UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSGRADO

DISTRIBUCIÓN Y PREVALENCIA DE *Bactericera cockerelli* (Šulc.) EN HOSPEDEROS SILVESTRES EN LA ZONA AGRÍCOLA DE GALEANA NUEVO LEÓN.

Tesis

Que como requisito parcial para obtener el grado de DOCTOR EN CIENCIAS EN PARASITOLOGÍA AGRÍCOLA

Presenta:

CAROLINA DELGADO LUNA

Saltillo, Coahuila Julio 2023

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Tesis

Elaborada por CAROLINA DELGADO LUNA como requisito parcial para obtener el grado de Doctor en Ciencias en Parasitología Agrícola con la supervisión y aprobación del Comité de Asesoría.

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Julio 2023

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A mi comité de tesis compuesto por los doctores Sergio René Sánchez Peña, Ernesto Cerna Chávez, Oswaldo García Martínez, Agustín Hernández Juárez y Moisés Felipe Victoriano por compartirme su conocimiento y apoyarme siempre que lo necesité.

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A mis padres Enrique Delgado González y Catalina Luna Hernández, por su apoyo incondicional y motivarme a cumplir mis sueños.

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Pero en especial, gracias a la persona que confió y vio en mí la capacidad de lograr lo que hoy está plasmado en esta tesis. Por brindarme su apoyo incondicional, ayudarme a creer en mí y en que soy capaza de lograr todo lo que me proponga.

Dedicatoria

A mis hijos: Enrique y Daniel

En este momento sus pequeñas mentecitas no logran comprender lo que su simple presencia significa en mi vida. Sin embargo, espero que para cuando sean capaces y miren atrás descubran la gran motivación que son para mí. Quiero poder ser su ejemplo, de que siempre se puede luchar y trabajar por lo que se quiere sin importar las circunstancias. Espero poder estar en cada paso que den y ser su apoyo.

Gracias por llegar a mi vida, llenarla de alegrías y mantenerme con los pies bien puestos en la tierra.

Gmail Carolina Delgado <delgadolunac29@gmail.com>

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1 mensaje

Sergio Sanchez <sanchezcheco@gmail.com> 8 de diciembre de 2021, 15:19
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2 mensajes

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Responder a: "Dr. Sriyanka Lahiri (she/her)" <lahiris@ufl.edu>
Para: Sergio Sánchez Peña <sanchezcheco@gmail.com>, Carolina Delgado-Luna <delgadolunac29@gmail.com>, Ernesto Cerna-Chavez <jabaly1@yahoo.com>, Alvaro Romero-Castillo <alv89@outlook.com>

Sergio Sánchez Peña, Carolina Delgado-Luna, Ernesto Cerna-Chavez, Alvaro Romero-Castillo :

We have reached a decision regarding your submission to Florida Entomologist, "A portable chamber for experimental observations of Bactericera cockerelli on plant seedlings and leaves ".

Our decision is to: Accept Submission

Sriyanka Lahiri, Ph.D.

Subject Editor, Florida Entomologist

Assistant Professor, University of Florida

Emma Weeks, Ph.D.

Editor-in-Chief, Florida Entomologist

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27-Mar-2023

Dear Dr. Sanchez-PENA:

Manuscript ID PDIS-02-23-0350-RE entitled "Physalis virginiana as a wild field host of Bactericera cockerelli (Hemiptera: Triozidae) and Liberibacter solanacearum", which you submitted to Plant Disease, has been reviewed. The comments of the reviewers are included at the bottom of this letter.

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Once again, thank you for submitting your manuscript to Plant Disease and I look forward to receiving your revision.

Sincerely,
Dr. Ana Maria Bocsanczy
Senior Editor, Plant Disease
anamariab@ufl.edu

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author
The paper by Delgado-Luna et al. is focused on the identification of a wild solanaceous plant Physalis virginiana grown in Northern Mexico as a potential reservoir host for the Candidatus Liberibacter solanacearum (Lso) and its vector potato/tomato psyllid (PTP). The authors identified P. virginiana as a host of both Lso and PTP in the natural environment in Mexico, and demonstrated survival and successful development of PTP on this host in the laboratory. Methodology used for sample collections, analysis, and laboratory tests was selected and used appropriately. The

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21 mensajes

Sergio Sanchez <sanchezcheco@gmail.com> 2 de diciembre de 2020, 18:06
Para: "Reyes Corral, Cesar Alejandro" <cesar.reyescorral@wsu.edu>, "Reyes Corral, Cesar (reye8940@vandals.uidaho.edu)" <reye8940@vandals.uidaho.edu>, "Reyes, Cesar - ARS" <cesar.reyes@usda.gov>
CC: Carolina Delgado <delgadolunac29@gmail.com>

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Subject: Plant Disease - Accept with revision: Manuscript ID PDIS-10-20-2240-RE
To: <SanchezCHECO@gmail.com>

02-Dec-2020

Dear Dr. Sanchez-PENA:

Manuscript ID PDIS-10-20-2240-RE entitled ""Candidatus Liberibacter solanacearum" infection of commercial tomatillo, Physalis ixocarpa Brot. (Solanales: Solanaceae) in Saltillo, Mexico", which you submitted to Plant Disease, has been reviewed. The comments of the reviewers are included at the bottom of this letter.

The reviewers have recommended publication, but also suggest some revisions to your manuscript. In addition the comments, I would like to see explanations or discussion on the following: 1) why 71% of symptomatic plants were infected with Lso, not all? 2) Have you sequenced the PCR products to confirmation? and 3) suggest adding PCR product gel figures. Therefore, I invite you to respond to the reviewers' comments and revise your manuscript.

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Once again, thank you for submitting your manuscript to Plant Disease and I look forward to receiving your revision.

Sincerely,
Dr. Shien Lu

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INTRODUCCIÓN

El psílido *Bactericera cockerelli* (Šulc. 1909) (Hemíptera: Triozidae) es nativo del noreste de México y suroeste de Estados Unidos (Munyaneza *et* al., 2007). Esté es probablemente el insecto plaga más importante en cultivos de solanáceas en el oeste de Estados Unidos y noreste de México, es vector del patógeno *Candidatus* Liberibacter solanacearum (Lso), agente causal de las enfermedades "zebra chip", "permanente del tomate" y "Variegado del Chile" en cultivos de papa, tomate y chile respectivamente (Reyes-Corral *et al*., 2020; Djaman *et al*., 2020).

Bactericera cockerelli fue reportada como plaga por primera vez en 1915 en *Solanum pseudocapsicum* L. (falsa cereza de Jerusalén) planta ornamental cultivada en el norte de California de los Estados Unidos (Compere, 1915; Prager and Trumble, 2018). En 1917 Essig la reportó como plaga de importancia económica en cultivos de chile y tomate, y para la década de 1920 se le asoció con la condición conocida como "amarillamiento del psílido" en plantas de papa y tomate (Binkley, 1929) ocasionando que el psílido se convirtiera en una mayor preocupación. En México en 1984, en el estado de Guanajuato se reportó por primera vez una enfermedad conocida como "permanente del tomate (PT)" (Garzón, 1984), y en 1994 en Saltillo, Coahuila, México se reportó por primera vez a nivel mundial el "zebra chip (ZC)" una enfermedad emergente en cultivos de papa (Secor y Rivera, 2004; Gudmestad y Secor, 2007). En 2007 mediante pruebas de campo-invernadero se determinó que *B. cockerelli* es vector de zebra chip (Munyaneza *et al*., 2007). Sin embargo, fue hasta 2009 que se logró confirmar molecularmente que las enfermedades de zebra chip y permanente del tomate son ocasionadas por la bacteria Lso (Liefting *et al*. 2009; Munyaneza *et al*., 2009).

Wallis (1955) elaboro un listado con un importante número de plantas donde se reproduce el psílido, así mismo agrego las especies donde habían sido capturados adultos; actualmente esté número de plantas se ha incrementado considerablemente. Conocer e identificar los hospederos de *B. cockerelli* que le permiten sobrevivir, reproducirse y desarrollarse en ausencia de cultivos de solanáceas es de gran importancia en el estudio de su ecología (Martin, 2008).

Burckhard *et al*. (2014) mencionan que a menudo la literatura denomina hospedero a plantas donde los psílidos se han encontrado accidentalmente sin confirmar el desarrollo del inmaduro, por lo que sugieren una terminología para aclarar las definiciones de plantas asociadas con psílidos. Concluyen que planta hospedera es aquella que le permite al psílido desarrollarse desde huevo hasta adulto (Burckhard *et al*., 2014). Las plantas hospederas reportadas para *B. cockerelli* pertenecen a las familias Solanaceae, Convolvulaceae y Lamiaceae (Martin 2008; Djaman *et al*., 2020), sin embargo, se tiene evidencia de que *B. cockerelli* se alimenta también de plantas que pertenecen a otras 20 familias, sin que le sea posible completar su desarrollo en éstas, a las que se llama plantas alimenticias (Martin, 2008; Reyes-Corral *et al.*, 2021).

Debido a que *B. cockerelli* es vector de Lso, los hospederos silvestres juegan un papel sumamente importante en la diseminación del patógeno. Se ha confirmado que muchos de los hospederos silvestres reportados para *B. cockerelli* también son susceptibles a Lso, y sirven como fuentes de dispersión de psílidos infectados, que posteriormente colonizan campos de cultivos de solanáceas (Wen *et al*. 2009; Torres-Glenda *et al*., 2015; Vereijssen *et al*. 2015; Cooper *et al*. 2019; Caicedo *et al*. 2020; Reyes-Corral *et al*. 2020; Reyes-Corral *et al*. 2021; Cooper *et al*., 2023). Las especies de plantas hospederas y alimenticias pueden variar de acuerdo con la zona geográfica (Cooper *et al*., 2019; Reyes-Corral *et al.*, 2021), y su importancia depende de su abundancia, cercanía a campos de cultivo, haplotipo del insecto, su preferencia alimenticia y planta en la que se desarrolló el psílido (Prager *et al*., 2014; Martin, 2008). La información disponible sobre las especies de plantas silvestres que sirven como hospederas de *B. cockerelli* y que además pueden albergar Lso es limitada (Cooper *et al*., 2019). Por lo anterior, el objetivo de esta investigación fue identificar los hospederos silvestres de *B. cockerelli,* y determinar el papel que juegan en su distribución y abundancia en campo en la zona agrícola de Galeana, Nuevo León, México.

REVISIÓN DE LITERATURA

Generalidades

Bactericera cockerelli es una plaga de gran importancia económica en solanáceas. Ocasiona daños directos al alimentarse de la planta e indirectos al ser vector del patógeno Lso, agente causal de las enfermedades conocidas como: zebra chip (ZC) de la papa, permanente del tomate (PT) y variegado del chile. Los síntomas característicos de ZC son amarillamiento, acucharamiento y necrosis en hojas, retraso en el crecimiento, tubérculos aéreos, entrenudos acortados, reducción en el tamaño y rendimiento de los tubérculos, así como la muerte temprana de la planta (Munyaneza *et al*., 2007; Sengoda *et al*.,2010). Además, los tubérculos con ZC se caracterizan por el oscurecimiento del tejido vascular interno, que al freírse se vuelven más oscuros exhibiendo patrones de rayas café oscuro, de ahí el término “zebra chip”. Esta enfermedad fue reportada por primera vez en cultivos de papa cerca de Saltillo, Coahuila, México, en 1994 (Gudmestad y Secor, 2007). Posteriormente Munyaneza *et al*., en 2007 relacionaron la bacteria Lso con ZC.

El cultivo de tomate también se ve afectado por *B. cockerelli* y Lso; las plantas afectadas presentan aborto de flores y frutos, amarillamiento, necrosis y acucharamiento en follaje, entrenudos cortos y rendimientos reducidos (Delgado-Ortiz *et al*., 2019). Como daño directo, *B. cockerelli* puede ocasionar una reducción mayor del 60% en rendimiento y calidad de tubérculos y más del 50% de perdidas en tomate fresco para mercado (Liu *et al*., 2006; Munyaneza *et al*., 2008). También se alimenta y reproduce en cultivos de chile (*Capsicum* spp.), tomatillo verde (*Physalis ixocarpa*) y berenjena (*Solanum melongena*), así como en varias especies de solanáceas silvestres que ya han sido reportadas como hospederos del psílido y Lso (Wallis 1955; Wen *et al*. 2009; Torres-Glenda *et al*., 2015; Caicedo *et al*. 2020; Reyes-Corral *et al*., 2020; Cooper *et al*., 2023).

Los hospederos silvestres permiten la sobrevivencia, reproducción y distribución del psílido en campo en ausencia de solanáceas cultivadas (Burckhard *et al*., 2014), estas especies varían de acuerdo con la zona geográfica y su importancia esta influenciada por su abundancia y cercanía a los campos de cultivo, además

del haplotipo del insecto, preferencia alimenticia y planta en que se desarrolla el insecto (Wallis, 1955; Martin, 2008; Prager *et al*., 2014). Henne *et al*. (2010) realizaron investigaciones sobre la importancia de los hospederos silvestres de *B. cockerelli* y como pueden contribuir en la epidemiología de Lso y servir de reservorio en ausencia de cultivos de solanáceas. Hasta el momento se ha estudiado la biología e historia de vida del psílido en papa, tomate, pimiento y berenjena (Yang y Liu 2009; Yang *et al*., 2010 y 2013). Sin embargo, la investigación en hospederos silvestres aun es muy limitada; solo hay estudios en *Physalis longifolia*, *Physalis virginiana* y *Solanum elaeagnifolium* (Thinakaran *et al*., 2015; Reyes-Corral *et al*., 2021; Delgado-Luna *et al*., 2023 en prensa).

Plantas hospederas

Los insectos muestran preferencia por especies de plantas, cultivares o etapas de cultivo respondiendo a ciertas señales olfativas o visuales (Hokkanen, 1991). Existe una relación específica entre psílidos y las plantas en que estos se desarrollan, conocidas comúnmente como “Plantas Hospederas” (Martin, 2008; Burckhard *et al*., 2014). Sin embargo, existe contradicción en los reportes de plantas hospederas para psílidos, ya que a menudo se reportaron plantas donde se encontró accidentalmente el psílido y no se observó el desarrollo del ciclo de vida. Los psílidos completan su ciclo de vida en un número de plantas hospederas restringidas a unas cuantas especies, debido a esto los psílidos se han ganado la reputación de ser altamente específicos (Burckhard *et al*., 2014). Además de hospederos cultivados y silvestres, también existen plantas que sirven como hospedero invernal; por ejemplo, *L. berlandieri* en el sureste de Estados Unidos y noreste de México (Delgado-Luna *et al*., 2022, 2023 (en prensa); Cooper *et al*., 2023). Debido al diferente uso que dan los psílidos a ciertas especies de plantas, Burckhard *et al*. (2014) proponen una terminología para las plantas donde se encuentran los psílidos basada en la relación psílido-planta.

Planta hospedera

Son todas las plantas que le permiten al psílido completar su ciclo de vida desde huevo hasta su etapa adulta (Burckhard *et al*., 2014).

Planta de hibernación o refugio

Se denomina planta de hibernación o refugio a las plantas donde los psílidos se refugian durante el invierno y se pueden alimentar de ellas (Burckhard *et al*., 2014).

Planta alimenticia

Las plantas alimenticias son aquellas donde psílidos adultos se alimentan, pero no se reproducen o desarrollan, por lo tanto, no pueden pasar un tiempo prolongado sobre estas (Burckhard *et al*., 2014).

Planta ocasional

Son plantas donde los psílidos se posan, pero no se alimentan, reproducen o desarrollan y no pasan un tiempo prolongado sobre estas (Burckhard *et al*., 2014).

Hospederos silvestres de Bactericera cockerelli susceptibles a Liberibacter solanacearum

Se han detectado especies de solanáceas y convolvuláceas silvestres que pueden albergar Lso (Thinakaran *et al.,* 2015; Cooper *et al*., 2019; Reyes-Corral *et al*., 2020) (Tabla 1), las cuales contribuyen en la epidemiologia de las enfermedades en cultivos de solanáceas. Reyes-Corral *et al*., (2020) reporta que rizomas de *Physalis longifolia* infectados sobreviven el invierno y pueden producir plantas con Lso durante la siguiente primavera. Así mismo, Thinakaran *et al*., (2015) reportaron que Lso puede sobrevivir el inverno en estolones de *S. elaeagnifolium*, y que psílidos que se colocaron y alimentaron de estas plantas se infectaron de Lso. Delgado-Luna *et al*., (2023) (en prensa) reporta que adultos de *B. cockerelli* emergidos de plantas de *Physalis virginiana* de campo con Lso

también fueron positivos a Lso. Especies de convolvuláceas evaluadas bajo condiciones de laboratorio y adultos también fueron positivos a Lso (Cooper *et al*., 2019). Esto indica que plantas hospederas silvestres donde *B. cockerelli* inverna y sobrevive en ausencia de hospederos cultivados pueden producir insectos infectados, los cuales posteriormente colonizan cultivos de solanáceas. Existen informes contradictorios sobre la transmisión de Lso en papa, un estudio indica que semillas de papa producen plantas infectadas (Pitman *et al*., 2011) y otro que tubérculos sintomáticos no lo hacen (Swisher-Grimm *et al*., 2020). Por lo tanto, es importante determinar que hospederos silvestres perenes pueden albergar Lso, producir planta e insectos con Lso.

Tabla 1. Hospederos de *B. cockerelli* positivos a *Liberibacter solanacearum*

Hospedero	**Liberibacter solanacearum**
Solanaceae	
Capsicum annuum	x
Chamaesaracha coronopus (Dunal)	
Datura stramonium L.	x
Hyoscyamus niger	x
Lycium andersonii A. Gray	
Lycium barbarum L.	x
Lycium berlandieri Dunal	x
Lycium exsertum Gray	
Lycium fremontii Gray	
Lycium halimifolium Mill.	
Lycium macrodon A. Gray	
Lycium pallidum Miers	
Lycium parishii Gray	
Lycium quadridichum C. L. Hitchcock	
Lycium torreyi Gray	
Nicandra physalodes (L.) Gaertn	
Nicotiana alata Moore	

Nicotiana attenuata Steud	x
Nicotiana glauca Graham	x
Nicotiana tabacum L.	
Nierembergia hippomanica Miers	
Solanum aviculare G. Forst	
Solanum betaceum Cav	x
Solanum bulbocastanum	
Solanum carolinens L.	
Solanum dulcamara L.	x
Solanum elaeagnifolium Cav.	x
Solanum jamesii Torr.	
Solanum Lycopersicum	x
Solanum Nigrum L.	
Solanum physalifolium Rusby	
Solanum pseudocapsicum Link.	
Solanum ptychanthum Dunal	
Solanum rostratum Dunal	x
Solanum triflorum Nutt.	x
Solanum tuberosum	x
Solanum Verrucosum Schltdl	
Physalis angulata L.	
Physalis comata Rydb.	
Physalis franchetii Mast.	
Physalis heterofhylla Nees	
Physalis lanceolata Michx.	
Physalis lobata Torr.	
Physalis longifolia Nutt.	x
Physalis mollis Nutt.	
Physalis peruviana L.	x
Physalis pruinosa L.	
Physalis rotundata Rydb.	

Physalis virginiana	x
Convolvulaceae	
Capsicum frutescens L.	
Convolvulus arvensis L.	x
Convolvulus tricolor L.	
Ipomoea alba L.	x
Ipomoea batatas (L.) Lam.	
Ipomoea cordatatotrilova Dennstedt	
Ipomoea hederaceae L.	
Ipomoea nil (L.) Roth	
Ipomoea ternifolia Torrey	
Lamiaceae	
Micromeria chamissonis (Beent)	

Bactericera cockerelli (Šulc)

Bactericera cockerelli también conocido como el psílido de la papa/tomate, salerillo, paratrioza o pulgón saltador, es nativo del noreste de México y suroeste de Estados Unidos. Es probablemente el insecto plaga de mayor importancia en solanáceas, ya que ocasiona daños directos al alimentarse de la planta e indirectos al ser vector de la bacteria fitopatógena Lso. Se alimenta principalmente del floema de plantas solanáceas y se desarrolla en más de 40 especies de plantas de esta familia. (Wallis, 1955). Además, tiene una amplia gama de plantas alimenticias que abarca alrededor de 20 familias: Convolvulaceae, Amaranthaceae, Apiaceae, Asclepiadaceae, Asteraceae, Brassicaceae, Cannabaceae, Caprifoliaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Menthaceae, Moraceae, Oleaceae, Pinaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Salicaceae, Scrophulariaceae, Ulmaceae, Urticaceae, Violaceae y Zygophyllaceace (Wallis, 1955; Butler y Trumble, 2012; Reyes-Corral *et al*., 2021; Reyes-Corral *et al*., 2021). *B. cockerelli* presenta metamorfosis incompleta, tarda entre 25 a 33 días

en completar su ciclo de vida, a una temperatura de 26 - 27°C y humedad relativa de 60-70%. Las hembras ovipositan en los bordes o envés de las hojas; una hembra puede depositar entre 300 a 500 huevos en 21 días (Abdullah, 2008; Yang y Liu, 2009).

Posición taxonómica

Borror *et al*. (1998) ubican taxonómicamente a *B. cockerelli* de la siguiente manera.

Reino: Animalia
Filo: Arthropoda
Clase: Insecta
Orden: Hemiptera
Suborden: Sternorrhyncha
Superfamilia: Psylloidea
Familia: Triozidae
Género: *Bactericera*
Especie: *B. cockerelli*
(Šulc, 1909)

Huevo

Los huevos recién ovipositados son transparentes, en uno de los extremos tienen un filamento que les permite adherirse a la hoja; tienen forma ovoide, corion brillante. Posteriormente adquieren una coloración amarillo-anaranjado. Los huevos son depositados individualmente en el envés y mayormente en los bordes de las hojas (Marín *et al*., 1995; Gómez *et al.*, 2008).

Ninfa

La ninfa presenta cinco estadios; con apariencia oval, con dorso-ventral aplanado, ojos bien definidos, antenas con sensilias placoides o rinarios (estructuras con función olfatoria y sensorial) las cuales aumentan en número y son más notorias conforme avanzan los diferentes estadios. Alrededor del cuerpo

presentan estructuras cilíndricas con un filamento ceroso, conocidas como sectosetas que forman un halo. El abdomen es semicircular con espiráculos en cada uno de los primeros cuatro segmentos; presentan un poro anal en el abdomen que secreta mielecilla o salerillo. Los primeros cuatro estadios presentan coloración amarillo-anaranjada, mientras que en el quinto estadio se puede observar una coloración verde claro. Los paquetes alares se pueden observar a partir del tercer estadio, mientras que en el quinto estadio se puede observar bien definida la segmentación entre la cabeza, tórax y abdomen (Marín *et al*., 1995; Gómez *et al.*, 2008).

Adulto

Los adultos recién emergidos conocidos también como adulto “teneral” presentan una coloración verde-amarillento; tienen poca movilidad, sus alas son blancas las que después de tres o cuatro horas se vuelven transparentes. Los adultos maduros tienen una coloración café oscura o negra, antenas filiformes y alas transparentes. Los machos tienen seis segmentos abdominales más el genital, el cual se encuentra plegado en la parte media dorsal del abdomen. Las estructuras genitales en forma de pinza se pueden observar dorsalmente y caracterizan a los machos. Las hembras presentan cinco segmentos abdominales, más el segmento genital con apariencia de cono. A diferencia de los machos, las hembras presentan en la parte superior del abdomen una mancha blanca en forma de “Y” invertida (Marín *et al*., 1995; Gómez *et al.*, 2008).

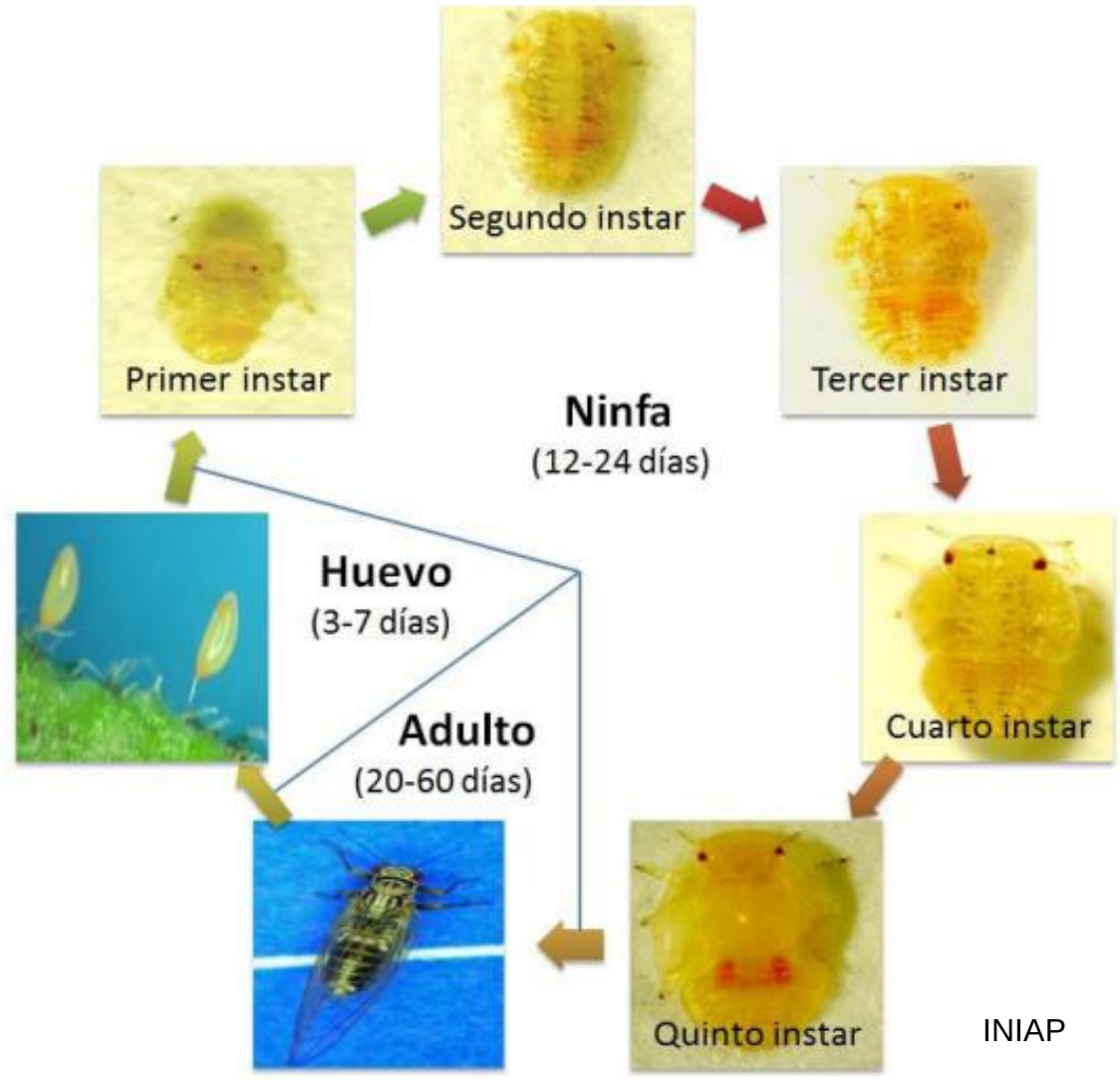

Figura 1. Ciclo de vida de *Bactericera cockerelli*.

Distribución de Bactericera cokerelli

A nivel mundial *B. cockerelli* se encuentra presente en Australia Occidental, Canadá, Ecuador, El Salvador, Estados Unidos, Guatemala, Honduras, Islas Norfolk, México, Nicaragua, Nueva Zelanda y Perú (EPPO, 2023). En México se encuentra presente en los estados de Aguascalientes, Baja California, Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Morelos, Nayarit, Nuevo León, Puebla, San Luis Potosí, Sinaloa, Tamaulipas y Zacatecas (Pletsch,1947; Vega et al., 2008; Cerna *et al*., 2021).

Liberibacter solanacearum

Liberibacter solanacearum (Lso) es transmitido por el vector *B. cockerelli*. Las enfermedades ocasionadas por Lso se caracterizan por largos periodos de latencia (Harrison *et al*., 2021). Los síntomas generalmente empiezan a desarrollarse tres semanas después de la infección (Munyaneza *et al*., 2007; Secor *et al*., 2009; Lévy *et* al., 2011; Mendoza-Herrera *et* al., 2018) y el desarrollo de la enfermedad es independiente de la densidad de psílidos en la planta (Rashed *et al*., 2012). Lso reduce significativamente la calidad del fruto y tubérculos su valor comercial en el mercado, además el control de la enfermedad y vector es casi insostenible debido a la inversión millonaria en productos químicos y aplicación (Gudmestad y Secor, 2007; Greenway y Rondon, 2018; Delgado-Ortiz *et al.*, 2019).

Posición taxonómica

Dominio: Bacteria

Filo: Proteobacteria

Clase: Alphaproteobacteria

Orden: Rhizobiales

Familia: Phyllobacteriaceae

Género: *Candidatus* Liberibacter

Especie: "*Candidatus* Liberibacter solanacearum" (sin. psyllaurous) (CAB International, 2015).

Descripción

Lso es una bacteria Gram-negativa, restringido al floema de las plantas con morfología de bacilo; mide aproximadamente 0.2-0.3 µm de ancho y 2-3 µm de largo, es considerado un parasito obligado no cultivable *in vitro* (Jagoueix *et al*., 1994; Bové, 2006; Tanaka *et al*., 2007). Esta bacteria puede ser transmitida por insectos vectores (psílidos) semilla e injerto (Crosslin y Munyaneza, 2009; Liefting *et al*., 2009; Secor *et al*., 2009; Camacho-Tapia *et al*., 2011; Bertolini *et al*., 2015).

Distribución de Lso

Actualmente Lso se ha reportado en; Australia Occidental, Canadá, Ecuador, El Salvador, Estados Unidos, Guatemala, Honduras, Isla Norfolk, México, Nicaragua y Nueva Zelanda (EPPO, 2022). En México los estados reportados con presencia de Lso son; Aguascalientes, Coahuila, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Nayarit, Nuevo León, Puebla, San Luis Potosí, Tamaulipas y Zacatecas (Cerna *et al*., 2021).

Importancia económica

Bactericera cockerelli y Lso en conjunto pueden provocar grandes pérdidas económicas, incluso ponen en riesgo la seguridad alimentaria (Gudmestad y Secor, 2007; Wang *et al*., 2020). En el cultivo de papa y tomate las perdidas en producción pueden llegar al 100%, mermando la calidad del fruto/tubérculo y valor comercial (Delgado-Ortiz *et al*., 2019). Para su control se invierte una gran cantidad de recursos económicos, principalmente por el desconocimiento de las fuentes de psílidos infectados con Lso. Este desconocimiento ha provocado el uso intensivo de plaguicidas, que a su vez ha ocasionado que el psílido desarrolle resistencia (Dávila-Medina *et al*., 2012), obligando a los productores a abandonar el cultivo (papa principalmente) debido a los altos costos de producción (CESAVEM 2007; Ramírez *et al*., 2013). Además, existe preocupación creciente por el impacto ambiental resultante del uso excesivo de plaguicidas (Wang *et al*., 2020).

Transmisión

Se ha demostrado que Lso se distribuye en todo el cuerpo de *B. cockerelli*: canal alimentario, intestino medio, glándulas salivales y bacteriomas; la transmisión es de forma circulativa (Cooper *et al*., 2013; Tang *et al*., 2020). Sengoda *et al*. (2013) reportaron que los niveles de Lso aumentan durante 15 días a partir de la adquisición del patógeno y posteriormente se mantienen constantes. Se tiene evidencia que las ninfas son menos eficientes que adultos para transmitir Lso, y

que el exponer plantas de papa a 20 psílidos adultos infectados durante una hora es suficiente para que desarrollen síntomas de ZC (Tang *et al*., 2020). También se ha demostrado que un solo adulto de *B. cockerelli* es capaz de infectar con Lso en plantas de papa en un periodo de 6 horas (Tang *et al*., 2020). El tiempo de adquisición puede variar dependiendo la etapa fisiológica del psílido; las ninfas requieren aproximadamente 15 minutos y los adultos 30 minutos en promedio para adquirir el patógeno (Garzón *et al*., 2009). Existen reportes de que los psílidos incuban el patógeno por un periodo de hasta 24 horas, y pueden transmitirlo en 15 minutos en promedio (Garzón *et al*., 2009). Sin embargo, otros autores reportan que los psílidos pueden transmitir Lso a plantas después de un periodo de incubación de dos semanas (Sengoda *et al*., 2014).

Control

El control de *B. cockerelli* es complicado debido a la dificultad de predecir cuándo y dónde psílidos infectados con Lso colonizan los cultivos desde hospederos silvestres y por la incapacidad de predecir el riesgo de infestación año tras año (Reyes-Corral *et al*., 2020). No existen métodos prácticos para controlar directamente Lso, por lo que el control del patógeno se basa en aplicaciones intensivas de plaguicidas dirigidas al insecto vector, principalmente de carbamatos, neonicotinoides, piretroides, organoclorados y organofosforados (Dávila-Medina *et* al., 2012; Cooper *et al*., 2019; Reyes-Corral *et al*., 2020). Para el control de Lso se recomienda eliminar focos de infección, por ejemplo: residuos de cosecha, plantas hospederas del psílido, plantas enfermas en los márgenes de los cultivos y lotes vecinos (Bujanos *et al*., 2005). El uso de enemigos naturales de *B. cockerelli* en sus diferentes etapas representa una alternativa en su control. Los hongos entomopatógenos *Metarhizium anisopliae*, *Beauveria bassiana* y *Isaria fumosorosea* han demostrado bajo condiciones de laboratorio y campo, ocasionar una mortalidad hasta100% en diferentes fases de desarrollo del psílido (Bujanos *et al*., 2005; Lacey *et al*., 2009, 2010; Sánchez-Peña *et al*., 2007). Entre los enemigos naturales más eficientes se encuentran los parasitoides *Tamarixia triozae* (Burks, 1943) (Psyllidae : Triozinae) y

Metaphycus psyllidis (Compere, 1943) (Hymenoptera; Encyrtidae), y larvas depredadoras de coccinélidos, crisopas, nábidos y sírfidos (Compere, 1943; Pletsch, 1947; Sarkar *et al*., 2023). También se han desarrollado variedades tolerantes o resistentes que ayudan a minimizar el riesgo de transmisión de Lso, reduciendo la alimentación y desarrollo del vector (Munyaneza *et al*., 2011).

CONCLUSION GENERAL

En la zona agrícola de Galeana Nuevo, León, México, se puede encontrar *Lycium berlandieri*, *Physalis virginiana*, *Solanum elaeagnifolium* y *Chamaesaracha coronopus*, así como plantas de papa voluntaria (plantas crecidas a partir de tubérculos dejados durante la cosecha de la temporada anterior), todos estos son hospederos de *Bactericera cockerelli*. Además, los hospederos silvestres mencionados tienen la capacidad de albergar a *Liberibacter solanacearum* a excepción de *C. coronopus* donde no se ha confirmado la preferencia de Lso. Los datos obtenidos permiten formular las siguientes hipótesis: Los hospederos silvestres son una fuente de refugio para *B. cockerelli*, que le permite alimentarse, reproducirse, desarrollarse e invernar en ausencia de hospederos cultivados. Durante el invierno el psílido sobrevive en *L. berlandieri*; posteriormente al inicio de la primavera comienzan a emerger plantas de papa voluntaria y *P. virginiana*, *S. elaeagnifolium* y *C. coronopus*. Cuando estos hospederos se encuentran presentes en campo *B. cockerelli* migra a éstos desde *L. berlandieri*, posteriormente entre junio-julio coloniza los cultivos de solanáceas. Posteriormente conforme se acerca la cosecha los psílidos comienzan a migrar nuevamente a los hospederos silvestres, principalmente a *L. berlandieri* donde inverna hasta el próximo ciclo.

Se ha comprobado que adultos emergidos de plantas de *P. virginiana* con Lso también son positivos a la bacteria.

Con base en estas observaciones podemos afirmar que un gran número de los psílidos que emergen de los hospederos silvestres son portadores del patógeno; los cuales posteriormente colonizan e infectan los cultivos.

Es importante realizar estudios de campo más extensos sobre el papel que juegan los hospederos silvestres en la epidemiologia de Lso, así como la función específica de cada especie de planta como hospedera silvestre, ya que dichas plantas pueden ser refugio y alimento de insectos con Lso.

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Chamaesaracha[1]: New Weed Host Plant Genus for *Bactericera cockerelli*[2] at the Potato-Growing Area of Northeastern Mexico

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Abstract. There is insufficient knowledge of wild plant hosts of the tomato/potato psyllid, *Bactericera cockerelli*, one of the worst pests of solanaceous crops. We found *B. cockerelli* can complete its life cycle in the field on the widespread, unreported plant host, greenleaf five eyes, *Chamaesaracha coronopus* (Dunal) A. Gray (Solanaceae) at Galeana, NL, Mexico. After 48 hours in a laboratory, adult survival of *B. cockerelli* on *C. coronopus* and *Chamaesaracha coniodes* was 73.3 and 6.6%, respectively ($p < 0.05$). There was no oviposition on *C. coniodes*. Some *Chamaesaracha* species might contribute to population dynamics of *B. cockerelli* in North America.

Resumen. No hay conocimiento suficiente sobre las plantas silvestres hospederas del psílido del tomate/papa, *Bactericera cockerelli* (Sulc), una de las peores plagas de las solanáceas cultivadas. Encontramos que *B. cockerelli* puede completar su ciclo de vida en el campo (Galeana, NL, México) en la planta hospedera ampliamente distribuida y no reportada *Chamaesaracha coronopus* (Dunal) A. Gray (Solanaceae). Después de 48 h en el laboratorio, la supervivencia de adultos de *B. cockerelli* sobre *C. coronopus* y *Chamaesaracha coniodes* (Moric. ex Dunal) Britton fue de 73.3 y 6.6%, respectivamente ($p < 0.05$). No hubo oviposición en *C. coniodes*. Algunas especies de *Chamaesaracha* podrían contribuir a la dinámica poblacional de *B. cockerelli* en América del Norte.

Identification of wild plant hosts is crucial for analysis of population dynamics of insect pests. The tomato/potato psyllid, *Bactericera cockerelli* (Sulc), is native to northern Mexico and the southwestern United States and is currently the most important insect pest of potato (*Solanum tuberosum* L.) in the western USA and in Mexico, because it is the vector of the bacterium *Liberibacter solanacearum* (Lso), the pathogen causing the diseases "zebra chip" of potato and "permanente" of tomato (*Solanum lycopersicum* L.) (Djaman et al. 2020, Reyes-Corral et al. 2021). Zebra chip was first observed in potatoes commercially produced in the agricultural region

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of Galeana, in the potato-growing area of Nuevo Leon State in Mexico and the neighboring area of southeastern Coahuila in 1994. Potatoes were cultivated there on close to 9,000 ha before occurrence of the disease. The impact of zebra chip has been catastrophic and probably is the main reason for periodical abandonment of about 70% of the original area grown to potatoes in the region (personal communication with potato producers). Reported wild and cultivated host plants of *B. cockerelli* (those appropriate for complete, normal development of the insect) belong mostly to Solanaceae (37 species), Convolvulaceae (three species), and Menthaceae (one species) (Martin 2008, Djaman et al. 2020). Adult *B. cockerelli* also have been reported feeding on plants of 20 other families; these are considered food plants not appropriate for complete development of the insect. Nevertheless, they aid survival of the insect in the absence of hosts (Reyes-Corral et al. 2021).

Host plant species of *B. cockerelli* vary according to geographical area (Cooper et al. 2019, Reyes-Corral et al. 2021). Among wild hosts reported for *B. cockerelli* in Mexico are species of the genera *Datura, Lycium, Physalis*, and wild species of *Solanum* such as silverleaf nightshade, *S. eleagnifolium* Cav.; and buffalo bur, *S. rostratum* Dunal (Thinakaran et al. 2015, Cooper et al. 2019). Recently, in Galeana, Nuevo León, we observed *B. cockerelli* eggs and nymphs on a widespread, unidentified wild plant that did not belong in the genera reported as hosts. Wild host plants are essential for development and survival of pest insects in the absence of cultivated hosts; therefore, it is important to identify the plants. The objectives of this work were to: 1) Identify this novel wild host of *B. cockerelli* in the potato-producing region of Galeana, Nuevo León and 2) Assess survival and oviposition of *B. cockerelli* adults in two species of plants of this novel genus of hosts.

On 16 June 2021, we sampled weeds in a field previously cultivated to potato (with volunteer plants present), at the municipality of Galeana, Nuevo León, Mexico (24°52'53.8' N 100°24'24.3' W, 1,878 m elevation). The area is semi-arid, with dry steppe climate, and average annual temperature of 19°C. We collected 37 plants from an unidentified species harboring nymphs and eggs of *B. cockerelli*; the plants were put individually into Ziploc® bags. Leaves with nymphs were put into chambers (1-liter plastic containers, with a fabric window on the lid and wet cotton on the fabric cover) to observe possible emergence of adults.

On 1 July 2021, plants similar to the one mentioned were collected at Saltillo, Coahuila State (25°21'44.0'N 101°01'51.2'W, 1,758 m above sea level), about 70 km from the previous area. Plants were taken to a laboratory and identified taxonomically using keys of Turner (2015). Insects were identified according to Kaur et al. (2018) and by comparison to a laboratory colony identified by high-resolution melting analysis and sequencing of the mitochondrial Cytochrome C Oxidase subunit 1-like gene (CO1) (Reyes-Corral et al. 2021).

Oviposition and survival tests were done using the two potential new host plants. Three sprouts per plant were used with five adults (three females and two males) each; they were placed separately inside a portable chamber (Delgado-Luna 2020). The insects were from a laboratory colony, established on tomato plants at UAAAN, from field populations that were >95% Central haplotype *B. cockerelli* (Reyes-Corral et al. 2021). Oviposition and survival were recorded at 48 hours. Data were analyzed by a Student's *t*-test in the InfoStat statistical package (Di Rienzo et al. 2011).

The plant colonized by nymphs of *B. cockerelli* at Galeana was identified as greenleaf five eyes, *Chamaesaracha coronopus* (Dunal) A. Gray (Fig. 1A). A prostrate or erect plant, it grows 30 cm tall, with slight to deeply lobed leaves; it has

branched trichomes on the leaves and stems; hanging yellowish-green flowers; fruit 4-8 mm wide, and brown alveolate seeds. It is distributed in the deserts of the south-western United States and north-central Mexico (Averett 2010, Turner 2015).

In field samples of *C. coronopus*, an average of 0.29 egg and 1.54 nymphs of *B. cockerelli* were found per plant (n = 37, Fig. 1B, C). Adults emerged from 24.5% of nymphs on leaves put into a moist chamber (n = 57 nymphs). The plant has not been reported as a host of potato-tomato psyllid.

Chamaesaracha coniodes (Moric. ex Dunal) Britton was identified in the Saltillo area. It is also widespread in warm deserts of North America. It grows 30 cm tall, with green to yellow flowers; it has alternate, sessile, or short petiolate leaves. The leaves and stems have sticky hairs; fruit is 4-8 mm wide and whitish, and hanging seeds are alveolate light brown. *Chamaesaracha* species share many morphological characteristics; leaf shape and pubescence are key to correct identification (Averett 2010, Turner 2015). Voucher specimens of both plant species were deposited in the herbarium of the Universidad Autónoma Agraria Antonio Narro (ANSM). *Chamaesaracha coniodes* was not observed colonized by *B. cockerelli* in the field; the insect is widespread in the Saltillo area (Reyes-Corral 2021).

After 48 hours, survival of *B. cockerelli* adults was significantly different (p = 0.02) between the two species of *Chamaesaracha*. An average of 73.3% of insects survived on *C. coronopus*, while only 6.6% did on *C. coniodes*. A mean of 9.6 eggs per replication (sprout) was laid on *C. coronopus*, while no eggs were laid on *C. coniodes*; however, this was not statistically significant.

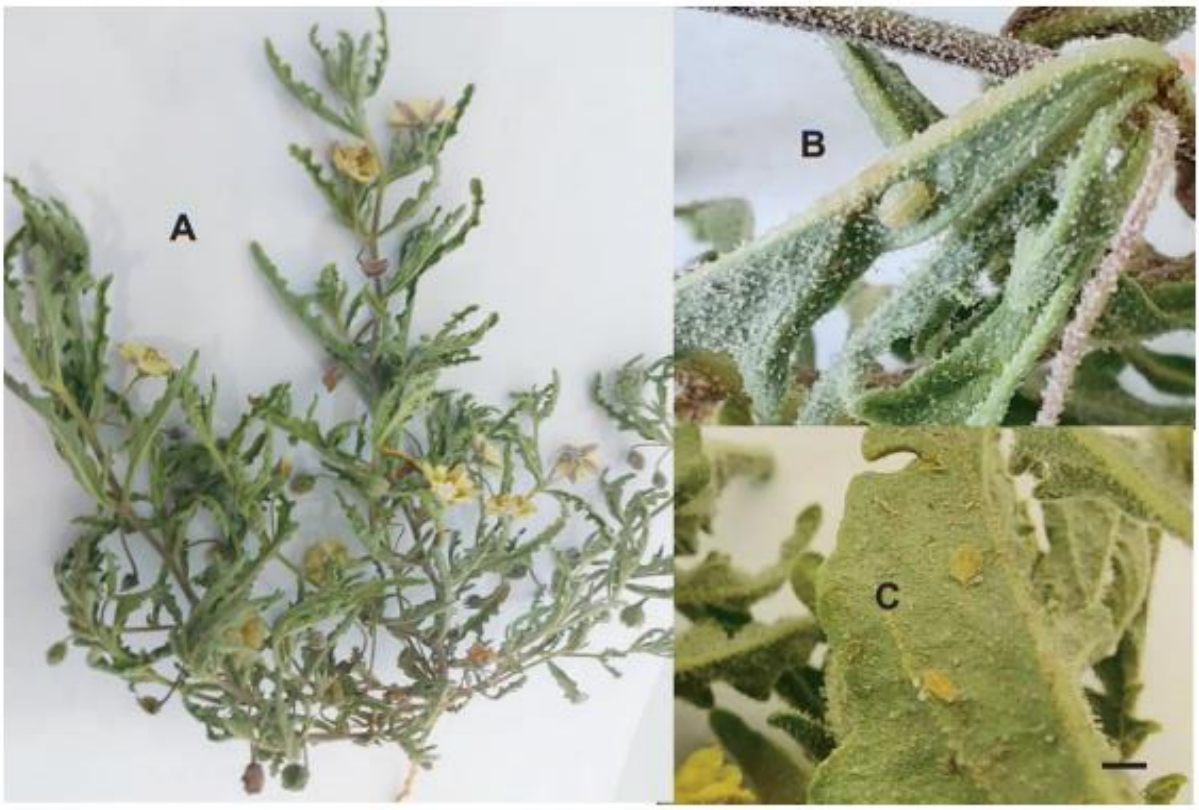

Fig. 1. A) *Chamaesaracha coronopus* from Galeana, NL. B) and C) nymphs of *Bactericera cockerelli* on leaves of *Chamaesaracha coronopus*. Scale bar for B) and C): 0.75 mm.

The presence of eggs and nymphs, and emergence of adults on *C. coronopus*, indicated the wild nightshade served *B. cockerelli* as a host. Our observations also suggest that the different species of *Chamaesaracha* might vary in suitability as hosts of the tomato/potato psyllid. Specimens of *C. coniodes* were conspicuously more hirsute than those of *C. coronopus*, and movement was difficult for *B. cockerelli* adults on *C. coniodes*. We suspect pubescence might be involved in differential survival and egg-laying on the two species of plants. Development of *B. cockerelli* on the different species of *Chamaesaracha* should be verified.

Stands of these plants might contribute to abundance of *B. cockerelli* in the region. *Chamaesaracha* species widespread across arid North America might support area-wide survival of *B. cockerelli*. The status of *Chamaesaracha* as a reservoir/host of *L. solanacearum* also should be clarified, to elucidate its role in disease dynamics.

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A Portable Chamber for Experimental Observations of Bactericera Cockerelli on Plant Seedlings and Leaves

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A portable chamber for experimental observations of *Bactericera cockerelli* on plant seedlings and leaves

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Bactericera cockerelli Šulc (Hemiptera: Triozidae), the potato-tomato psyllid, is the pest of most concern in solanaceous crops, as a vector of *Liberibacter* (Hyphomicrobiales: Rhizobiaceae), causal agents of severe diseases. Laboratory research on biology, behavior, and physiology of *B. cockerelli* is required (Reyes-Corral et al. 2021). Experimental methods with live insects should be adaptable, simple, and repeatable. Insect bioassays often are performed on complete plants (Liu & Trumble 2005; Echegaray et al. 2016). On detached leaves (Lehman 1930; Wagan et al. 2018), observations can be done for only a few d due to leaf decay. With the clip chamber technique (insects confined in small chambers attached to leaves on whole plants) observations may be difficult because manipulation is cumbersome (Liu & Trumble 2005; Echegaray et al. 2016; Szczepaniec et al. 2019).

A portable chamber was produced to facilitate laboratory observation of processes like oviposition, hatching, and development of *B. cockerelli*, and the effect of variables like plant material (leaflets or rootless seedlings) and substrate (peat moss or water).

This chamber was based on the design of author Romero-Castillo. On Figure 1, one can see that it is composed of: two bottoms (A and B) of transparent polyethylene Petri dishes (100 mm diam × 15 mm deep); a 5 mL cryovial tube (D) with screw cap (C) (Corning PD1013, Corning, New York, USA); organza cloth; Parafilm® (Bemis, Mexico City, Mexico), silicone bars (Modatelas, Saltillo, Mexico); and non-toxic modeling clay (Baco, Mexico City, Mexico). Each bottom (A and B) has a 1.5 cm diam hole (a) for ventilation, covered with organza fabric sealed with silicone. The bottom A has a lateral hole of diameter equal to the tube cap (b). The cap (C) of this tube has a hole (c) 0.7 mm in diam. Assemblage: the cap (C) of the cryovial tube was inserted in hole (b) of bottom A, and the contact of cap and bottom sealed with clay. The tube has water or substrate added, then covered with the perforated cap.

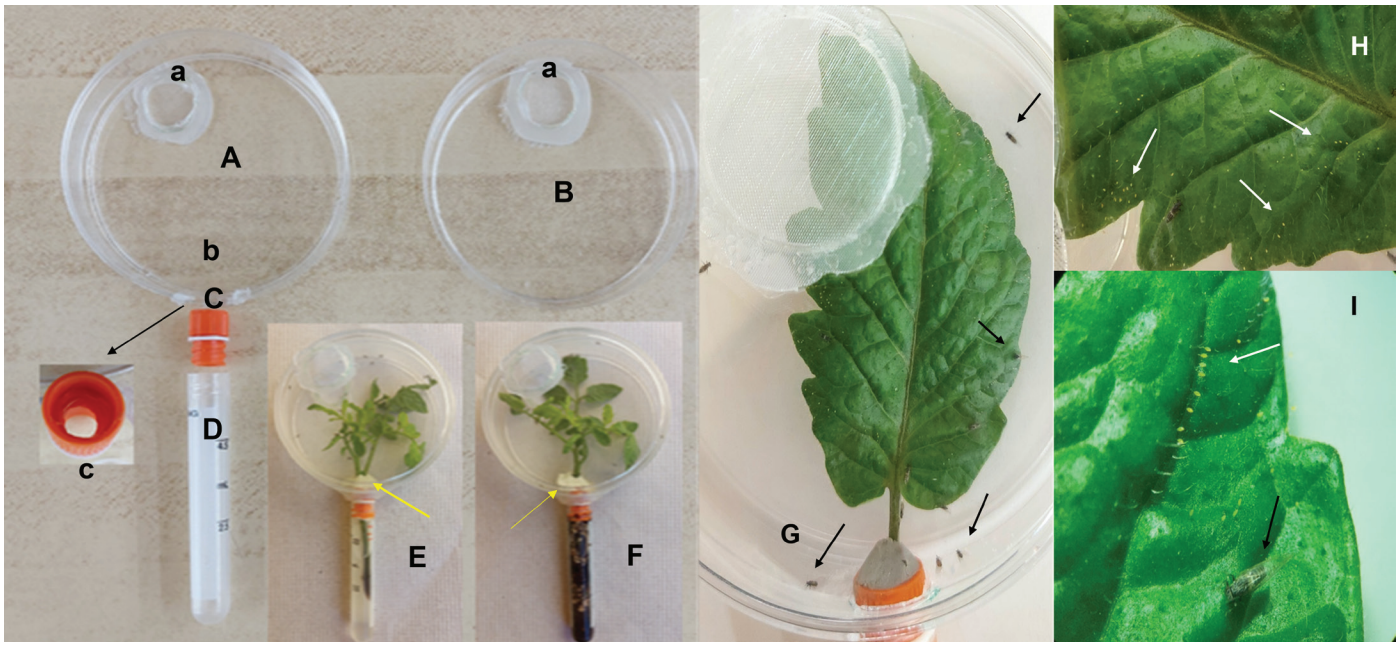

Fig. 1. Portable chamber. Bottoms A and B of chamber, with holes (a) and (b); cryovial cap (C) positioned in hole b; cap C with perforation (c); cryovial tube (D). Two chambers complete with plants, with water (E) or substrate (F) and modeling clay (yellow arrows) sealing the plant in tube. Adults (G) and eggs (H, I) (black and white arrows, respectively) of *Bactericera cockerelli*, and tomato leaflet inside chamber.

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Table 1. Mean ± SE of *Bactericera cockerelli* oviposition and egg hatching.

Treatments	Eggs per chamber Mean ± SE	Eggs per female Mean ± SE	Hatching % Mean ± SE
Leaflets in water	67.3 ± 18.2 a (13.2–170.3)	13.3 ± 3.6 a (2.6–34.2)	66.8 ± 9.2 a (46.1–94.7)
Plants in water	75.8 ± 7.4 a (38.1–111.3)	15.2 ± 1.5 a (7.6–22.2)	78.9 ± 6.1 a (30.8–95.5)
Plants in peat moss	68.4 ± 10.0 a (22.2–128.4)	13.7 ± 2.0 a (4.4–25.8)	73.28 ± 3.7 a (54.71–98.2)

*means within columns followed by the same letter are not significantly different (α = 0.05). SE = Standard error. Range of observations in parentheses.

Terminal leaflets (6–7 cm) of tomato leaves or seedlings (with cut root) were used. The petiole or stem was inserted in hole (c) of the cap, and the contact of plant and cap was sealed with clay to prevent leaks and insect escape. Bottoms A and B were placed together and the union sealed with Parafilm®, forming the chamber (Fig. 1).

There were 2 sets of observations on *B. cockerelli*. The first observations used 10 replicates (chambers) with tomato leaflets and the petiole in water. To ensure the use of live, vigorous leaflets, these were incubated in chambers for 24 h before starting observations. After this acclimatization period, adult insects (2 males and 3 females) were introduced per chamber, which was quickly assembled to prevent insect escape; the union of bottoms A and B was sealed with Parafilm®. After 48 h adults were removed, and the number of eggs, and eventually nymphs and adults, were recorded over several d. Leaflets were changed twice (14 and 21 d after experiment setup) since nymphs (about 30 per leaflet) caused rapid deterioration of plant material.

In the second set of observations, on tomato (Rio Grande variety), we compared the effect of 3 treatments on oviposition: leaflets in water, plants in water, and plants in moistened substrate (Premier® sphagnum peat moss; Premier, Quakertown, Pennsylvania, USA). Plants were first acclimatized for 24 h as before. Ten *B. cockerelli* adults (5 females and 5 males) were introduced per chamber as described with 10 replicates (chambers) per treatment. Adults were confined in the chamber for 48 h, then removed. After 5 d, we recorded the number of unhatched and hatched eggs. Data (second observations) were processed by analysis of variance and means separation (Tukey's HSD) in the statistical package InfoStat (Di Rienzo et al. 2017).

In the first observations, females oviposited a mean of 15.3 eggs per leaflet. In the second observations, means were 13.3 eggs on leaflets in water, 13.7 on plants in moistened substrate, and 15.2 on plants in water (Table 1). Twelve eggs were observed in a clutch by Lehman (1930).

In the first observations (leaflets with water), the life cycle of *B. cockerelli* took on average 20.2 d (range: 20–27 d). On potted plants in the laboratory, it took 18.7 d (range: 17–27 d) (Yang et al. 2013). In the second observations, 85.9% of eggs hatched, and 74% of eggs became adults across treatments. No significant differences were observed in oviposition (P = 0.8585) or egg hatching (P = 0.7164) between treatments (leaflets in water, seedlings in water, and plant with substrate) (Table 1).

Plants remained in good condition until the second experiment was finished. Both leaflets and plants kept growing and often developed roots in vials. This root development may be useful for experimental evaluations. Leaflets with few (near 10) nymphs remained viable for at least 3 wk; the plants (both in water and substrate), for up to 5 wk.

Kaur et al. (2020) observed *B. cockerelli* on potato leaves, in an arena composed of a plastic cup (29.5 mL) and a 1.5 mL vial; however, its shape and size make observations under the stereoscope difficult. Flat, clear Petri dishes of a small size, and easy manipulation of our chamber allow observations under the stereoscope; leaflets or plants can be used with water or solid substrates. Also it is useful for the following: *B. cockerelli* on potato leaflets, and pepper (*Capsicum annuum* L.; Solanaceae) and nightshade (*Solanum elaeagnifolium* Cav.; Solanaceae) twigs; Asian citrus psyllid (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae), on orange (*Citrus* × *sinensis* Osbeck; Rutaceae) and orange jessamine (*Murraya paniculata* L. Jack; Rutaceae); and western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae), and on bean (*Phaseolus vulgaris* L.; Fabaceae) leaves.

This chamber is easy to implement and replicate; it allows examination under the stereoscope of oviposition, hatching, development time, and mortality of insects. It is a simple option for observation of *B. cockerelli* and other insects. These results can be a baseline for experimental analysis of phytophagous insects and plants.

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Summary

We describe a portable, economical plastic chamber for observations on development and reproduction of potato-tomato psyllid, *Bactericera cockerelli* for up to a few wk on plant material under controlled conditions. It can be used for other insects as well.

Key Words: insect; phytophagous; laboratory; Solanaceae

Sumario

Se describe una cámara de plástico portátil y económica para observaciones durante semanas del desarrollo y reproducción de el psílido de la papa y tomate, *Bactericera cockerelli* en material vegetal bajo condiciones controladas. Esta cámara también se puede utilizar para otros insectos.

Palabras Clave: insecto; fitófago; laboratorio; Solanaceae

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Physalis virginiana as a wild field host of *Bactericera cockerelli* (Hemiptera: Triozidae)[1] and *Liberibacter solanacearum*[2]

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Keywords: Psyllid, wild host, Solanaceae, vector.

Abstract

The potato/tomato psyllid, *Bactericera cockerelli* (Šulc), is among the most important pests of solanaceous crops, as vector of the pathogen *Candidatus* Liberibacter solanacearum (Lso). Lso-infected psyllids often arrive in crop fields from various wild species of Solanaceae and Convolvulaceae, especially those that provide early-season hosts for the vector. *Physalis* species are perennial plants within the Solanaceae with often broad geographical distributions that overlap those of *B. cockerelli*, yet the status of many *Physalis* species as hosts for *B. cockerelli* or Lso remains unknown. Our objective was to determine whether wild *Physalis* species that occur in the potato-growing region of Galeana, Nuevo Leon, Mexico, host *B. cockerelli* populations, and whether they are also susceptible to Lso. Sampling was carried out at the potato-growing zone of Galeana, Nuevo León, Mexico, where unidentified *Physalis* sp. are common. In March–October 2021, a wild plant identified as *Physalis virginiana* was observed; eggs, nymphs, and adults of *B. cockerelli* were observed on these plants throughout the growing season, and nymphs completed development on these plants under laboratory conditions. Lso was also detected in 22 of the 93 (23.7%) wild *P. virginiana* plants using conventional PCR, while 13.3% of *B. cockerelli* adults that emerged from *P. virginiana* cuttings harbored the pathogen. This is the first report that *P. virginiana* is a host for *B. cockerelli* and for Lso. These results suggest that *P. virginiana* is a likely source of Lso-infected psyllids colonizing solanaceous crops in northeastern Mexico. The importance of *P. virginiana* and other wild hosts on the population

40 dynamics of vector and pathogen should be investigated to assist on pest

41 management decision-making.

42 **Key words:** Psyllid, wild host, Solanaceae, vector.

Introduction

The potato/tomato psyllid *Bactericera cockerelli* (Šulc.) (Hemiptera: Triozidae) is the most important insect pest of solanaceous crops in the agricultural region of arid northeastern Mexico (Buchman *et al*. 2011, Delgado-Luna *et al*. 2022). There is worldwide concern for this insect as it is vector of the plant pathogenic bacterium informally called *Liberibacter solanacearum* Liefting *et al.* 2009 (= "*Candidatus* Liberibacter solanacearum", = "*Candidatus* Liberibacter psyllaurous") (Lso), which causes devastating diseases known as "zebra chip" in potatoes and "permanente" in tomatoes (Djaman *et al*. 2019). *Bactericera cockerelli* is native to western North America. Several distinct haplotypes of *B. cockerelli* occur that differ in geographic distribution, presence of endosymbionts, host plant use, and physical characteristics (Cooper et al. 2019, Horton et al. 2014, Prager et al. 2014, Cooper et al. 2015, Mustafa et al. 2015, Fu et al. 2020, Cooper et al. 2022).

Lso also occurs as several haplotypes, with Lso haplotypes A and B commonly infecting Solanaceous crops (Lin et al. 2012). Haplotype B causes more severe symptoms in plants than does Haplotype A and it is the prevailing haplotype in the southwestern USA and northern Mexico (Swisher-Grimm et al. 2020, Medoza-Herrera et al. 2018, Harrison et al. 2019). There are no methods to

directly control Lso, so management of this pathogen relies upon the use of insecticides to suppress populations of the vector.

A major challenge in the control of *B. cockerelli* and Lso is that infected psyllids often arrive in crop fields from non-crop weedy hosts (Cooper et al. 2022). *Bactericera cockerelli* develops on a diversity of wild species within the Solanaceae and Convolvulaceae, and the composition of wild hosts varies among the different geographic areas (Thinakaran *et al*. 2015, Kaur *et al*. 2018, Martin. 2018, Cooper *et al*. 2019, Reyes-Corral *et al*. 2020, Reyes-Corral *et al.* 2021, Delgado-Luna *et al*. 2022). Many of these wild hosts are also susceptible to Lso and serve as non-crop sources of Lso-infected psyllids that colonize crop fields (Wen *et al*. 2009, Vereijssen *et al*. 2015, Cooper *et al*. 2019, Reyes-Corral *et al*. 2020, Reyes-Corral *et al.* 2021, Caicedo *et al*. 2020). One group of wild host species that has been largely overlooked includes species within the genus *Physalis.* Over 70 species of *Physalis* occur in Mexico and 17 occur in the United States and Central America (Martínez 1993, Santiaguillo *et al*., 2010). Reyes-Corral (2020, 2021) found that *Physalis longifolia* is a potential source of Lso-infected psyllids in the U.S. Pacific Northwest (Washington, Oregon, and Idaho). This plant, like other species of *Physalis* and Solanaceae*,* are perennial herbaceous plants. They overwinter as below-ground fleshy tap roots, rhizomes, or tubers. The vegetative growth that ensues from Lso-infected plants can develop into new Lso-infected plants the following spring. Research on other non-cultivated species of *Physalis* as hosts of *B. cockerelli* and Lso is lacking.

The goal of our study was to survey wild species of *Physalis* in the agricultural region of northeastern Mexico for *B. cockerelli* and Lso. We had two specific objectives: 1) survey wild stands of *Physalis* for presence of *B. cockerelli* eggs, nymphs, and adults, 2) determine whether *Physalis* are susceptible to Lso and whether psyllids that complete develop on these plants harbor the pathogen. Lso epidemics have severely impacted production at the potato-growing area of southern Nuevo León (NL) and Coahuila states, Mexico, mostly in the municipality of Galeana, NL (historically, 9000 ha or more). Therefore, it is crucial to identify which wild plants have relevant roles in the distribution and abundance of *B. cockerelli* and the epidemiology of Lso. This information may lead to development of risk or prediction models for areawide integrated pest management of Lso.

Materials and Methods

Field survey for presence of *B. cockerelli*.

Sampling was carried out from December 2020 to March 2022, in Galeana, state of Nuevo León, Mexico, at three localities: San Rafael (25°06'18"N 100°36'002'W, 2057 meters above sea level); Seis de Enero (24°52'53.8"N 100° 24'24.3"W, 1878 masl) and La Trinidad (24°52'58"N 100°25'40"W, 1879 masl). The average annual temperature is 19°C; there are freezes every year and brief intense cold spells (-10°C) in some years. The prevailing ecosystems are agricultural areas and secondary vegetation derived from a temperate arid steppe with short grasses and sparse *Juniperus* sp. and thickets of wolfberry, *Lycium berlandieri* (Dunal); creosote bush (*Larrea tridentata*); allthorn (*Koeberlinia* sp.), and others.

The potato-tomato psyllid is native to the area (Djaman et al. 2019). In March-October 2021 a wild species of *Physalis* was noted harboring eggs and nymphs of *B. cockerelli*. The plants were collected at the three points on sampling dates, based on resources and accessibility; in addition, the distribution of this plant is heterogeneous, therefore sampling was variable (Table 1). Plants were identified by JAVQ using Sullivan (2004) and Waterfall (1967), and vouchers were deposited in the Herbarium of the Universidad Autónoma Agraria Antonio Narro (ANSM).

Development of *B. cockerelli* on *P. virginiana*.

Plants were placed in Ziploc bags (SC Johnson Mexico, Toluca) and checked in the laboratory for presence of psyllids. *Physalis* leaves with *B. cockerelli* nymphs on them were placed in a humid chamber (one-liter plastic containers, with an organza fabric lid and moist cotton on the lid) to monitor adult emergence. Insects were identified with Kaur *et al*. (2018).

Fifteen adults were randomly taken from those emerging from leaves in humid chambers to determine if they were infected with Lso. Samples were analyzed for the presence of Lso using real-time PCR. DNA was extracted samples using modified CTAB precipitation protocols modified for insects (Zhang et al. 1998). The quality and quantity of DNA was assessed using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA), and samples were diluted to 50 ng/µl. Real-time PCR was performed using primers LsoF (GTC GAG CGC TTA TTT TTA ATA GGA) (Li et al. 2009) and HLBr (GCG TTA TCC CGT AGA AAA AGG TAG) (Li et al. 2006). Each 20 ul reaction included 10ul of Roche SYBR Green taq polymerase (Roche Applied Science, Indianapolis, IN) and

130 5uM of each primer. Real-time PCR was performed on a Light Cycler 480 (Roche
131 Applied Science) under the following conditions: 45x, 95°C for 10s, 60°C for 10°s,
132 and 72°C for 30s. Melting analysis was performed after amplification cycles to
133 confirm amplification of the target gene. Melting conditions included 95°C for 1:00
134 with a ramp rate of 4.4°C/s, 40°C for 1:00 with a ramp rate of 2.2°C/s, 60°C for
135 0.02 with a ramp rate of 1, and 95°C with a continuous acquisition at 25
136 acquisitions/C. PCR conditions for cooling, 40°C for 0:10 with a ramp rate of
137 2.2C/s.

138 Potato psyllid haplotypes were determined using high resolution melt
139 analysis of the cytochrome oxidase I gene (CO1) according to Swisher et al.
140 (2012). The analysis of the samples was conducted using a Lightcycler 480
141 (Roche Applied Science, Indianapolis, IN) using 20 µl reactions consisting of 10 µl
142 of LightCycler 480 HRM Master Mix, 0.5 µl of each 20 µM primer, 5.8 µl of DNA-
143 free water, 1.6 µl of 25 mM MgCl2, and 1.6 µl of DNA template. Two different sets
144 of primers were used (Chapman et al. 2012, Swisher et al. 2012). The first set of
145 primers CO1 F3 (5'-TAC GCC ATA CTA GCA ATC GG-3') and CO1 meltR (5'-TGA
146 AAT AGG AAT CAA-3') distinguish the central haplotype from northwestern,
147 western and southwestern haplotypes. The second set of primers CO1 meltF (5'-
148 GGA TTC ATT GTT TGA GCA CAT C-3') and CO1 meltR distinguish the western
149 haplotype from the northwestern and central haplotype. Cycling temperatures for
150 both primers included a preincubation step of 95°C for 10 min with a 4.4°C/s ramp
151 rate following 35 amplification cycles consisting of 95°C for 10 s with 4.4°C/s ramp
152 rate, 60°C with a second target of 53°C at a step size of 0.5°C with one cycle step

153 delay for 15 s with 2.2°C/s ramp state and 72°C single acquisition for 25s with
154 4.4°C/s ramp rate. Melting conditions were set at 95°C for 1 s with a ramp rate of
155 4.4°C/s, 40°C for 1 s with a ramp rate of 2.2°C/s, 60°C for 20 s with a ramp rate of
156 1°C, 95°C for continuous acquisition at 25 acquisitions/°C with a cooling step of
157 40°C for 10 s with a ramp rate of 2.2°C/s. Results were analyzed using the
158 LightCycler 480 Gene Scanning Software (Roche Applied Science) and manually
159 classifying each sample based on melting curves from known haplotype standard
160 samples.
161
162 **Presence of Lso in *Physalis virginiana.***
163 One cm of root was taken randomly from 93 field-collected plants (Table 1
164 lists sampling points and dates). Samples were analyzed for the presence of Lso
165 using real-time PCR. DNA was extracted from samples using modified CTAB
166 precipitation protocols modified for plants (Munyaneza et al. 2010). The quality
167 and quantity of DNA was assessed using a Nanodrop 2000 spectrophotometer
168 (ThermoFisher Scientific, Waltham, MA), and samples were diluted to 50 ng/µl.
169 Real-time PCR was performed as described for insects.
170 Lso positive samples were classified as haplotypes A or B based on simple
171 sequence repeat (SSR) markers (Lin et al. 2012). PCR for haplotyping was
172 performed in 25µl reactions, each containing 0.5 µl of Advantage Taq polymerase
173 (Takara Bio, Mountain View, CA), 2.5 µl of Advantage buffer, 0.5 µl of 10 µM
174 dNTPs, 0.5 µl of 100 nM of each primer, and 5 µl of DNA template. Primers were
175 Lso-SSR-1F (TTA TTT TGA GAT GGT TTG TTA AAT G) and Lso-SSR-1R (TAT

TAT CAT TCT ATT GCC TAT TTC G). PCR conditions included 40 cycles of 94°C for 10 s, 58°C for 10 s and 72°C for 15 s. The presence and size of amplicons associated with Liberibacter haplotypes A (240 bp) or B (180 bp) was observed on 1.5% agarose gels.

Results

We consistently observed eggs and nymphs of *B. cockerelli* on one species of *Physalis* in Galeana Mexico. This plant was identified as Virginia ground-cherry or wild tomatillo, *Physalis virginiana* Mill. (Figure 1). Plants are herbs or small forbs, 15-45 cm tall; they have lanceolate leaves 5-8 cm long and 1-2 cm wide. Flowers are solitary in the axils of the leaves, yellow or greenish yellow, with five light brown spots; fruits are fleshy, orange in color. Figure 1 shows a plant growing under favorable conditions in the field. In less favorable, drier habitats, many plants appear stunted and only have one or two small stems less than 15 cm tall, and few or no fruits (Figure 2). This plant has not been previously reported as a host for *B. cockerelli* or *L. solanacearum*. In Galeana, the plant is widespread, herbaceous, perennial over several years, surviving the winter as deep rhizomes that sprout in the spring. It is found in grasslands, fields, open woods, and disturbed habitats. It has a transcontinental distribution that includes the central, eastern and southeastern United States and northern and central Mexico (Sullivan 2004, Waterfall 1967).

The percentage of adults emerged from nymphs in moist chambers on 7 April was 9.71%; in 2 June this was 7.8 and 3.71%, while in 11 September nymphs

but no emergence of adults was observed. It is likely that first and second instar nymphs collected were not able to finish their life cycle on decaying cut plants inside the humid chamber, while 4th and 5th instar nymphs managed to reach their adult stage; therefore, the number of adults produced per plant reported here (Table 1) likely underestimates the number of *B. cockerelli* that could emerge from these plants in the field.

Two of the 15 (13.3%) insects analyzed were also positive for Lso; these insects emerged from plants with undetermined Lso status. These insects were identified as central haplotype (Swisher et al. 2012), which is the predominant haplotype found in this region (Reyes-Corral et al. 2020).

Plant and insect samples were tested for presence of Lso using real-time PCR. A total of 93 plants were analyzed, and 23.7% of the plants were positive for Lso (Table 2). These Lso-positive plants were found at the Trinidad and Seis de Enero sampling locations. Infections were caused by Lso haplotype B, which has largely displaced haplotype A in most growing regions.

Discussion

P. virginiana was first observed in the field from February and until October. Potato, pepper and tomatillo crops had recently been established between 7 April and 29 May 2021. Therefore, *P. virginiana* is available as a host of several *B. cockerelli* generations (potentially three or more), 10 to 12 weeks before the emergence of solanaceous crops grown in the area each year. *B. cockerelli* were observed on *P. virginiana* plants from April to June, but were rare or absent on this

host from July to October. Senescence of *P. virginiana* was observed in October; the plant was not observed in the field from December 2020 to February 2021, and from October 2021 to February 2022. This plant has not been previously reported as a host for *B. cockerelli* or *L. solanacearum*.

Reyes-Corral *et al*. (2020) report that the central, western and northwestern haplotypes of *B. cockerelli* preferred to settle on the wild host *Physalis longifolia* than on potato (*Solanum tuberosum*) and produced up to three times more offspring on the former; *P. longifolia* is a better host for the northwestern than for the western haplotypes of *B. cockerelli* (Cooper *et al*. 2019). It remains unknown whether *B. cockerelli* also prefer *P. virginiana* over potato.

The genus *Physalis* comprises a large number of species that occur within certain potato, tomato, tomatillo, and pepper growing regions of western North America that are often challenged by Lso vectored by *B. cockerelli*. As mentioned, many *Physalis* species are vegetative perennials that overwinter as below-ground rhizomes that emerge in early to late spring the next year. Here, we provide the first report that *Physalis virginiana* Mill. is a host for *B. cockerelli* and Lso.

Lso has been reported in field samples of *Physalis ixocarpa* (tomatillo) in Mexico and *Physalis peruviana* in Ecuador (Caicedo *et al*. 2020, Reyes-Corral *et al.* 2021). It has also been experimentally determined that *P. longifolia* is susceptible to Lso, but Lso has thus far not been detected in wild stands of *P. longifolia* in the Pacific Northwest U.S. (Reyes-Corral *et al*. 2020). Many wild solanaceous plants in the region reported herein are also perennial herbs through deep rhizome-like roots or stolons (Delgado-Luna et al. 2022; unpublished

observations). Thinakaran *et al.* (2015) reported that Lso-infected silverleaf nightshade or trompillo (*Solanum elaeagnifolium*) plants retained the pathogen under field conditions throughout the year. The pathogen was confirmed from leaves, roots and, importantly, from stolons collected one year after infecting these perennial plants (Thinakaran *et al*. 2015). Reyes-Corral *et al*. (2020) report that Lso also overwintered in *P. longifolia* rhizomes, which show brown streaks (a symptom analogous to potato "zebra chip") when sectioned. Lso-infected *P. longifolia* rhizomes also overwinter and produce infected plants the following spring (Reyes-Corral *et al*. 2020). Henne *et al*. (2010) report that up to 44% of Lso-infected potato tubers remain viable, but develop into weak, uncompetitive plants with spindly shoots. Therefore, it is important to determine whether Lso-infected *P. virginiana* rhizomes produce infected plants the following spring. We consider likely that *P. virginiana* plants do not lose their Lso infections over the winter months. However, our data indicate that infection levels of Lso in *P. virginiana* are not near 100% (Table 2); further research is needed on the overwintering and across-years fluctuation of Lso across in individual specimens of these perennial hosts.

We consider *P. virginiana* to be a high-risk source for Lso-infected psyllids because it is widely distributed in the United States and Mexico; also, because its infected foliage is available in the field before solanaceous crops (potato, pepper, and tomatillo) are planted in NE Mexico. Also, we verified that *P. virginiana* plants infected with Lso can produce infected insects. Based on our observations, we can propose that in the region of study, *B. cockerelli* appears to overwinter in situ on wild hosts, mainly on wolfberry, *L. berlandieri* (Cooper *et al*., 2022; personal

observations) and subsequently infests *P. virginiana* plants and other wild hosts such as five eyes, *Chamaesaracha* spp. (Delgado-Luna *et al*. 2022) and *S. elaeagnifolium* (Thinakaran *et al*. 2015) in the spring; later, it migrates to solanaceous crops established in the surroundings. This information can support decision-making in regional agricultural systems at risk of Lso infection, helping predictions on the risk of *B. cockerelli* infestation and Lso infection in agricultural areas where wild hosts are present. Potato production in the region continues to subsist at the expense of tremendous pesticide inputs (essentially daily fumigation of potato fields with concoctions of highly toxic chemicals), relying on destruction of insect pests as they land on the crop and increasing both economic and environmental costs in the agricultural system. In this region, production costs including pesticides have caused near abandonment of potato production over the last decade. It is critical to develop sustainable pest management programs for the region. Therefore, it is relevant to carry out extensive field studies on the role of wild hosts as a source of infected adult psyllids.

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441

442 **Table 1.** Eggs/plant, nymphs/plant and percentage of adults emerged from nymphs
443 collected on *P. virginiana*, by date and sampling location (San Rafael, La Trinidad,
444 Seis de Enero; municipality of Galeana, NL, Mexico).

Date	Place	plants examined	eggs/plant	nymphs/plant	Emerged adults
			Mean ± SE	Mean ± SE	(% emerged)
21-Mar-21	La Trinidad	0	0 ± 0	0 ± 0	0
07-Apr-21	San Rafael	15	0 ± 0	0 ± 0	0
	La Trinidad	40	10.2 ± 2.04	13.9 ± 2.42	54 (9.71%)
29-May-21	La Trinidad	*	*	*	*
02-Jun-21	La Trinidad	90	0.3 ± 0.12	3.7 ± 1.09	26 (7.8%)
16-Jun-21	San Rafael	15	0 ± 0	0 ± 0	0
	La Trinidad	40	10.2 ± 2.05	10.1 ± 2.42	15 (3.71%)
07-Jul-21	La Trinidad	30	0 ± 0	0 ± 0	0
	Seis de Enero	20	0 ± 0	0 ± 0	0
10-Aug-21	La Trinidad	0	0 ± 0	0 ± 0	0

11-Sep-21	La Trinidad	20	0 ± 0	0.2 ± 0.19	0
28-Sep-21	Seis de Enero	25	0 ± 0	0 ± 0	0
23-Oct-21	La Trinidad	0	0 ± 0	0 ± 0	0
25-Mar-22	La Trinidad	0	0 0	0 0	0

445

446 * Samples lost due to severe weather.

447

448 *

449

450 **Table 2.** Number of Lso positive *Physalis virginiana* plants by date and sampling

451 point in Galeana, Nuevo León, Mexico.

Date	Place	plants analyzed	N° plants positive (%)
02-jun-21	La Trinidad	34	4 (11.8%)
16-jun-21	La Trinidad	14	7 (50.0%)
	6 de enero	8	6 (75%)
11-sep-21	La Trinidad	11	2 (18.2 %)
28-sep-21	6 de enero	26	3 (11.5%)

452

453 **Figure 1.** 20 cm tall plant of *Physalis virginiana* Mill. a) flower, b) fruit.

454 **Figure 2.** Clusters of *Physalis virginiana* plants at the Seis de Enero, Galeana, NL
455 locality (September 10, 2022).

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'*Candidatus* Liberibacter solanacearum' Infection of *Physalis ixocarpa* Brot. (Solanales: Solanaceae) in Saltillo, Mexico

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Abstract

The potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) is a pest of solanaceous crops (order Solanales), including potato (*Solanum tuberosum* L.) and tomato (*S. lycopersicum* L.). Feeding by high populations of nymphs causes psyllid yellows while adults and nymphs are vectors of the plant pathogen '*Candidatus* Liberibacter solanacearum'. Foliar symptoms that were consistent with either '*Ca.* L. solanacearum' infection or psyllid yellows were observed in 2019 on tomatillo (*Physalis ixocarpa* Brot.; family Solanaceae) grown within an experimental plot located near Saltillo, Mexico. This study had three primary objectives: 9i) determine whether the foliar symptoms observed on tomatillo were associated with '*Ca.* L. solanacearum' infection, (ii) identify the haplotypes of '*Ca.* L. solanacearum' and potato psyllids present in the symptomatic plot, and (iii) use gut content analysis to infer the plant sources of '*Ca.* L. solanacearum'-infected psyllids. Results confirmed that 71% of symptomatic plants and 71% of psyllids collected from the plants were infected with '*Ca.* L. solanacearum'. The detection of '*Ca.* L. solanacearum' in plants and psyllids and the lack of nymphal populations associated with psyllid yellows strongly suggests that the observed foliar symptoms were caused by '*Ca.* L. solanacearum' infection. All infected plants and insects harbored the more virulent '*Ca.* L. solanacearum' haplotype B but one psyllid was also coinfected with haplotype A. The potato psyllids were predominantly of the central haplotype but one psyllid was identified as the western haplotype. Molecular gut content analysis of psyllids confirmed the movement of psyllids between noncrop habitats and tomatillo and indicated that '*Ca.* L. solanacearum' infection of psyllids was associated with increased plant diversity in their diet.

Keywords: bacteria, *Bactericera cockerelli*, etiology, field crops, Hemiptera, Liberibacter, pathogen detection, pathogen diversity, *Physalis ixocarpa*, potato psyllid, prokaryotes, zebra chip

'*Candidatus* Liberibacter solanacearum' (syn. '*Ca.* L. psyllarous') is a phloem-limited bacterial plant pathogen that is transmitted among plants by the potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) in North America (Hansen et al. 2008; Liefting et al. 2009; Munyaneza et al. 2007). This pathogen infects a diversity of plants within the family Solanaceae (order Solanales) and, in most susceptible plants, causes foliar symptoms characterized by leaf yellowing, reduced yield, and plant dieback (Cooper et al. 2019b). In potato, the infection by '*Ca.* L. solanacearum' can result in development of striped patterns in potato tubers known as zebra chip disease (Munyaneza 2012). The disease was first observed in harvested potato tubers from Saltillo, Mexico in the 1990s, and now occurs in nearly all potato- and tomato-growing regions of western North America (Munyaneza 2012). '*Ca.* L. solanacearum' occurs as several distinct haplotypes based upon 16S and simple sequence repeat (SSR) sequences (Swisher Grimm et al. 2018). These haplotypes appear to differ in severity of visible disease symptoms that they induce in infected plants. '*Ca.* L. solanacearum' haplotypes A and B commonly occur in cultivated crops, and haplotype B appears to be more virulent in potato, tomato, and longleaf groundcherry (*Physalis longifolia*) (Harrison et al. 2019; Mendoza-Herrera et al. 2018; Reyes Corral et al. 2020; Swisher Grimm et al. 2018). '*Ca.* L. solanacearum' haplotypes G and F have also been detected on species of Solanaceae in North America but both appear to be less common than haplotypes A and B (Mauck et al. 2019; Swisher Grimm and Garczynski 2019). The remaining haplotypes occur in Europe and infect crops within the family Apiaceae, including carrot and celery (Nelson et al. 2011).

Feeding by large populations of potato psyllid nymphs causes leaf yellowing and senescence that is nearly indistinguishable from foliar symptoms caused by '*Ca.* L. solanacearum' infection (Munyaneza 2012). Like '*Ca.* L. solanacearum', potato psyllids occur as several distinct haplotypes based upon variations in cytochrome oxidase I (CO1) genes—central, western, northwestern, and southwestern—that differ in certain biological traits, including fertility rates, development time, host plant use and preference, and presence of the bacterial endosymbiont *Wolbachia* (Cooper et al. 2019a; Horton et al. 2014; Mustafa et al. 2015a, b; Swisher et al. 2013b). All of the currently known psyllid haplotypes can acquire and transmit '*Ca.* L. solanacearum' to plants (Mustafa et al. 2015b). Potato psyllids complete development on a wide range of crop and noncrop plants within the Solanaceae and Convolvulaceae families, and often colonize crop fields from noncrop hosts (Cooper et al. 2019a; Horton et al. 2015a, b; Kaur et al. 2018; Knowlton and Thomas 1934; Wallis 1955; Wenninger et al. 2019). A major challenge in managing potato psyllids and '*Ca.* L. solanacearum' is that we currently do not know which plant species serve as primary sources of '*Ca.* L. solanacearum' inoculum that is acquired by potato psyllids prior to crop colonization. This uncertainty is exacerbated by the regional variations in diversity of species of Solanaceae and Convolvulaceae that occur throughout the potato psyllid's geographic range, which spans most of western North America, parts of Central and South America, Australia, and

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New Zealand (Castillo-Carrillo et al. 2019; Munyaneza 2012). Recently, a molecular gut content approach was developed to identify the dietary history of potato psyllids (Cooper et al. 2016, 2019c). This approach allows researchers to investigate how variations in regional plant biodiversity affect the dietary history of potato psyllids that colonize potato and identify epidemiologically important plant species that serve as primary sources of '*Ca.* L. solanacearum' inoculum.

Tomatillo (*P. ixocarpa* Brot., family Solanaceae; synonym *P. philadelphica* Lam. var. *immaculata* Waterf.) (Solanales: Solanaceae) or Mexican groundcherry is an economically important crop in Mexico, with about 771,000 metric tons produced annually. Although commercial tomatillo is a documented host of potato psyllid and '*Ca.* L. solanacearum' (Contreras-Rendón et al. 2020; Silva-Rojas et al. 2016), the literature on epidemiology of '*Ca.* L. solanacearum' in tomatillo is scarce and symptoms of '*Ca.* L. solanacearum' infection in tomatillo have not been thoroughly described. In November 2019, a tomatillo plot in Saltillo, Coahuila, Mexico that was heavily infested with *B. cockerelli* exhibited severe yellowing symptoms typical for either psyllid yellows or infection by '*Ca.* L. solanacearum'. This agricultural region is the location where zebra chip disease of potato was first observed in 1994 (Munyaneza 2012) and is associated with a diversity of potential noncrop overwintering, food, or hosts plants of potato psyllids and '*Ca.* L. solanacearum'. In this report, we confirm that diseased plants in this tomatillo plot were infected with '*Ca.* L. solanacearum', and that potato psyllids collected from this plot were also infected with the pathogen. We then determined the haplotypes of the pathogen and psyllids infesting this plot. Finally, we performed gut content analysis to infer whether '*Ca.* L. solanacearum'-infected potato psyllids present in the tomatillo plot had arrived there from other crops or from noncrop weedy hosts.

Materials and Methods

Experimental tomatillo plot. *B. cockerelli* and *P. ixocarpa* samples were collected from an experimental plot located at the Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico (coordinates: 25.352561, −101.032276) on 15 November 2019. The experimental tomatillo plot was first established on 2 July 2019. Scattered plants or plots of other cultivated species of Solanaceae, including potato, pepper, and tomato, occurred within dozens or hundreds of meters from the sampled tomatillo plot. On 5 October 2019, foliar symptoms similar to '*Ca.* L. solanacearum' infection or psyllid yellows were observed and recorded on individual tomatillo plants. Plants were sampled for *B. cockerelli* by randomly selecting 10 plants, which were gently shaken five to seven times over a 43-by-28-cm white paper sheet, and counting the number of potato psyllid adults present. These plants and additional plants were also visually examined for the presence of potato psyllid nymphs and adults. A subsample of 21 potato psyllid adults was collected into 96% ethanol using a fine brush. Partially expanded and fully expanded leaves were excised from symptomatic plants, cut longitudinally into three sections, and placed in a vial with 96% EtOH as a preservative. The samples were processed at the United States Department of Agriculture laboratory in Wapato, WA for molecular analyses.

'*Ca.* L. solanacearum' detection and haplotype identification. DNA was purified from each psyllid and leaf sample using a cetyltrimethylammonium bromide precipitation method and was resuspended in 50 and 100 µl of nuclease-free water, respectively (Crosslin et al. 2011; Munyaneza et al. 2010). Conventional PCR using primers for the 16S ribosomal RNA gene of '*Ca.* L. solanacearum' (OA2 and OI2c) was used to confirm the presence or absence of the bacterium (Crosslin et al. 2011; Jagoueix et al. 1996). PCR conditions included an initial denaturation step of 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s; with a final extension at 72°C for 5 min. Each 20-µl reaction contained Amplitaq Gold 360 PCR Master Mix (Invitrogen, Carlsbad, CA, U.S.A.), 5 µM each primer, and DNA template (10 to 20 ng/ml for insect samples and 50 to 150 ng/ml for plant samples).

When '*Ca.* L. solanacearum' was detected in insect or plant samples, subsequent analyses were performed to identify the haplotype present based on the amplicon size of SSR markers (Lin et al. 2012). PCR for SSR markers was performed in 25 µl containing 0.5 µl of Advantage Taq polymerase (Takara Bio, Mountain View, CA, U.S.A.), 2.5 µl of 10× Advantage buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 100 nM each SSR primer ('*Ca.* L. solanacearum'-SSR-1F and '*Ca.* L. solanacearum'-SSR-1R), and 5 µl of DNA template. Temperature conditions for '*Ca.* L. solanacearum' haplotyping consisted of an initial denaturation at 94°C for 5 min; followed by 40 cycles of 94°C for 10 s, 58°C for 10 s, and 72°C for 15 s; with a 72°C final extension step for 5 min. Samples were electrophoresed using a 1.5% agarose gel and 5 µl of amplification product to discern '*Ca.* L. solanacearum' haplotypes A and B, based on amplicon size. Haplotype F, which is known to cause zebra chip disease of potato in Oregon, produces an amplicon size similar to that of haplotype A. Therefore, haplotypes were further confirmed from a subset of samples by excising SSR amplicons from gels and cloning them using a TOPO TA cloning kit with TOP10 *Escherichia coli* chemically competent cells (Invitrogen). Plasmid DNA was then extracted from selected colonies using a QIAprep spin mini prep kit (Qiagen, Valencia, CA, U.S.A.) and was sequenced by MC Laboratories (San Francisco, CA, U.S.A.).

The haplotype of each psyllid was identified using high-resolution melting analysis of the mitochondrial cytochrome C oxidase subunit 1-like gene (CO1) using a Lightcycler 480 (Roche Applied Science, Indianapolis, IN, U.S.A.) (Swisher et al. 2012, 2014). Each reaction consisted of 10 µl of Lightcycler 480 HRM Master Mix, 0.5 µl of each 20 µM primer, 5.8 µl of DNA-free water, 1.6 µl of 25 mM $MgCl_2$, and 1.6 µl of DNA template. Two different sets of primers (CO1 F3/CO1 meltR and CO1 meltF/CO1 meltR) were used to identify psyllid haplotypes (Chapman et al. 2012; Swisher et al. 2012). The first set CO1 F3 (5′-TAC GCC ATA CTA GCA ATC GG-3′) and CO1 meltR (5′-TGA AAT AGG AAT CAA-3′) distinguished the central haplotype from northwestern, western, and southwestern haplotypes. The second set CO1 meltF (5′-GGA TTC ATT GTT TGA GCA CAT C-3′) and CO1 meltR distinguished the western and southwestern haplotypes from the northwestern and central haplotypes. Programs for both of the primer sets started with a preincubation step of 95°C for 10 min with a 4.4°C/s ramp rate following 35 amplification cycles consisting of 95°C for 10 s with a 4.4°C/s ramp rate, 60°C with a second target of 53°C at a step size of 0.5°C with one cycle step delay for 15 s with a 2.2°C/s ramp rate, and 72°C single acquisition for 25 s with a 4.4°C/s ramp rate. Melting conditions were set at 95°C for 1 s with a ramp rate of 4.4°C/s, 40°C for 1 s with a ramp rate of 2.2°C/s, 60°C for 20 s with a ramp rate of 1°C, and 95°C for continuous acquisition at 25 acquisitions/°C with a cooling step of 40°C for 10 s with a ramp rate of 2.2°C/s. Results were then analyzed using the LightCycler 480 Gene Scanning Software (Roche Applied Science) and each sample was classified based on melting curves from known haplotype standard samples.

Gut content analysis. Gut content analysis of the plant-derived internal transcribed spacer (ITS) and the chloroplast *trnF* gene was used to infer the likely plant sources of psyllids collected from tomatillo. The primers for trnF were B49873-e (5′-GGT TCA AGT CCC TCT ATC CC-3′) and A50272-F (5′-ATT TGA ACT GGT GAC ACG AG-3′) (Taberlet et al. 1991) and the primers for a region of ITS2 were ITS2F (5′-ATG CGA TAC TTG GTG TGA AT-3′) and ITS3R (5′-GAC GCT TCT CCA GAC TAC AAT-3′) (Chen et al. 2010). Separate asymmetric, barcoded forward and reverse primers (Pacific Biosciences [PacBio]) were used for each individual psyllid. PCR controls included DNA from a potato psyllid reared on potato under laboratory conditions, a blank sample included in the DNA extraction procedure, a no-template control, and single primer controls. PCR conditions for reactions using both primer pairs included an initial denaturation step of 94°C for 10 min; followed by 39 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. Each 50-µl reaction contained Invitrogen Amplitaq Gold 360 PCR Master Mix (Invitrogen), 250 nM each primer, and 5 µl of DNA template. The presence of 400- to 600-bp amplicons was observed on 1% agarose gels stained with ethidium bromide.

PCR products were pooled into a single sample, shipped to the Washington State University Laboratory for Biotechnology and Bioanalysis, and sequenced directly using the PacBio sequencing

platform. Pooled barcoded amplicons were ligated to the hairpin SMRT bell adapters using PacBio Template kit v 1.0, and library purified using AMPureXP beads (Beckman-Coulter). Libraries were quantified and bound to the P6 polymerase, magbead loaded into a single SMRT cell, and observed for 6 h using C4 chemistry on a PacBioRSII. Raw movies were processed into reads, reads were processed into high-quality reads of interest, and barcodes were separated using SMRT Portal version 2.3. Average read length was 24.6 Kb, which produced an average single molecule coverage of 33×. This resulted in nearly all of the data being very high quality, with Phred scores between 35 and 45 (99.95 and 99.995 accuracy). Sequences were grouped into operational taxonomic units (OTUs) using the de novo assembly alignment tool of Geneious R10 using the custom sensitivity setting, with a minimum overlap identity of 98% and 98% maximum mismatches per read. Each OTU was then analyzed using the BLAST function of the NCBI database to putatively identify taxa to the lowest possible taxonomic unit (Altschul et al. 1990). Single unique reads were not assembled into OTUs and, therefore, were discarded. For analyses, plant taxa that are known to support complete nymphal development of *B. cockerelli* (namely, species within the families Solanaceae or Convolvulaceae) were considered "host plants" (Burckhardt et al. 2014). Plant taxa that were identified from psyllids but are not known to support development of *B. cockerelli* were considered "food plants" (Burckhardt et al. 2014). Patterns between psyllid infection status and plant taxa identified from gut content analysis were assessed through cluster analysis using the CLUSTER procedure of SAS 9.4 while specifying the Ward's minimum variance clustering method.

Results

Severe yellowing, cupping of leaves, darkening of leaf veins, and plant dieback were observed on 90% of tomatillo plants grown in a 12-week-old experimental plot near Saltillo, Mexico (Fig. 1). These symptoms were observed evenly throughout the entire plot without an obvious edge effect. All plants examined harbored potato psyllid adults but potato psyllid nymphs were not observed. On average, 7.2 adult potato psyllids were collected from each sampled plant within this plot. Our counts of psyllid adults are conservative because some psyllids jumped from the sheet before being accurately counted.

In the present work, PCR for '*Ca.* L. solanacearum' identification was performed on two different gene regions (16S and SSR), and amplification of both genes was consistent. Results of PCR revealed that '*Ca.* L. solanacearum' was detected in 71% of symptomatic plants and in 71% of psyllids collected from the plants (Table 1).

Based on the variations in size of SSR amplicons, all '*Ca.* L. solanacearum'-infected psyllids and leaves harbored '*Ca.* L. solanacearum' haplotype B, and one psyllid was coinfected with haplotypes A and B (Table 1). The haplotype identities were further confirmed by direct sequencing amplicons from a subset of samples, including the single psyllid harboring both '*Ca.* L. solanacearum' haplotypes. Most potato psyllids were identified as central haplotype but a single psyllid was identified as western haplotype and was infected with '*Ca.* L. solanacearum' haplotype B.

High-throughput sequencing of plant DNA amplified from 21 surface-sterilized potato psyllids produced more than 155,000 sequences after filtering reads for size and quality and after removing nontarget sequencings, including bacteria (Table 2). PCR amplicons were not observed for blank, no-template, or single-primer controls. Although more sequence reads were obtained for trnF than for ITS,

Table 1. '*Candidatus* Liberibacter solanacearum'-infection rates occurring in samples of symptomatic *Physalis ixocarpa* plants and potato psyllids collected from these plants

Sample	*Physalis* sp.	Potato psyllids
'*Ca.* L. solanacearum' infected[a]	5/7	15/21
Haplotype A	0/5	1/15[b]
Haplotype B	4/5	15/15
Undetermined	1/5	1/15

[a] Visible amplicons produced by PCR with primers to amplify a portion of 16S and simple sequence repeat regions.
[b] Coinfected with Liberibacter haplotype B. Results were confirmed by direct sequencing.

Table 2. Number of produced plant sequences from gut content analysis of psyllids using universal PCR primers for trnF and internal transcribed spacer (ITS), and number of operational taxonomic units (OTUs)

Parameters	trnF	ITS
Total number of sequence reads[a]	151,149	4,795
Mean ± standard error number of sequence reads per psyllid	7,198 ± 1,124.6	228 ± 57.5
Total number of OTUs[b]	20	28

[a] Number of trnF and ITS sequences included in gut content analysis after removing low-quality sequence reads and nontarget sequences, including those identified as bacteria.
[b] Number of OTUs, defined as groups of sequence reads sharing at least 95% sequence identity. Consensus sequences of OTUs were identified to the nearest possible plant taxonomy by BLAST analysis.

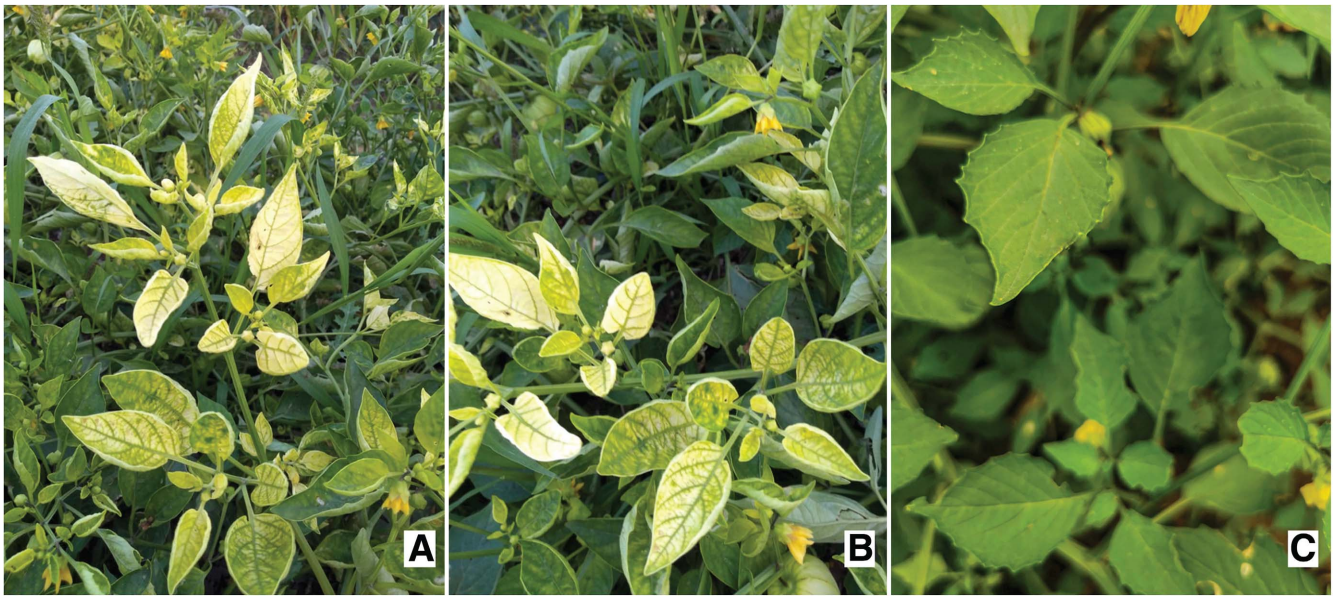

Fig. 1. *Physalis ixocarpa* plants. **A** and **B,** Close-up of *P. ixocarpa* exhibiting severe yellowing, chlorosis, and curling (cupped-up leaves) symptoms in an experimental plot at the Universidad Autonoma Agraria Antonio Narro in Saltillo, Mexico during November 2019. **C,** Healthy plant with green, flat, noncupped-up leaves.

a more diverse dietary history was detected by sequencing the ITS gene (Table 2). Generally, results of trnF and ITS were consistent (Fig. 2). Dietary plant classification follows Burckhardt et al. (2014). Many sequences aligned with species of Solanaceae but with low sequence alignment to specific solanaceous taxa. These OTUs were classified as unidentified members of Solanaceae with reference to the nearest sequence homology available in NCBI. Plants that are not suitable for nymph development of potato psyllid (plants that are not within the Solanaceae or Convolvulaceae families) were grouped by plant family, yet these plants may allow adults to survive periods without a development host (Burckhardt et al. 2014). *Physalis* spp. and an unidentified Solanaceae species with the closest sequence homology to *Solanum dulcamara* were both detected from all psyllids (Fig. 2).

Cluster analysis of dietary plant taxa grouped the psyllids into three clades (Fig. 3). All clades included *Physalis* spp. and *S. dulcamara*-like signatures. Additionally, clade 1 was primarily associated with two distinct *S. americanum*-like sequences, *S. tuberosum*-like sequences, *S. tuberosum* (potato), and species within the Asteraceae family (Fig. 3). Although two OTUs aligned most closely with NCBI entries identified as *S. americanum,* these two OTUs shared just 87.1% sequence identity, suggesting that they are from two different species of Solanaceae. Psyllids within clade 1 sampled an average of two nonhost plants (Fig. 3). Clade 2, which included most of the uninfected psyllids, was characterized by a relative absence of plant taxa other than *Physalis* spp. and *S. dulcamara*-like sequences (Fig. 3). Psyllids within this clade sampled an average of 0.4 nonhost food plant taxa. Clade 3 was characterized by *S. americanum*-like sequences and species within the Asteraceae family, and an average of three nonhost food plants per psyllid.

Discussion

Tomatillo plants within an experimental plot located near Saltillo, Mexico developed foliar symptoms in 2019 which were conspicuously similar to those produced by either '*Ca.* L. solanacearum' infection of potato or tomato, or psyllid yellows. PCR confirmed that the plants and psyllids collected from these plants harbored '*Ca.* L. solanacearum'. The detection of '*Ca.* L. solanacearum' in plants and psyllids and the lack of nymphal populations associated with the development of psyllid yellows suggests that the observed foliar symptoms were likely caused by '*Ca.* L. solanacearum' infection. The failure to detect

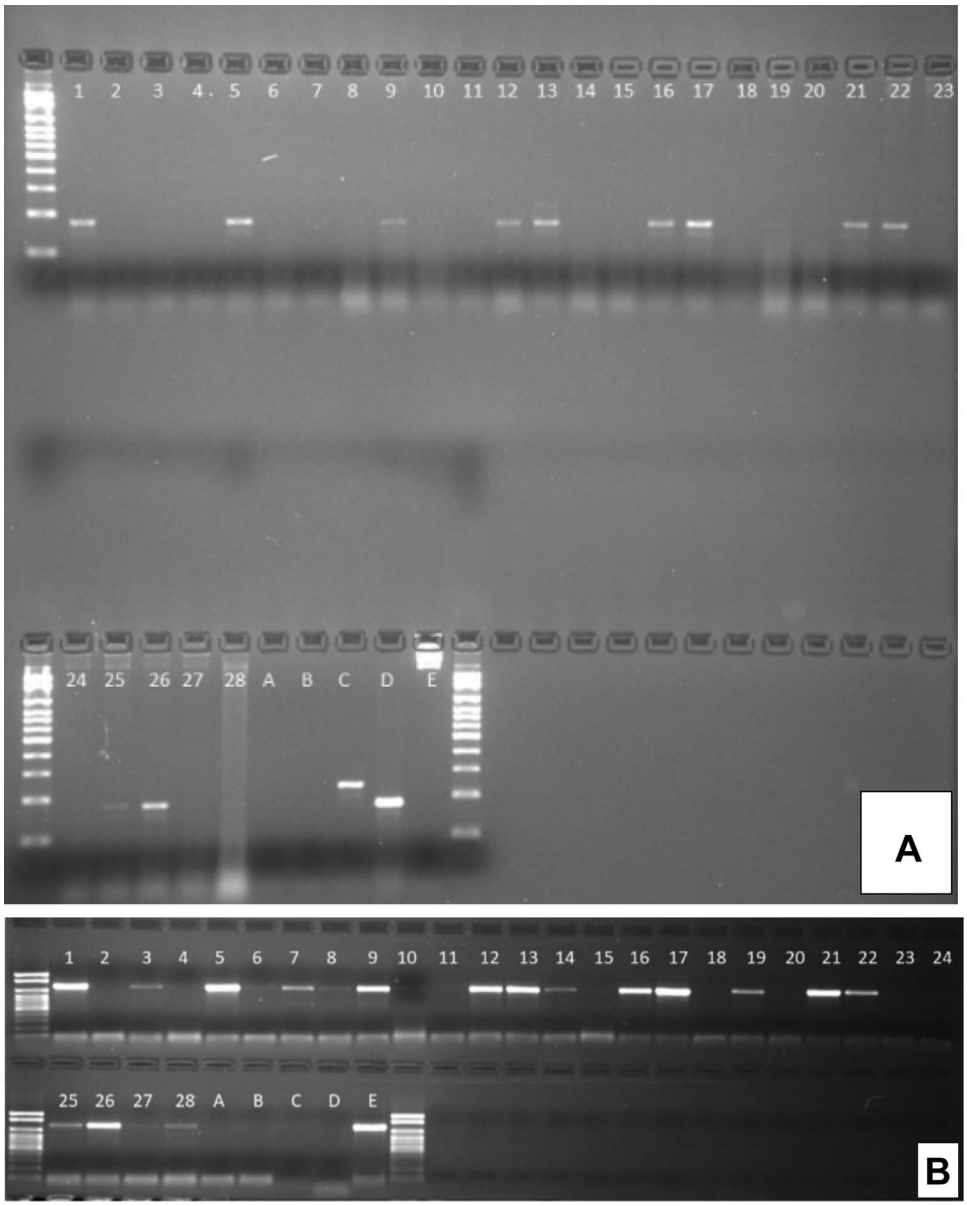

Fig. 2. PCR products for '*Candidatus* Liberibacter solanacearum' detection and characterization. **A,** OA2/OI2c ('*Ca.* L. solanacearum' +/−) Lane A, Blank; lane B, no-template control; lane C, minus forward primer; lane D, minus reverse primer; and lane E, '*Ca.* L. solanacearum'+. **B,** Simple sequence repeat (for characterization of '*Ca.* L. solanacearum' haplotypes A and B) Lane A, Blank; lane B, no-template control; lane C, '*Ca.* L. solanacearum' A+; lane D, '*Ca.* L. solanacearum' B+; and lane E, '*Ca.* L. solanacearum' A+ (from vector). In both figures, lanes 1 to 21 are insects (*Bactericera cockerelli*) and 22 to 28 are tomatillo plants.

'*Ca.* L. solanacearum' in 29% of symptomatic plants may be due to low titers in sampled tissues, and underscores the challenge in detecting '*Ca.* L. solanacearum' in plants (Buchman et al. 2011; Crosslin and Munyaneza 2009; Levy et al. 2011; Li et al. 2009).

At least four haplotypes of '*Ca.* L. solanacearum' have been identified in North America but only two (haplotypes A and B) commonly infect solanaceous crops. All infected plant samples and infected psyllids collected from the symptomatic tomatillo plot harbored '*Ca.* L. solanacearum' haplotype B, and one psyllid harbored both '*Ca.* L. solanacearum' haplotypes A and B. Both haplotypes have been detected in central Mexico, with haplotype A primarily found in chili or natural habitats (Nelson et al. 2011; Rojas-Martinez et al. 2016; Swisher et al. 2018). Compared with '*Ca.* L. solanacearum' haplotype A, haplotype B produces more severe foliar symptoms in tomato and potato, and more severe zebra chip symptoms in potato tubers (Harrison et al. 2019; Mendoza-Herrera et al. 2018; Swisher Grimm et al. 2018). Haplotype B also appears to cause more severe symptoms than haplotype A in the wild Physalis, *P. longifolia* (Reyes Corral et al. 2020). Haplotype A was more common in crop fields prior to 2006 but has since been largely displaced by haplotype B in the western United States (Dahan et al. 2019; Wen et al. 2013).

Nearly all potato psyllids collected from the tomatillo plot were of the central haplotype, which predominately occurs in the central United States and Mexico (Liu et al. 2006; Swisher et al. 2013a). However, a single psyllid was classified as western haplotype, which predominately occurs in the western United States and Baja, Mexico. These results are consistent with a previous survey conducted in Durango and Zacatecas, Mexico, which identified both psyllid haplotypes, with the central haplotype being more common (Swisher et al. 2018). The western and central haplotypes are biologically similar in most traits that have been assessed (Cooper et al. 2015; Horton et al. 2014; Mustafa et al. 2015a, b) but psyllids of the central haplotype have a smaller body size, lower fecundity, and longer development times on certain host plants than do those of the western haplotype (Mustafa et al. 2015a, c).

Molecular gut content analysis can be used to identify the dietary history of psyllids and to infer their landscape-level dispersals prior to their capture (Cooper et al. 2016, 2019c). This information can then be used to identify the likely sources of infective psyllids

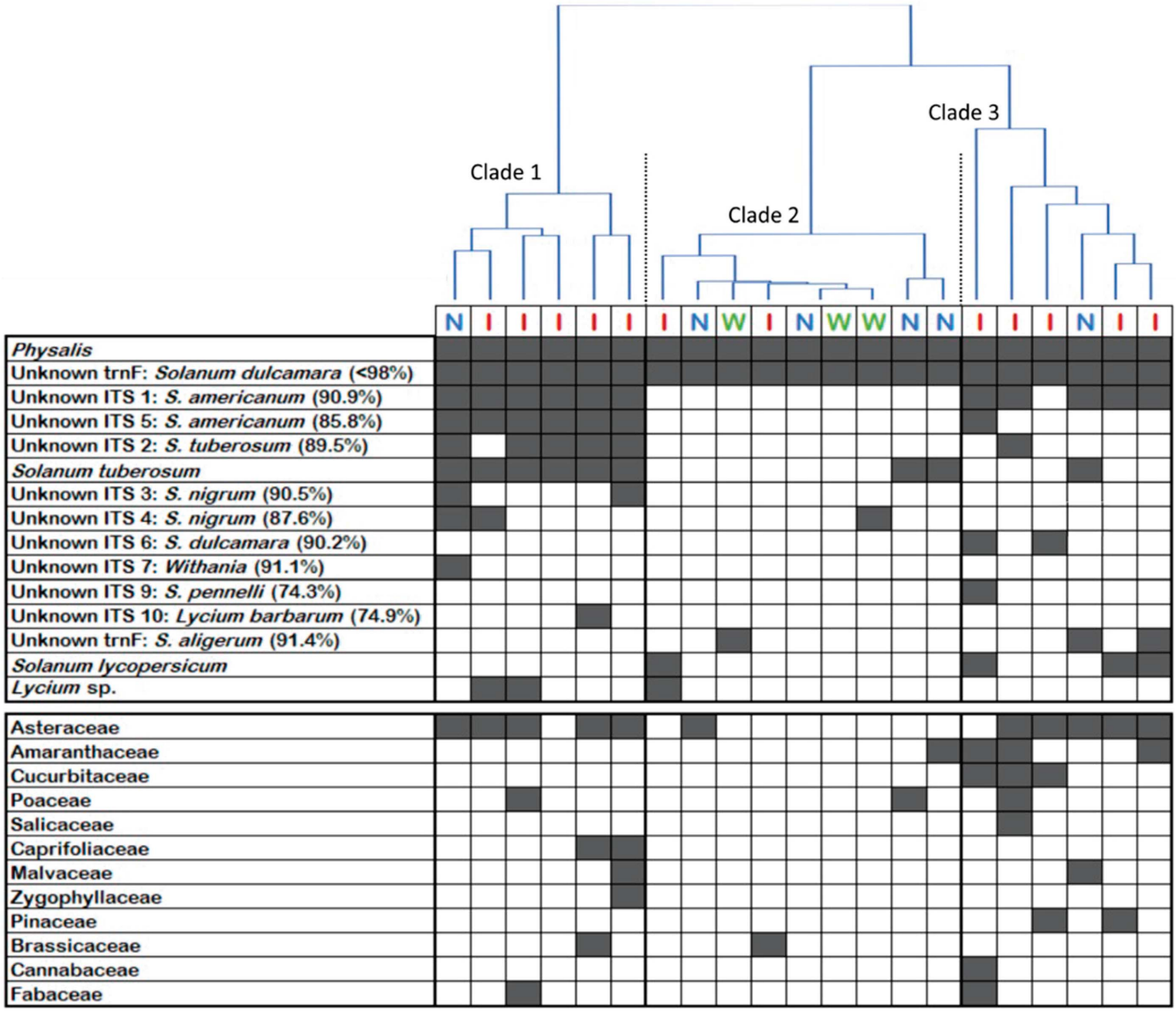

Fig. 3. Cluster analysis of plant taxa identified from psyllids collected from tomatillo. The relationship between psyllid infection status and plant taxa identified from analysis of gut content were assessed through cluster analysis using the CLUSTER procedure of SAS 9.4, specifying the Ward's minimum variance clustering method. Percentages after each plant taxa indicate identity after BLAST at the NCBI database. Each column represents an individual psyllid, while black shading indicates that the plant taxa was identified from the psyllid. '*Candidatus* Liberibacter solanacearum'-infected psyllids are indicated by a red "I", noninfected psyllids are indicated by a blue "N", and psyllids from which only weak PCR amplicons for '*Ca.* L. solanacearum' were observed are indicated by a green "W".

colonizing crop fields. *Physalis* sequences were detected in all psyllids tested, confirming that the psyllids had fed upon a tomatillo plant. Potato, tomato, and noncrop Solanaceae sequences were also detected in many psyllids, indicating movement of potato psyllids between crop and noncrop host plants. Unfortunately, it was not possible to confidently identify most of the noncrop Solanaceae samples to species because the sequences did not share close identity with any NCBI accessions. All psyllids had fed upon a plant with sequences most closely similar to those of *S. dulcamara*, which does not occur at this latitude. *S. triquetrum* (Texas nightshade or hierba mora) of the Dulcamaroid clade may occur in this region (Knapp 2013) but field surveys are required to confirm its presence. Abundant *Datura stramonium* (Jimsonweed) plants infested with *B. cockerelli* adults were observed within 20 to 50 m from the *P. ixocarpa* field (S. R. Sanchez-Peña, personal observation) but the plant was not identified from gut content sequences. In addition, a diversity of species of Convolvulaceae occurs in this region, yet taxa from this family were not detected in gut content analysis.

Cluster analysis revealed patterns among dietary history and '*Ca.* L. solanacearum' infection status of the psyllids (Fig. 2). Dietary history of psyllids clustered into three primary clades (Fig. 2, clades 1 to 3), with uninfected psyllids occurring primarily in clade 2. Strong and obvious PCR amplicons for 16S of '*Ca.* L. solanacearum' were observed for most of the 18 '*Ca.* L. solanacearum'-infected psyllids; however, 3 psyllids produced only faint 16S PCR amplicons despite using equal amounts of template DNA in PCR assays, and equal amounts of PCR amplicon for electrophoresis. These three samples also produced only faint amplicons following PCR with the haplotyping primers. Comparing '*Ca.* L. solanacearum' titer among psyllids using qPCR was not a goal of our study but the obvious differences in amplicon strength for both genes may suggest large differences in pathogen titers, and that the psyllids may have acquired the pathogen shortly before capture and, therefore, were likely not yet infective (Sengoda et al. 2014). These three psyllids exhibiting weak '*Ca.* L. solanacearum' amplicons clustered within clade 2 along with most of the uninfected psyllids (Fig. 2). It seems likely that these psyllids arrived uninfected in tomatillo and acquired the pathogen from infected plants within the plot. Most of the '*Ca.* L. solanacearum'-infected psyllids grouped within clusters 1 and 3, which were characterized by a larger diversity of plant taxa than cluster 2. Although obvious differences in dietary history differentiated psyllids within clades 1 and 3, all psyllids in both clades had fed upon a plant characterized by an *S. americanum*-like sequence and sequences of plants within the family Asteraceae. These results indicate that '*Ca.* L. solanacearum' infection is consistent with increasing dietary diversity and movement of psyllids from noncrop habitats.

Results of this study confirm that commercial tomatillo (*P. ixocarpa*) is susceptible to infection by '*Ca.* L. solanacearum' and is possibly a suitable host for *B. cockerelli*. Disease symptoms caused by '*Ca.* L. solanacearum' in *P. ixocarpa* are similar to those observed in other solanaceous crops and weeds (Cooper et al. 2019a; Munyaneza 2012). Results also indicate that the potato psyllid central haplotype and '*Ca.* L. solanacearum' haplotype B are present in Saltillo, Mexico. Finally, results of gut content analysis provide evidence that psyllids colonize wild relatives of *P. ixocarpa* within the family Solanaceae, and that '*Ca.* L. solanacearum' infection status correlates with increased diversity in plant hosts visited by the psyllids. These findings warrant more extensive field surveys for psyllid and '*Ca.* L. solanacearum' infestations in commercial tomatillo, and also warrant the development of a custom plant sequence library to identify which weed species are important for '*Ca.* L. solanacearum' epidemiology in this region.

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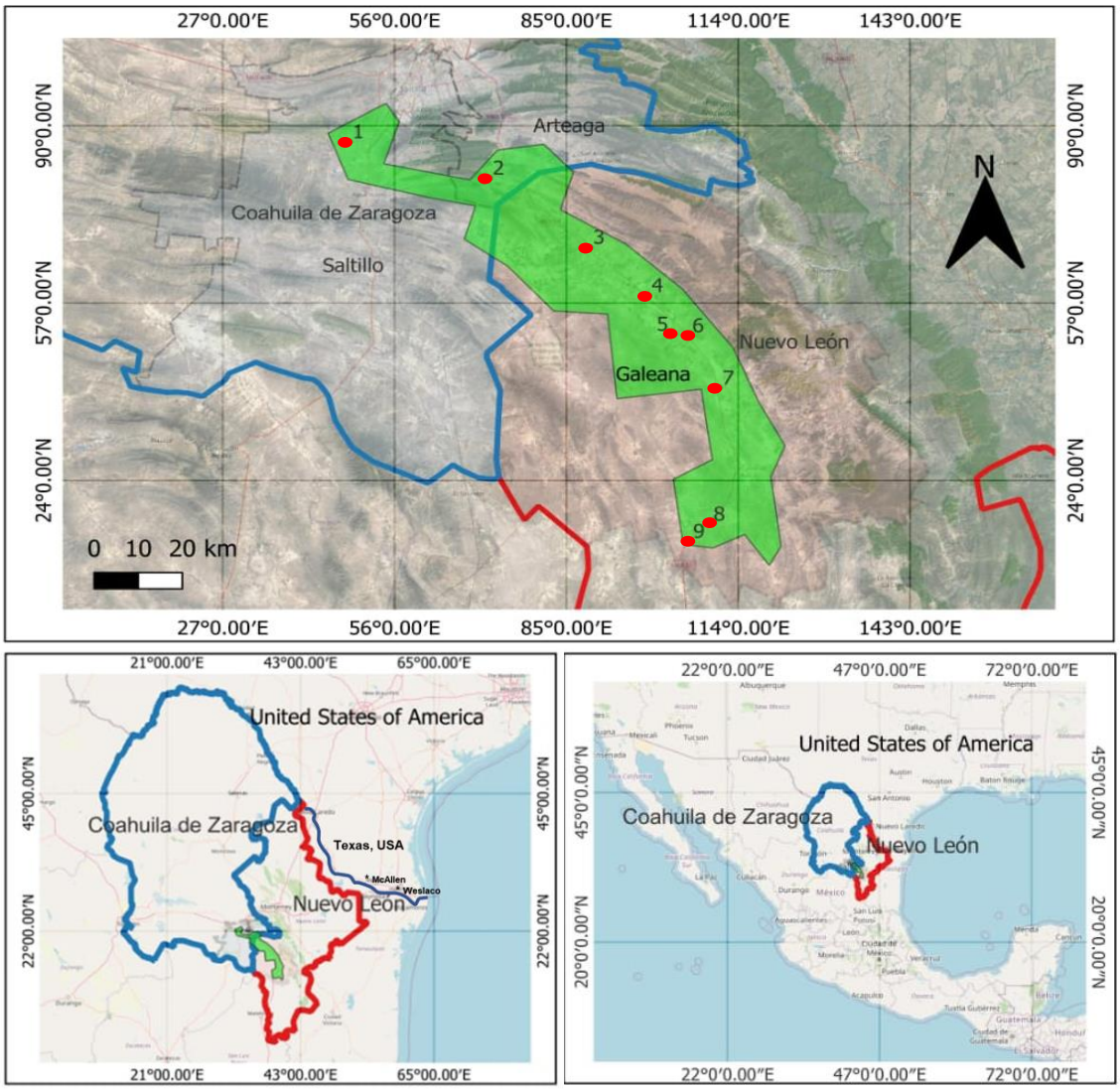
Anexos



Zona de estudio ubicada entre los estados de Coahuila de Zaragoza y Nuevo León, México. Puntos rojos en el mapa superior indican puntos de muestreo.

Muestreo de *Bactericera cockerelli* en hospederos silvestres

Procesamiento de muestras en laboratorio

Lycium berlandieri importante hospedero de *Bactericera cockerelli* en la zona agrícola de Galeana Nuevo León México

Arbustos de *Lycium berlandieri* en los bordes de un campo de papa

Physalis virginiana nuevo reporte como hospedero de *Bactericera cockerelli*

Papa voluntaria y *Physalis virginiana* en un campo de papa abandonado

Ninfas de *Bactericera cockerelli* sobre hoja de *Solanum elaeagnifolium*

Chamaesaracha coronopus nuevo reporte como hospedero de *Bactericera cockerelli* en la zona agrícola de Galeana Nuevo León México

Muestra de *Lycium berlandieri* con un importante número de ninfas de *Bactericera cockerelli* (flecha roja indica parasitoide *Tamarixia triozidae*)