; 69 (66-73) ventral annuli microtuberculated. Seta *c2* 38 (34-45) on ventral annulus 2 (2-4); seta *d* 49 (45-55) on ventral annulus 17 (10-20), seta *e* 40 (36-46), 23 (22-26) apart, on ventral annulus 35 (30-40); seta *f* 37 (32-43), 24 (21-26) apart, on ventral annulus 56 (49-62). Seta *h1* minute; *h2* 42 (40-44). **External genitalia** 23 (21-25) long, 28 (27-29) wide, coverflap with short irregular dashes, genual setae *3a* 13 (8-13) long. **Internal genitalia** with anterior genital apodeme trapezoidal, with an oblique apodeme under the anterior genital apodeme; and a pre-spermathecal tube joined to the longitudinal bridge; spermathecal tube directed towards the anterior region forming a steep angle with a leaf shaped spermatheca.

**MALE: (n=11).** Body fusiform, 234 (227-237), 84 (78-88) wide, yellowish to brownish. **Gnathosoma** 55 (51-58), projecting downwards, pedipalp coxal seta *ep* 2 (1-3), dorsal pedipalpal genual seta *d* 10 (6-13); chelicerae 53 (50-58), auxiliary stylets 53 (47-58), oral stylets 55 (50-58). **Prodorsal shield** 34 (30-38), 48 (44-53) wide, broad-based and apically rounded frontal lobe 1 (1-2), 3 (2-4) wide; scapular tubercles ahead of rear shield margin, scapular seta (*sc*) 1 (1-2). **Coxigenital region** with complete annuli 6 (6-7). **Coxisternal shield** with incomplete lines and small granulations transversally aligned. Prosternal apodeme fused. Antero lateral seta on coxisternum I *lb* 32 (30-39); proximal seta on coxisternum I *la* 10 (10-16); proximal seta on coxisternum II *2a* 12 (9-18). **Legs** with all segments; seta *bv* absent on both legs. **Leg I** 38 (34-45), femur 12 (7-16); genu 5 (5-6), antaxial genual setae (*l'*) 28 (25-33); tibia 12 (10-15); tarsus 8 (6-11), paraxial, fastigial tarsal setae (*ft'*) 16 (12-22), antaxial, fastigial tarsal setae (*ft''*) 20 (16-26), paraxial, unguinal tarsal setae (*u'*) 4 (4-5), empodium 6 (6-7), divided, (15) rayed\*, solenidion  $\omega$  7 (6-8), slightly curved and knobbed. **Leg II** 37 (33-43); femur 13 (11-16); genu 4 (3-5), antaxial genual setae (*l''*) 20 (16-23); tibia 11 (9-13); tarsus 7 (6-8), antaxial, fastigial tarsal setae (*ft''*) 20 (15-25), paraxial, fastigial tarsal setae (*ft'*) 20 (16-26), paraxial, unguinal tarsal setae (*u'*) 5 (4-7), tarsal empodium (*em*) 6 (6-7)\*, divided, – rayed (15)\*; tarsal solenidion ( $\omega$ ) 8 (7-11), slightly curved and knobbed. **Opisthosoma** with 68 (66-69) dorsal annuli, with minute microtubercles on rear margin of each annulus, 63 (61-66) ventral annuli microtuberculated. Seta *c2* 40 (38-44), on ventral annulus 2 (2-3); seta *d* 45 (42-49), on ventral annulus 17 (15-20); seta *e* 46 (40-49), 22 (21-23) apart, on ventral annulus 34 (30-38); seta *f* 40 (35-44), 22 (21-22) apart, on ventral annulus 54 (50-58). Seta *h1* minute; *h2* 45 (42-49). External genitalia 18 (17-18) long, coverflap with short irregular dashes, genital setae *3a* 10 (8-13) long.

1 Table 1 Measurements of *Diptacus rubus* n.sp. mites from *Rubus* sp. (L—length, W—width).

Characteristic	Female (n=16)		Male (n=11)		Larva (n=5)		Nymph (n=5)	
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max
Idiosoma L	231.7±1.1	225-237	234.5±1	227-237	160±0.6	158.2-161.4	183.4±0.9	180.7-186.5
Idiosoma W	77.2±1.1	69.4-83.5	84.8±1	78-88	45.6±1.5	41-49	45.5±0.9	42-47
Prodorsal shield L	35.2±0.3	33-37.8	34.5±0.7	30.9-38.8	28.3±0.8	26.3-30.4	29.8±1.1	27-32.7
Prodorsal shield W	45.6±0.7	39.6-54	48±1.02	44-53.7	43.6±0.9	42-47.2	44.2±0.9	41.8-46.6
Scapular seta (sc) L	1.9±1.1	1.2-2.5	1.8±0.1	1-2.4	1.1±0.02	1.1-1.2	1.5±0.1	1.1-1.7
Frontal lobe L	2 ± 0.17	1.5-2.8	1.7±0.07	1.5-1.7	1.7±0.07	1.5-2	1.7±0.7	1.5-2
Frontal lobe (base) W	3.3 ± 0.19	3-4	3±0.3	2-4	3±0.3	2-4	3±0.3	2-4
Gnatosoma L	55.1±0.6	48-60	55.8±0.6	51-58	45.6 ±1.5	41-49	45.4±0.9	42-47
Dorsal pedipalp genual seta (d)	10.3±0.6	7-15	10.8±0.5	6.9-13	6.3±0.4	5.2-8	7.6±0.4	6.5-9
Pedipalp coxal seta (ep)	2.3±0.07	1.7-2.9	2±0.1	1.5-2.9	1.7±0.1	1.2-1.9	1.9±0.1	1.7-2.3
Chelicerae L	55.9±0.4	52-60	53.9±0.9	50-58	46.4±0.8	44.4-48.9	50.1±1.4	46-53.9
Auxiliary stylet L	53±0.6	46-60	53.4±1	47.1-58.2	44.1±1.4	40.8-47.8	43.5±0.7	41.7-46.1
Oral stylet L	52±0.7	42-54	55.5±1	50.5-58.9	40.6±1.3	37-45.4	41.3±0.7	39-43.1
Leg I L	42±0.3	40-44.5	38.4±1.1	34.5-45	26.1±0.3	25.2-27.2	25.9±1.2	23.5-29.8
Femur I L	13.5±0.2	12.5-15	12.7±0.7	7.3-16	11.3±0.3	10.3-12.4	10.3±0.7	9.1-12.5
Genu I L	6.6±0.2	4.6-7.9	5.3±0.1	5-6.7	4.1±0.2	3.6-4.8	4.3±0.2	3.9-5.4
Antaxial genual I(I'')	36±0.6	32.5-39	28.8±0.8	25.4-33.8	21.3±1.1	18.2-23.8	22.1±1.3	18.6-25.6
Tibia I L	13.4±0.2	11-15	12.1±0.4	10.1-15	5.5±0.02	4.9-5.9	4.9±0.5	3.7-6.4
Tarsus I L	8.2±0.2	6.2-9.5	8.2±1.2	6.2-11.2	5.2±0.1	4.6-5.6	6.1±0.3	5.2-7.4
Paraxial fastigial tarsal seta (ft') L	14±0.8	11-23	16.3±1	12.2-22.6	8.9±0.72	7.4-10.7	10.8±0.4	9.4-11.8
Antaxial fastigial tarsal seta (ft'') L	16±0.7	16-25	20.3±1	16.8-26	9.9±0.18	9.5-10.4	16.5±0.6	14.7-18.7
Paraxial unguinal (u') L	5.1±0.2	4-7	4.8±0.1	4.1-5.8	4±0.15	3.6-4.4	4±0.2	3.4-4.6
Tarsal solenidion I L	7±0.1	5.4-8			5.5±0.1	4.9-5.9		
Empodium I L	7±0.08	6.5-7.5	6.8±0.1	6.3-7.5	4.5±0.3	4-6	5±0.4	3.9-6.1
Empodium I rays.	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)
Leg II	39.8±0.5	36-43	37.7±0.9	33.7-43.2	22.7±1.5	17.5-27	25.3±0.7	23.5-27.4
Femur II L	13±0.1	12.3-14.5	13.6±0.4	11.6-16	10.1±0.5	9-12.2	10.2±0.9	8.4-12.9
Genu II L	5.9±0.2	4.6-7.7	4.8±0.2	3.9-5.6	3.5±0.1	3.1-3.9	3.7±0.04	3.6-3.9
Antaxial genual II (I'')	11.6±0.2	9.9-13.8	20.4±0.7	16.5-23.7	5.3±0.2	4.7-5.8	13.3±0.3	12-14
Tibia II L	12.4±0.2	10.4-13.6	11.6±0.4	9.6-13.2	4.3±0.1	4.1-4.8	5.2±0.1	4.8-5.6
Tarsus II L	8.1±0.2	6.6-10	7.5±0.2	6.1-8.7	5.7±0.2	5.3-6.2	6.1±0.3	5.2-7.2
Paraxial fastigial tarsal (ft')	16±0.7	13-22.6	20.3±1	16.8-26.2	13.7±1.0	11.1-17.3	18.6±0.3	17.8-19.5
Paraxial fastigial tarsal (ft'')	20±0.8	15-25	20.4±1	15.7-25.5	14.02±1.0	12.4-18	16.4±0.6	14.7-18.7
Paraxial unguinal II (u') L	4.5±0.2	4-7	5.1±0.3	4-7	4.3±0.3	3.3-5	4.4±0.1	4.4-4.7
Tarsal solenidion II L	7±0.2	6.6-10	-	-	6±0.2	5.5-6.7	-	-
Empodium II L	7±0.2	6.5-7.6	6.8±0.1	6.3-7.5	3.8±0.1	3.5-4.4	4.6±0.3	4-5.5

Table 2 Measurements of *Diptacus rubus* n.sp. mites from *Rubus* sp. (L—length, W—width).

Empodium rays.	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)	15(2)
Coxal seta I (1b) L	14.5±0.6	11-19.6	32.7±0.9	30-39	4.8 ± 0.4	3.9-6.4	5.3±0.2	4.6-6
Distance between seta I (1b)	11.1±0.2	10.3-11.7	9.2±0.1	9-10	7.9±0.6	6.8-9.1	9.2±0.1	9-10
Seta I (1a) L	11.8±0.2	10-13.3	10±0.8	10.1-16	7.1 ± 0.3	6-7.8	4.6±0.3	3.9-6
Distance between seta I (1a)	10.1±0.2		9.7±0.2	0-10.3	11.3±0.2	10.9-11.6	9.7±0.2	9-10.3
Seda II (2a) L	35±0.9	30.5-40.9	12.4±1.0	9-18.2	11±0.5	9.5-12.3	13.6±0.8	12-17
Distance between seta II (2a)	30.9±0.8	30-31.9	26.4±0.1	26.2-26.6	22.8±1.1	22-24	21.2±1.1	20-23.5
Coxisternal incomplete annuli	3.6±0.2	3-4	3.6±0.3	3-4	3	3	3.6±0.3	3-4
Coxisternal complete annuli	6.6±0.2	6-7	6.3±0.3	6-7	5.3±0.3	5-6	6.3±0.3	6-7
Genitalia L	23	21-25						
Genitalia W	28.4	27.7-29	17.8±0.2	17-18.8	-	-	-	-
Oblicuo apodeme	5.5	5.2-5.9	-	-	-	-	-	-
Longitudinal bridge	6.6	6-7.2	-	-	-	-	-	-
Seta (3a) L	10.2±0.4	8-13.4	9.7±0.3	8-11.4	4.3±0.1		5±0.4	4.3-6.4
Seta (c2) L	38.2±0.8	34.8-45.9	40.9±0.6	38-44	12.2±0.9	10-14.6	14.7±0.5	13-16.2
Seta (c2) on annuli	2±0.2	2-4	2.5±0.2	2-3	7.8±0.2	7-8	8.6±0.4	8-10
Ventral seta I (d) L	49.8±0.8	45-55	45.4±0.8	42-49	35.6±0.8	23.4-27.3	16.4±0.4	15.3-18
Distance between seta (d)	46.3±1.5	42-51	38.5±0.6	37.4-39.5	36.6±0.5	36.2-37.2	36.6±0.3	35.9-37
Seta (d) on annuli	17±0.9	10-20	17.4±0.6	15-20	18±0.4	17-19	19.4±0.6	18-21
Ventral seta (e) L	40±0.7	36-46	44.6±1	40-49	16.4±0.2	16.4-17.9	15.5±1.2	11.9-19.3
Distance between seta (e)	22.8±	20-26	21.9±0.4	21.2-22.7	20.3±0.0	20.2-20.4	20.4±0.1	20.3-20.7
Seta (e) on annuli	35±0.8	30-40	34.1±1	30-38	27.6±0.5	26-29	30.2±1.02	28-33
Ventral seta (f) L	37±0.9	32.2-43	40±0.8	35.6-44.9	18±0.9	15.3-20.1	14.7±1.2	11.6-20
Distance between seta (f)	24.1±0.7	21-26	25.1±0.05	25-25.2	25.1±0.0	25-25.2	21.6±0.4	21.1-22.6
Seta (f) on annuli	56±1.1	49-62	54.5±1	50-58	39.6±0.7	37-41	45.2±1.8	40-50
Number of dorsal annuli	74.5±0.7	72-77	68.1±1	66-69	57.5±0.8	55-59	63.5±1.2	60-66
Number of ventral annuli	69±1.0	66-73	63.5±0.7	61-66	50.7±1.1	53-58	53.7±0.6	52-55
Seda (h2) L	42.3±0.3	40-44	45.3±0.8	42.2-49.9	24±0.9	22.1-26.2	28.8±0.7	26-30



**NYMPH:** (n=5). Body fusiform, 183 (180-187), 45 (42-47) wide, yellowish to brownish. **Gnathosoma** 45 (45-47), projecting downwards; pedipalp coxal seta *ep* 2 (1-2), dorsal pedipalp genual seta *d* 7 (6-9); chelicerae 50 (46-54), auxiliary stylet 43 (41-46). **Prodorsal shield** 29 (27-32), 44 (41-47) wide, broad based and apically rounded frontal lobe 1 (1-2), 3 (2-4) wide; scapular tubercles rear shield margin, scapular seta (*sc*) 2 (1-2). **Coxigenital region** 3 (3-4) with complete annuli. Shield with incomplete lines and small granulations transversally aligned; prosternal apodeme fused. Antero lateral seta on coxisternum I *Ib* 5 (4-6); proximal seta on coxisternum I *Ia* 4 (3-6); proximal seta on coxisternum II *2a* 13 (12-17). **Legs** with all segments; seta *bv* absent on both legs. **Leg I** 25 (23-30), femur 10 (9-13); genu 4 (3-5), antaxial genual setae (*l''*) 22 (18-26); tibia 5 (3-6); tarsus 6 (5-7), paraxial, fastigial tarsal setae (*ft'*) 10 (9-12), antaxial, fastigial tarsal setae (*ft''*) 16 (14-19), paraxial, unguinal tarsal setae (*u'*) 4 (3-5), empodium, divided, (15) rayed\*, solenidion  $\omega$  6 (4-6), slightly curved and knobbed. **Leg II** 25 (23-27); femur 10 (8-13); genu 3 (3-4), antaxial genual setae (*l''*) 18 (17-20); tibia 5 (4-6); tarsus 6 (5-7), antaxial, fastigial tarsal setae (*ft''*) 18 (17-20), paraxial, fastigial tarsal setae (*ft'*) 16 (14-19), paraxial, unguinal tarsal setae (*u'*) 4 (4-5), tarsal empodium 4 (4-5), divided, – rayed (15)\*; tarsal solenidion ( $\omega$ ) 5 (5-6), slightly curved and knobbed. **Opisthosoma** with 45 (40-50) dorsal annuli, with minute microtubercles on rear margin of each annulus, 53 (52-55) ventral annuli microtuberculated. Seta *c*2 14 (13-16), on ventral annulus 8 (8-10); seta *d* 16 (15-18), 36 (35-37) apart, on ventral annulus 19 (18-21); seta *e* 15 (11-19), 20 (20-21) apart, on ventral annulus 30 (28-33); seta *f* 15 (11-20), 22 (21-22) apart, on ventral annulus 45 (40-50). Seta *h*1 minute; *h*2 29 (26-30).

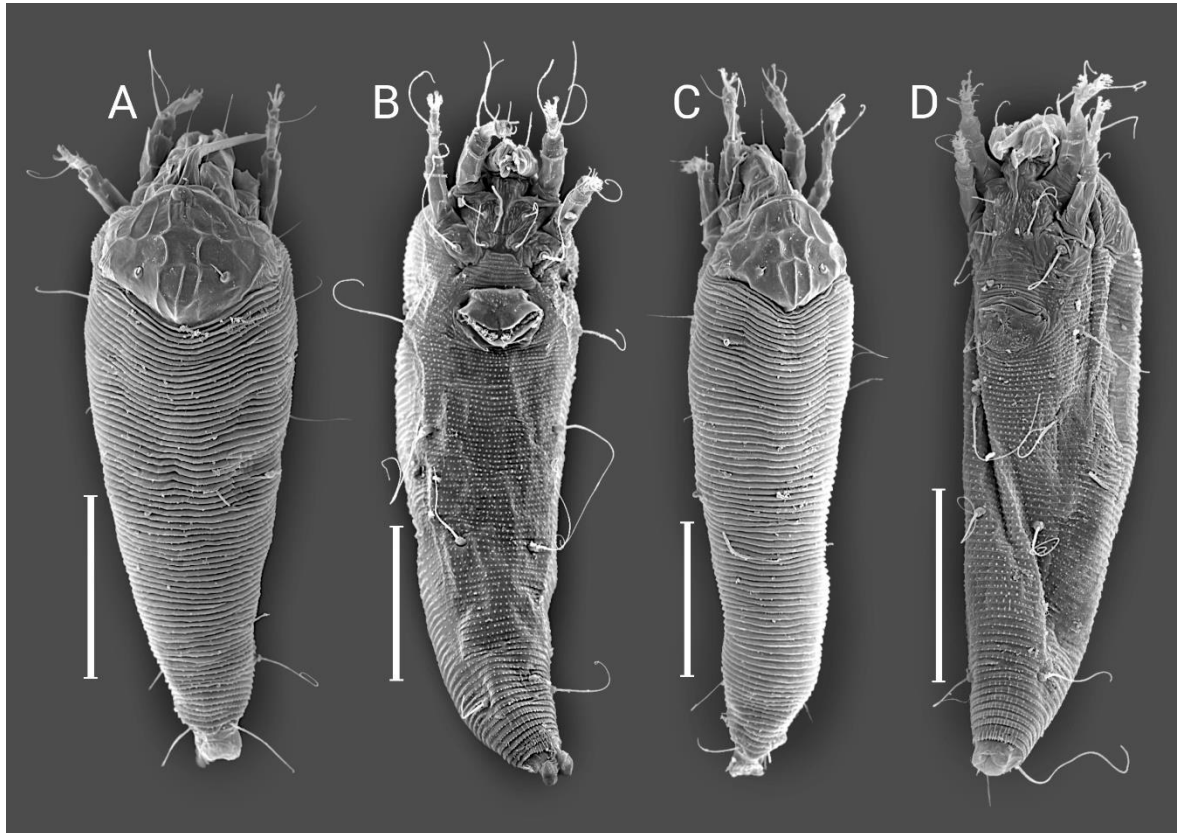
**LARVA:** (n=5). Body fusiform, 160 (158-161), 45 (41-49) wide, yellowish to brownish. **Gnathosoma** 45 (41-29), projecting downwards; pedipalp coxal seta (*ep*) 1.7 (1-2), dorsal

pedipalp genual seta (*d*) 6 (5-8); chelicerae 46 (44-49), auxiliary stylet 44 (40-48). **Prodorsal shield** 45 (41-49), 28 (26-31) wide, broad based and apically rounded frontal lobe 2 (1-2), 3 (2-4) wide; scapular tubercles rear shield margin, scapular seta (*sc*) 1 (1-2). **Coxigenital region** (3) with complete annuli. Shield with incomplete annuli and small granulations transversally aligned; posternal apodeme fused. Antero lateral seta on coxisternum I *Ib* 4 (3-6), 8 (6-9) apart; proximal seta on coxisternum I *Ia* 7 (6-7), 7 (6-8) apart; proximal seta on coxisternum II *2a* 11 (9.12), 23 (22-24) apart. **Legs** with all segments; seta *bv* absent on both legs. **Leg I** 26 (25-27), femur 11 (10-12); genu 4 (3-6), antaxial genual setae (*l''*) 21 (18-24); tibia 5 (4-6), paraxial tibial setae (*l'*) 22 (19-25); tarsus 5 (4-6), paraxial, fastigial tarsal setae (*ft'*) 9 (7-11), antaxial, fastigial tarsal setae (*ft''*) 9 (9-11), paraxial, unguinal tarsal setae (*u'*) 4 (3-4), empodium 4 (4-6), divided, (15) rayed \*, solenidion  $\omega$  5 (4-5), slightly curved and knobbed. **Leg II** 22 (17-27); femur 10 (9-12); genu 4 (3-4), antaxial genual setae (*l''*) 5 (4-7); tibia 4 (4-5); tarsus 6 (5-6), paraxial, fastigial tarsal setae (*ft'*) 13 (11-17), antaxial, fastigial tarsal setae (*ft''*) 14 (12-18), paraxial, unguinal tarsal setae (*u'*) 4 (3-5), tarsal empodium, divided, (15) rayed\*; tarsal solenidion ( $\omega$ ) 6 (5-7), slightly curved and knobbed. **Opisthosoma** with 57 (55-59) dorsal annuli, with minute microtubercles on rear margin of each annulus, 50 (53-58) ventral annuli microtuberculated. Seta *c2* 8 (7-8), on ventral annulus 8 (7-8); seta *d* 35 (23-27), 36 (36-37) apart, 18 (17-19); seta *e* 16 (16-18), 20 (20) apart, on ventral annulus 27 (26-29); seta *f* 18 (15-20), 25 (25) apart, on ventral annulus 39 (37-41). Seta *h1* minute; *h2* 24 (22-26).

**Type material.** Specimens were collected in the locality of Los Palillos (19° 33'25.4" N, -102° 28'34.5" E), municipality of Los Reyes de Salgado, Michoacan. Female holotype and 28

paratypes, 15 females and 13 males. All mounted specimens were deposited in the Acarology collection of the Crop Protection Department, Colegio de Postgraduados, Mexico.

Figure 1 *Diptacus rubus* n.sp. Dorsal (A) and ventral (B) view of the female. C y D) Ventral (C) and lateral (D) view of a male.



### **Biology of *Diptacus rubus* n.sp.**

*Diptacus rubus* had a simple life cycle with the same developmental stages typical of the Eriophyiodea superfamily: egg, larva, nymph and adult. They had two quiescent stages; the first was between the larval stage and the nymphal stage (nymphochrysalis), and the second was between the nymphal stage and the adult stage (imagochrysalis). The crystalline colour of the egg became yellow and translucent as it took on a semi-spherical shape. Fertilized females oviposited

on leaf surfaces, particularly along the secondary ribs. The embryo developed inside the egg before hatching. The mean duration of the egg stage was  $56.5 \pm 2$  h. Larvae were crystalline in colour and became translucent before the first quiescent stage; the mean duration of this stage was  $56.5 \pm 2$  h. The first quiescent stage, or nymphochrysalis, was white in colour with a mean duration of  $21.8 \pm 1.5$  h. There was only one nymphal stage, white in colour, and showed greater mobility and feeding activity than the larva; the mean duration of this stage was  $41.3 \pm 1.9$  h. The second quiescent stage, or imagochrysalis, was white in colour, and occurred just before the adult stage and had a mean duration of  $19.7 \pm 1.3$  h. The adult was white in colour and the most active stage with a mean longevity of  $114.5 \pm 2.7$  h.

## 2.5.DISCUSSION

Our study provides further knowledge about the morphology and biology of eriophyid mites from the genus *Diptacus* that are associated with *Rubus* sp. in Mexico. *Diptacus rubus* **n.sp.** is the first species from the family Diptilomiopidae to be reported on an economically important crop in Mexico. We did not find *Acalitus essigi* or *A. orthomera*, which have been reported previously on *R. ulmifolius* (Ayala-Ortega *et al.* 2019), despite sampling all plant structures and on six different occasions. It is unclear why we only found *D. rubus* **n.sp.** as the only eriophyid mite species, but it would be important to take more samples during different seasons and geographical regions in order to confirm our results.

We based our statement that *D. rubus* is a different and new species based on the following; *D. rubus* had 15 empodial rays, which was different to *D. gigantorubra*, *D. chizhouensis* and *D. caseius* all of which have five empodial rays (Xin & Dong 1983; Wang *et al.* 2009; Domes 2000); the oral stylet in *D. rubus* was four times larger (approx. 52  $\mu$ m) (Table 1) than reported for *D.*

*rubusculosum* (approx. 11  $\mu\text{m}$ ) (Trinidad *et al.* 2018); *D. rubus* had scapular setae that were ten times smaller (approx. 1.9  $\mu\text{m}$ ) than reported for *D. gigantorubra* (17  $\mu\text{m}$ ; Xing & Dong 1983), *D. chizhouensis* (14  $\mu\text{m}$ ; Domes 2000) and *D. caseius* (20  $\mu\text{m}$ ; Wang *et al.* 2009); the external genitalia of *D. rubus* had microtubercles without the longitudinally organized rings typical of *D. caseius* and *D. chizhouensis* (Domes 2000; Wang *et al.* 2009); the internal genitalia of *D. rubus* had a spermathecal tube joined to the longitudinal apodeme and the pre-spermathecal tube was swollen and directed to the anterior region of the body forming an steep angle, which was different to reports for other species in the family Diptilomiopidae (Chetverikov *et al.* 2015).

*Diptacus rubus* **n.sp.** had a simple life cycle with a protogyne female, which is a characteristic attribute to the mites known as ‘vagrants’ (Xiao-Feng & Xiao-Yue 2015). Female *D. rubus* deposited their eggs close to the secondary leaf ribs, presumably for better access to nutrients (Royalty & Perring 1996). The average duration from egg to the end of the adult stage was 13 days at  $25^{\circ}\text{C} \pm 2$ , with two quiescent stages between larva and nymph and between nymph and adult (Hall 1967; Manson & Olfield, 1996; Abou-Awad *et al.* 2010), known as nymphochrysalis and imagochrysalis respectively (Sternhcht & Goldenberd 1971). None of the active stages of *D. rubus* caused visible damage when feeding on plants as reported for *D. caseius* on *Rubus caeseius* (Domes 2000). It has been reported that the effect of eriophyid mites, in most cases, is non-symptomatic where mechanical damage caused by feeding activity are normally insignificant (Lindquist & Oldfield 1996). Potential explanations for the lack of visible damage to the plant tissues are that either chloroplast particles and cell contents are not fully extracted during feeding, or that the cell wall repairs itself after the mite’s mouthparts are removed (Lindquist & Oldfield 1996). The lack of visible damage has also been suggested as a result of low population densities of the mite, making damage to the plants negligible (Petanović & Kielkiewicz 2010).

In conclusion, we only found one species of eriophyid mite on the blackberry plants we sampled. This was *D. rubus* **n.sp.** which is adapted to live on the leaves as a vagrant with a simple life cycle comprising egg, larva, nymphochrysalis, nymph, imagochrysalis and adult with a protogyne female. This represents the first report of a species from the family Diptilomiopidae associated with an economically important crop in Mexico.

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## CONCLUSIONES GENERALES

Las especies más abundantes y dominantes en manejo orgánico y convencional fueron *Tetranychus urticae* y *Diptacus rubus* n.sp.

El manejo agronómico en el sistema productivo de zarzamora en Los Reyes de Salgado, Michoacán, no influye en la estructura y dinámica poblacional de los ácaros fitófagos y depredadores ya que en ambos casos la diversidad fue la misma. Esto puede estar determinado por la cercanía entre huertos, lo que puede influir negativamente en el número de especies por la deriva de agroquímicos.

La estructura y fluctuación poblacional de ácaros fitófagos y depredadores en el sistema productivo de zarzamora se asocia a la disponibilidad de follaje y está determinado por el manejo fisiológico también conocido como “forzado de la planta”.

La única especie que se encontró en zarzamora de Los Reyes de Salgado, Michoacán, fue *D. rubus* n.sp., un ácaro de hábito vagabundo que se desarrolla sobre las hojas. Tiene un ciclo de vida simple y pasa por huevo, larva, ninfocrisálida, ninfa, imagocrisálida y el adulto con una hembra protoginia. Es el primer acaro de la familia Diptilomiopidae en zarzamora de México.