Article

# *Bactericera cockerelli* vector of *Candidatus* Liberibacter solanacearum, morphometrics and haplotypes in populations from Mexico

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## Abstract

*Bactericera cockerelli* is a pest of economic importance in Solanaceae in Mexico, due to the yellowings that causes in crops, as well as the transmission of *Candidatus* Liberibacter solanacearum. Genetic variants of this insect are described, which are related to its ability to serve as a vector. In Mexico, the distribution of *B. cockerelli* is very wide and information about its morphological and genetic characteristics is lacking. The objective of this research was to characterize *B. cockerelli* morphologically and genetically and detect the presence of *Ca.* L. solanacearum in populations of *B. cockerelli* from Solanaceae-producing areas in Mexico. For which 35 locations from 13 states were sampled, on chili, tomato, eggplant and potato crops, under different production systems. The variables body length (LC), abdomen length (LAB), and abdomen width (AAB) were measured in insects of each population, the presence of *Ca*. L. solanacearum was detected in the 13 sampled states, where males had the highest percentage of positive insects. The presence of *Ca.* L. solanacearum was not influenced by the host or production system, but by the presence of *B. cockerelli*.

**Keywords:** *Ca*. L. solanacearum, eggplant, haplotype, potato psyllid.

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## Introduction

*Bactericera cockerelli* (Hemiptera: Triozidae), also known as salerillo, pulgón saltador or psilido de la papa (potato psyllid), is a pest of economic importance in Mexico, the United States of America and New Zealand (Munyaneza, 2010). Its importance lies on the damage generated of toxin type and by the transmission of procaryotes (Ramírez *et al.*, 2012), such *as Candidatus* Liberibacter solanacearum, a pathogen of economic importance in solanaceous crops in Mexico, Honduras, Guatemala, Belize, the United States of America, New Zealand, Europe, Norway, Nicaragua, Spain, France, Sweden, Finland and Canada (Munyaneza, 2013; Hong and Civerolo, 2014; Loiseau *et al*., 2014; Haapalainen *et al*., 2018; Delgado-Ortiz *et al*., 2019; Henrickson *et al*., 2019).

Infections by *Ca*. L. solanacearum have caused million-dollar losses in the industry by reducing crop production and quality (Gudmestad and Secor, 2007). In addition to potato, this pathogen affects tomato, chili pepper and other species of solanaceous cultures (Liefting *et al*., 2009). In tomato, reductions in production have been generated by up to 60% if presented alone or up to 100% if presented together with phytoplasmas, reducing the quality of the fruit and its commercial value (Delgado-Ortiz *et al*., 2019). It causes an increase in the costs of crop management and vector control (Gudmestad and Secor, 2007; Greenway and Rondon, 2018).

In Mexico, the crops that have been affected by *Ca*. L. solanacearum are potato, chili and tomato in the states of Sinaloa, Coahuila, Michoacán and in the State of Mexico (Camacho-Tapia *et al*., 2011; Munyaneza *et al*., 2007, 2009a, 2009b; Rubio *et al*., 2011). Studies on genetic variability between populations of *B. cockerelli* from the USA have revealed the presence of two biotypes that coincide with the geographical separation of the populations analyzed, so they were called Western biotype (found in Southern California) and Central biotype (present in Colorado and Nebraska), belonging to the same species (Liu *et al*., 2005; Jackson *et al*., 2009).

The separation of biotypes into insects is attributed to the variation between populations of the same species, which may include morphological, genetic and physiological characteristics: such as the ability to survive, reproduce or cause diseases in different hostesses (Shufran and Payton, 2009). A more specific level in the classification of *B. cockerelli* is that of haplotypes; which has been used in order to understand the temporal and spatial population dynamics of the psyllid through genetic analysis of a single individual (Swisher *et al*., 2012), defining a haplotype as the genetic form that differs in any other way by variations in its DNA sequence in at least one nucleotide (Templeton, 2006), where specifically, four haplotypes of *B. cockerelli* have been described (Western, Central, Northwestern and Southwestern) in North and Central America (Swisher *et al*., 2012; 2013; 2014).

The distribution of *B. cockerelli* in Mexico is very wide and several specimens have been collected in various areas of agricultural importance of Durango, Tamaulipas, Mexico City, Michoacán, San Luis Potosí, Morelos, Coahuila, Chihuahua, Nayarit, Jalisco, Baja California, Guanajuato, Aguascalientes, Zacatecas and Sinaloa (Pletsch, 1947; Vega *et al*., 2008), without any record of its morphological and genetic characteristics.

Therefore, the objective of this research was to analyze individuals of *B. cockerelli* from various populations, from Solanaceae-producing areas in Mexico, to obtain their morphological and genetic characterization, as well as to detect the presence of *Ca.* L. solanacearum.

## Materials and methods

A tour was made through the states of Aguascalientes (Ags), Zacatecas (Zac), Jalisco (Jal), Guanajuato (Gto), Hidalgo (Hgo), Puebla (Pue), San Luis Potosí (SLP), Nayarit (Nay), Nuevo León (NL), Durango (Dgo), Michoacán (Mich), Coahuila (Coah) and Tamaulipas (Tamps) from June to September 2017, collecting specimens of *B. cockerelli* present in Solanaceae crops, under different production systems (Table 1).

**Table 1. Collection sites of *B. cockerelli*, host and production system.**

| Population | Locality | State | Crop | Production system |
|---|---|---|---|---|
| 1 | Ojocaliente | Zac | Tomato | Greenhouse |
| 2 | 1/2 kilo | Ags | Eggplant | High tunnel |
| 3 | La finca de Adobe, El Taray | Ags | Chili | Open field |
| 4 | Los Laureles, Villa Hidalgo | Jal | Tomato | Open field |
| 5 | El Reparito, Villa Hidalgo | Jal | Chili | Greenhouse |
| 6 | Potrero de Vaquerías, Calvillo | Ags | Chili | Open field |
| 7 | Potrero de Vaquerías, Calvillo | Ags | Tomato | Open field |
| 8 | Potrero de Vaquerías, Calvillo | Ags | Chili | Open field |
| 9 | Rancho Nuevo de la Luz, León | Gto | Chili | Open field |
| 10 | San Francisco del Rincón | Gto | Potato | Open field |
| 11 | Rancho Nuevo de la Luz, León | Gto | Chili, tomato | Open field |
| 12 | Ciudad Guzmán | Jal | Tomato | Greenhouse |
| 13 | San Agustin Metzquititlan | Hgo | Chili | Open field |
| 14 | San Cristóbal | Hgo | Chili | Open field |
| 15 | La gallera, Tlaola | Pue | Chili | Open field |
| 16 | San Juan Tianguismanalco | Pue | Chili | Greenhouse |
| 17 | San Juan Tianguismanalco | Pue | Chili | Open field |
| 18 | La víbora, Villa de Arista | SLP | Chili | Open field |
| 19 | Bocas | SLP | Tomato | Open field |
| 20 | Santa Fe, Moctezuma | SLP | Chili | High tunnel |
| 21 | Campechana, Villa de Cos | Zac | Chili, tomato | Open field |
| 22 | Las Catarinas, Fresnillo | Zac | Potato | Open field |
| 23 | La Laborcilla, Calera de Víctor Rosales | Zac | Tomato | Open field |
| 24 | Santa María del Oro | Nay | Chili, tomato | Greenhouse |

| Population | Locality | State | Crop | Production system |
|---|---|---|---|---|
| 25 | San Rafael | NL | Potato | Open field |
| 26 | Navidad | NL | Potato | Open field |
| 27 | San Rafael | NL | Tomato | Shade net |
| 28 | Labor de Abajo, Poanas | Dgo | Chili | Open field |
| 29 | La Borrega, Poanas | Dgo | Chili | Open field |
| 30 | Héctor Marquez, Poanas | Dgo | Tomato | Greenhouse |
| 31 | Refugio, Durango | Dgo | Chili | Open field |
| 32 | Cocucho, Charapan | Mich | Potato | Open field |
| 33 | Saltillo | Coah | Tomato | Greenhouse |
| 34 | Hidalgo | Tamps | Chili | Open field |

The capture of the insects was carried out using a handheld vacuum, while in the places where the presence of adult insects was limited, foliage infested with nymphs and eggs of *B. cockerelli* was collected, being placed in tubes with 70% ethanol.

For the morphometric characterization of *B. cockerelli*, an average of 36 adults of *B cockerelli* collected randomly from each population were individually analyzed, to determine its external dimensions, the variables: body length (LC), abdomen length (LAB), abdomen width (AAB), wing length (LA), wing width (AA) and antenna length (LAN) were taken with the help of the Dino-Capture 2 program in a SMZ-711 stereoscopic microscope (Motic®).

Preparation of adult insects preserved in 70% ethanol consisted of placing them in a mixture of 90% glycerol to be mounted on slides exposing the parts of the insect to be measured. The data obtained were analyzed by sex and a comparison of means between the two sexes, with the program R Studio version 3.3, performing a comparison of means using a Tukey test, $p \leq 0.05$.

While for the determination of haplotypes of *B. cockerelli*, from the insects whose body was previously measured and including nymphs from some populations, DNA was extracted individually using the technique described by Doyle and Doyle (1990) modified. The haplotypes of *B. cockerelli* were determined using the initiators CO1 F3 (‘5-TACGCCATAC TAGCAATCGG-3’) and CO1 R3 (‘5-GAGTAACGTCGTGGTATTCC-3’) that amplify a 500 pb region of the mitochondrial gene Cytochrome C Oxidase subunit I (Swisher *et al*., 2012) using endpoint PCR, 4 µl of Taq&Go$^{T}$ Mastermix (MP Biomedicals), 0.5 µl of each first to 10 µl and 1 µl (50 ng) of DNA were used in the PCR reaction.

The reaction program was an initial denaturation of 98 °C for 30 s, followed by 30 cycles of 98 °C for 10 s, alignment at 56 °C by 20 s and extension at 72 °C by 30 s, followed by a final extension of 72 °C for 7 min, in a thermocycler Therm 1000 MaxyGeneTM, Axygen®. The generated amplicons were sequenced in both ways by Macrogene, USA, the sequences obtained were aligned with the Bio Edit programan and compared with the BLAST® program of the National Center for Biotechnological Information (NCBI).

For the detection of *Candidatus* Liberibacter solanacearum in *B. cockerelli*, the initiators Lso TX 16/23 F (5'-AATTTTAGCAAGTTCTAAGGG-3') and Lso TX 16/23 R (5'-GGTACCTCCCATATCGC-3') were used, which amplify a preserved region of 383pb between the 16S and 23S of the ribosomal DNA of '*Ca.* L. solanacearum' (Ravindran *et al.*, 2011). The PCR reaction for Lso TX 16/23 F/Lso TX 16/23 R was carried out with 4 µl of Taq&Go$^{T}$ Mastermix (MP Biomedicals), 0.5 µl of each initiator at 10 µl and 1 µl of DNA (50 ng), adjusting to a final reaction volume of 20 µl.

The reaction was performed under the following thermocycler conditions (Therm 1000 MaxyGeneTM, Axygen®): initial denaturation of 98 °C for 30 s; followed by 35 cycles of 98 °C by 10 s, 56 °C by 20 s for alignment and an extension of 72 °C by 30 s, followed by a final extension of 72 °C for 7 min. PCR products representative of each study population were selected to sequence in Macrogene USA to support the results obtained.

## Results and discussion

For the morphometric characterization of *B. cockerelli*, a total of 28 populations were analyzed, from which the body dimensions of the six selected variables (LC, LAB, AAB, LA, AA, LAN) were obtained. In the analysis of variables for females (Table 2), it is highlighted that the largest records in the LC, LAB, LA, AA and AAB variables were determined in populations 2, 6 and 8, while for the LAN variable, the largest dimensions were recorded in populations 23 and 26. With the population 34, collected in Hidalgo, Tamaulipas, standing out, which recorded the smallest dimensions in the variables LC, LAB, AAB and AA, while the population 14, was the one with the lowest LA, as well as the population 20 (Moctezuma, SLP) that presented the lowest LAN.

**Table 2. Morphometrics of females of *B. cockerelli* collected in the field.**

| Population* | Morphological feature** | | | | | |
|---|---|---|---|---|---|---|
| | LC | LAB | AAB | LA | AA | LAN |
| 2 | 2.05 a | 1.048 a | 0.72 b | 2.649 ab | 1.025 bc | 0.84 bcde |
| 3 | 1.969 abcde | 0.977 ab | 0.682 bc | 2.649 ab | 1.002 bc | 0.879 bcd |
| 4 | 1.973 abcd | 0.972 ab | 0.663 bc | 2.604 abc | 1.035 bc | 0.862 bcd |
| 5 | 1.801 def | 0.861 bcd | 0.585 bcd | 2.606 abc | 1.001 bc | 0.889 bc |
| 6 | 2.012 ab | 0.958 ab | 0.701 b | 2.665 a | 1.058 b | 0.912 b |
| 7 | 1.956 abcde | 0.942 ab | 0.632 bcd | 2.622 abc | 1.027 bc | 0.855 bcd |
| 8 | 1.749 def | 0.894 bcd | 0.943 a | 2.378 bc | 1.321 a | 0.832 bcde |
| 9 | 1.951 abcdef | 0.946 ab | 0.654 bc | 2.618 abc | 1.013 bc | 0.802 cde |
| 10 | 1.89 abcdef | 0.952 ab | 0.681 bc | 2.428 bc | 0.959 bc | 0.787 cde |
| 11 | 1.82 cdef | 0.893 bcd | 0.611 bcd | 2.413 bc | 0.933 bc | 0.778 de |
| 12 | 1.834 bcdef | 0.89 bcd | 0.626 bcd | 2.443 bc | 0.928 bc | 0.789 cde |
| 13 | 1.711 ef | 0.831 bcd | 0.614 bcd | 2.331 bc | 0.912 bc | 0.766 de |
| 14 | 1.817 cdef | 0.935 ab | 0.608 bcd | 2.281 c | 0.879 c | 0.718 de |

| Population* | Morphological feature** | | | | | |
|---|---|---|---|---|---|---|
| | LC | LAB | AAB | LA | AA | LAN |
| 15 | 1.556 ef | 0.552 d | 0.529 cd | 2.412 bc | 0.915 bc | 0.717 de |
| 16 | 1.849 bcdef | 0.917 b | 0.614 bcd | 2.461 bc | 0.948 bc | 0.806 cde |
| 17 | 1.869 abcdef | 0.933 ab | 0.607 bcd | 2.406 bc | 0.929 bc | 0.766 de |
| 18 | 1.802 def | 0.879 bcd | 0.634 bcd | 2.383 bc | 0.912 bc | 0.773 de |
| 19 | 1.848 bcdef | 0.903 bcd | 0.606 bcd | 2.414 bc | 0.931 bc | 0.726 de |
| 20 | 1.808 def | 0.844 bcd | 0.596 bcd | 2.427 bc | 0.95 bc | 0.667 e |
| 22 | 1.981 abcd | 0.929 ab | 0.681 bc | 2.637 abc | 1.038 bc | 0.712 de |
| 23 | 1.926 abcdef | 0.905 bc | 0.673 bc | 2.573 abc | 1.006 bc | 1.79 a |
| 26 | 2.001 abc | 0.963 ab | 0.68 bc | 2.641 abc | 1.019 bc | 1.83 a |
| 27 | 1.595 ef | 0.64 cd | 0.511 cd | 2.662 ab | 1.002 bc | 0.788 cde |
| 28 | 1.802 def | 0.805 bcd | 0.53 cd | 2.469 abc | 0.897 bc | 0.813 bcde |
| 30 | 1.956 abcdef | 0.904 bcd | 0.647 bcd | 2.648 abc | 1.051 bc | 0.85 bcde |
| 32 | 1.919 abcdef | 0.876 bcd | 0.624 bcd | 2.608 abc | 1.011 bc | 0.759 de |
| 34 | 1.47 f | 0.587 d | 0.409 d | 2.308 bc | 0.88 c | 0.7 de |
| 35 | 1.9 abcdef | 0.842 bcd | 0.551 cd | 2.508 abc | 0.962 bc | 0.732 de |

*= populations 1, 21, 24, 25, 29, 31 and 33 were not included in the analysis as adults were not available for data collection. **= the means (mm) with the same letter within each variable show no significant difference (Tukey, $p \leq 0.05$).

The variables LC, LAB, AAB and LAN in females of *B. cockerelli* had greater separation of statistically similar groups, with variations of up to 0.5 mm between populations. The first three variables are directly related, as LAB influences the size of LC, while the variables LAB and AAB relate to the eggs load in females at the time of capture; regarding LAN, this variable responds to another stimulus, since no behavior that corresponds to the size of LC is observed, so LAN may be influenced by food availability rather than by the size of the insect, finding smaller LANs in insects with high LCs or vice versa.

For the variables LA and AA, the group separation was smaller, so it is determined as a stable size variable between the populations of females analyzed. Differences between morphometrics, biological cycle development and physiology in *B. cockerelli* populations, in addition to climatic and feeding factors, can also be influenced by exposure to insecticides or sex, where the largest female size and short life cycles correspond to more susceptible populations (Cerna-Chávez *et al*., 2018; Hardstone *et al*., 2010).

In the analysis of male populations (Table 3), population 34 matches the analysis of females since it records the smallest dimensions in the variables LC, LAB and LAN. While the LC variable was shown to be a more homogeneous variable, with populations 2 and 6 registering the largest dimensions in the variables LAB, AAB, LA and AA, resulting in populations 23 and 26 with the highest LAN in the males analyzed.

**Table 3. Morphometrics of males of *B. cockerelli* collected in the field.**

| Population* | Morphological variable** | | | | | |
|---|---|---|---|---|---|---|
| | LC | LAB | AAB | LA | AA | LAN |
| 2 | 2.001 a | 0.995 a | 0.615 a | 2.608 a | 0.991 ab | 0.912 bc |
| 4 | 1.894 a | 0.871 ab | 0.535 ab | 2.56 ab | 0.996 ab | 0.903 bc |
| 5 | 1.708 ab | 0.779 ab | 0.514 b | 2.561 ab | 0.978 c | 0.89 bc |
| 6 | 1.968 a | 0.941 ab | 0.667 a | 2.614 a | 1.086 a | 0.852 bc |
| 7 | 2.014 a | 0.973 a | 0.571 ab | 2.557 abc | 0.988 ab | 0.867 bc |
| 8 | 1.92 a | 0.915 ab | 0.565 ab | 2.536 abc | 0.989 ab | 0.81 c |
| 9 | 1.939 a | 0.926 ab | 0.593 a | 2.477 abc | 0.999 ab | 0.864 bc |
| 10 | 1.803 a | 0.868 ab | 0.554 ab | 2.349 c | 0.917 c | 0.803 c |
| 11 | 1.766 a | 0.849 ab | 0.502 b | 2.252 c | 0.933 c | 0.815 c |
| 12 | 1.639 abc | 0.706 bc | 0.457 bc | 2.314 c | 0.867 c | 0.846 bc |
| 13 | 1.686 ab | 0.783 ab | 0.506 b | 2.315 c | 0.897 c | 0.807 c |
| 14 | 1.778 a | 0.85 ab | 0.522 b | 2.286 c | 0.862 c | 0.744 c |
| 15 | 1.45 bc | 0.573 c | 0.458 bc | 2.293 c | 0.9 c | 0.763 c |
| 16 | 1.821 a | 0.868 ab | 0.492 b | 2.386 bc | 0.908 c | 1.135 b |
| 17 | 1.736 ab | 0.797 ab | 0.511 b | 2.301 c | 0.8765 c | 0.835 bc |
| 18 | 1.709 ab | 0.812 ab | 0.511 b | 2.311 c | 0.881 c | 0.839 bc |
| 19 | 1.783 a | 0.829 ab | 0.456 bc | 2.356 bc | 0.878 c | 0.725 c |
| 20 | 1.769 a | 0.792 ab | 0.462 bc | 2.36 bc | 0.977 c | 0.755 c |
| 22 | 1.94 a | 0.899 ab | 0.523 ab | 2.575 ab | 0.994 ab | 0.741 c |
| 23 | 1.912 a | 0.865 ab | 0.543 ab | 2.546 abc | 0.964 c | 1.755 a |
| 26 | 1.899 a | 0.895 ab | 0.57 ab | 2.513 abc | 0.949 c | 1.731 a |
| 27 | 1.985 a | 0.946 ab | 0.584 ab | 2.559 abc | 1.005 ab | 0.716 c |
| 28 | 2.082 a | 1.096 a | 0.729 a | 2.255 c | 0.856 c | 0.577 c |
| 30 | 1.643 abc | 0.714 abc | 0.539 bc | 2.507 abc | 0.968 c | 0.808 c |
| 32 | 1.753 ab | 0.789 ab | 0.451 ab | 2.476 abc | 0.94 c | 0.798 c |
| 34 | 1.436 c | 0.575 c | 0.409 c | 2.277 c | 0.866 c | 0.733 c |
| 35 | 1.787 a | 0.793 ab | 0.469 bc | 2.43 bc | 0.928 c | 0.724 c |

*populations 1, 3, 21, 24, 25, 29, 31 and 33 were not included in the analysis as adults were not available for data collection. **The means (mm) with the same letter within each variable show no significant difference (Tukey, $p \leq 0.05$).

In a comparison between the dimensions registered by insects of both sexes, females registered larger size for the variables analyzed Anova (F27, 658; $p \leq 0.05$): LC (1.870a, 1.824b); LAB (0.898a, 0.851b); AAB (0.635a, 0.532b); LA (2.509a, 2,441b); AA (0.986a, 0.949b) and LAN (0.888a, 0.895a), for females and males respectively.

In the variables analyzed the females had a larger size (±0.1 mm), with the antenna length (LAN) being the only variable where no significant difference between the sexes was observed, Vargas *et al*. (2013) determined that the length of the antennae is affected in the insect according to the host in which it develops, while they were unable to associate the length of the antennas with sex.

Geographic location and environmental conditions are not considered as a determinant in the body size of the populations of *B. cockerelli* evaluated, since within the same state there are populations in two or three of the main groups mentioned, besides that those conditions are affected by the production system under which they develop.

However, morphometrics is influenced by the host, where the highest body proportions are placed in insects fed in eggplant cultivation, while in potato, chili and tomato, no particular behavior is observed. There has been an increase in fertility, survival rate, as well as less time to complete its life cycle when *B. cockerelli* develops in eggplant than in pepper under laboratory conditions (Yang and Liu, 2009).

Adults of *B. cockerelli* are small, varying from 1.3-1.9 mm (Liu and Trumble, 2007), but this characteristic may vary depending on the host plant, the geographic origin of the populations, sex and whether the measurement was performed with field or laboratory individuals (Butler and Trumble, 2012), Vargas *et al*. (2013) demonstrated that the morphometrics of adults and nymphs of *B. cockerelli* is affected by the tomato cultivar in which it develops.

Morphometrics studies are a tool that has proved to be highly effective in analyzing the variation resulting from the physiology of individuals (variation in size), typical of population-and probably genetic (variation of form) (Jaramillo *et al*., 2002), the morphometric variation can be used to discriminate 'phenotypic populations', defined as groups with similar growth, mortality and reproduction rates (Cadrin, 2000).

In the determination of haplotypes of *B. cockerelli*, in all PCR products the expected amplicons of 500 bp (Figure 1) were generated, after sequencing, an identical base sequence was obtained between the samples analyzed. Following the comparison of sequences in the NCBI database, a 100% similarity was obtained with the Central haplotype (Swisher *et al*., 2012).

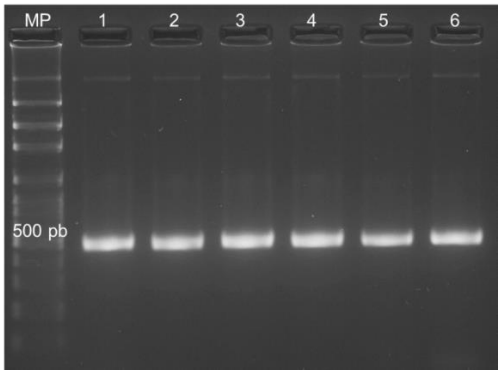


**Figure 1. Amplified obtained from *B. cockerelli.***

Product at a weight of 500 bp with CO1 F3/CO1 R3 initiators. MP: 100 bp, band 1: population 8; band 2: population 18; band 3: population 24; band 4: population 28; band 5: population 32 and band 6: population 34; where each product represents only one individual from each population.

The Central haplotype has been found in the states of Wyoming, Nebraska, Texas and Washington in the USA; Honduras and Nicaragua in Central America; in Mexico, it has been found in the states of Toluca and Querétaro (Swisher *et al*., 2013). The Central haplotype of *B. cockerelli* differs in only one nucleotide (SNP) from the Western haplotype in residue 51 where it presents a cytosine in place of a thymine, as well as 16 SNPs from the Northwestern (Swisher *et al*., 2012) and two SNPs from the Southwestern haplotype (Swisher *et al*., 2014).

In this study, the presence of the Central haplotype is detected in the states of Aguascalientes, Jalisco, Durango, Guanajuato, Hidalgo, Puebla, Michoacán, Zacatecas, Nuevo León, Coahuila, Nayarit, San Luis Potosí and Tamaulipas. Liu *et al*. (2005) report two biotypes of *B. cockerelli* in the central and western United States of America to which Chapman *et al.* (2012) separated in Central and Western through high-resolution melt analysis and DNA sequencing of the *mt*COI gene, these biotypes had a variation of a nucleotide at position 297 of an amplified of 421 base pairs, where the Central biotype presents a guanine (G) and Western biotype an adenine (A), declaring the Central biotype the native biotype and the Western as the invasive biotype.

According to studies conducted by Montiel *et al*. (2016), the Central biotype (Texas and Nebraska) and Southwestern haplotype are closely related, while the Western biotype diverges from these populations, but relates to an apparent new northwest population, which may have emerged from this biotype.

For the determination of *Ca L.* solanacearum in the populations of *B. cockerelli*, a total of 862 insects were analyzed; 348 females, 415 males and 99 nymphs (obtained from 35 populations of 13 states), of which 80.17% of females were positive to *Ca.* L. solanacearum, while in males the presence of the bacteria was determined in 84.57% (Table 4).

**Table 4. Detection of *Ca* L. solanacearum in populations of *B. cockerelli*.**

| Population | State | Percentage of insects positive for '*Ca.* L. solanacearum' | Insects* | |
|---|---|---|---|---|
| | | | H | M |
| 1 | Zacatecas | 5/5 (100) | N | |
| 2 | Aguascalientes | 33/33 (100) | 18/18 | 15/15 |
| 3 | Aguascalientes | 3/3 (100) | 3/3 | |
| 4 | Jalisco | 35/35 (100) | 14/14 | 21/21 |
| 5 | Jalisco | 0/36 (0) | 0/18 | 0/18 |
| 6 | Aguascalientes | 35/35 (100) | 16/16 | 19/19 |
| 7 | Aguascalientes | 36/36 (100) | 12/12 | 24/24 |
| 8 | Aguascalientes | 32/32 (100) | 10/10 | 22/22 |
| 9 | Guanajuato | 36/36 (100) | 11/11 | 25/25 |
| 10 | Guanajuato | 35/35 (100) | 12/12 | 23/23 |

| Population | State | Percentage of insects positive for '*Ca.* L. solanacearum' | Insects* | |
|---|---|---|---|---|
| | | | H | M |
| 11 | Guanajuato | 36/36 (100) | 20/20 | 16/16 |
| 12 | Jalisco | 27/36 (75) | 4/9 | 23/27 |
| 13 | Hidalgo | 16/16 (100) | 10/10 | 6/6 |
| 14 | Hidalgo | 36/36 (100) | 7/7 | 29/29 |
| 15 | Puebla | 13/13 (100) | 1/1 | 12/12 |
| 16 | Puebla | 0/14 (0) | 0/6 | 0/8 |
| 17 | Puebla | 28/36 (77.7) | 20/23 | 8/13 |
| 18 | SLP | 26/26 (100) | 14/14 | 12/12 |
| 19 | SLP | 24/24 (100) | 17/17 | 7/7 |
| 20 | SLP | 17/36 (47.2) | 13/22 | 4/14 |
| 21 | Zacatecas | 23/23 (100) | N | |
| 22 | Zacatecas | 13/18 (72.2) | 6/10 | 7/8 |
| 23 | Zacatecas | 36/36 (100) | 17/17 | 17/19 |
| 24 | Nayarit | 13/13 (100) | N | |
| 25 | Nuevo León | 12/12 (100) | N | |
| 26 | Nuevo León | 23/23 (100) | 15/15 | 8/8 |
| 27 | Nuevo León | 36/36 (100) | 16/16 | 20/20 |
| 28 | Durango | 3/3 (100) | 2/2 | 1/1 |
| 29 | Durango | 3/4 (75) | N | |
| 30 | Durango | 0/5 (0) | 0/3 | 0/2 |
| 31 | Durango | 0/6 (0) | N | |
| 32 | Michoacán | 17/17 (100) | 12/12 | 5/5 |
| 33 | Coahuila | 36/36 (100) | N | |
| 34 | Tamaulipas | 12/35 (34.2) | 4/13 | 8/22 |
| 35 | Puebla | 24/36 (66.6) | 5/17 | 19/19 |

* H= females; M= males; N= nymphs.

The nymphs of *B. cockerelli* turned out to be excellent carriers of *Ca.* L. solanacearum, with 100% positive populations where adult insect capture was not possible. In all sampled states, at least one population of *B. cockerelli* positive for *Ca.* L. solanacearum was obtained, which provides a broad overview of the distribution and relationship between the vector and '*Ca.* L. solanacearum'.

In relation to the host on which the populations of *B. cockerelli* were collected; 92.3% of the populations from tomato resulted positive for *Ca.* L. solanacearum; while 83.3% of samples from chili resulted positive for the bacteria. For populations collected in potato cultivation, 100% of the samples were positive.

While the populations that resulted negative for *Ca.* L. solanacearum under open field production systems were population 16, 30 and 31 (Puebla and Durango, respectively) and population 5 collected in greenhouse. Thinakaran *et al*. (2015) have reported the preference of the Central Haplotype in the field over different hostesses, being favoritism greater in potato and tomato over pepper, eggplant and *Solanaum elaeagnifolium* (nightshade), where spawning is favored in potato cultivation, while under laboratory conditions the preference for eggplant over tomato cultivation and the rest of the hostesses is superior.

Cadena (1993) reported the presence of the purple tip of the potato in commercial lots of Guanajuato, Nuevo León, Coahuila, Valle de Toluca (Estado de México), Michoacán, Tlaxcala, Hidalgo and Puebla. Coinciding with the populations of *B. cockerelli* positive for *Ca.* L. solanacearum of the states of Guanajuato, Nuevo León, Coahuila, Michoacán, Hidalgo and Puebla in this study, indicating that the disease has been present in these areas for decades, with the possibility of having dispersed to other regions and crops, which is supported by the rest of the positive populations of *B. cockerelli* collected in the states of Zacatecas, Aguascalientes, Jalisco, Durango, Nayarit, San Luis Potosí and Tamaulipas.

Research by Secor and Rivera (2004) documented the symptoms of the purple tip in the potato-producing region of Saltillo, Coahuila, whose etiological agent was determined by Munyaneza *et al*. (2007), who also related it to the presence of the vector and whose report includes the state of Nuevo León, while Rubio *et al.* (2011) detected *Ca*. L. solanacearum in the potato-producing region of Toluca, where they also found the presence of the vector. In tomato cultivation, *Ca.* L. solanacearum had already been reported in the states of Michoacán (Hernández, 2013) and Sinaloa (Munyaneza *et al*., 2009a) and chili pepper in Sinaloa, Mexico (Munyaneza *et al*., 2009b).

The degree of technification in crop production generally influences the incidence of pests and diseases and the severity of the attack (Jirón-Rojas *et al*., 2016) and according to the results of this study, it can be inferred that both the vector and the bacterium are able to adapt to any production system to which they have access. Being both vector and pathogen present in open field production lots, shade net, high tunnel and greenhouse.

The population from the town of Potrero de Vaquerías, Calvillo, Aguascalientes in open field chili cultivation, which was negative for *Ca.* L. solanacearum, which was found adjacent to a tomato field, whose level of infestation was considerably higher than in chili, decreasing the possibility that the captured insects carried the bacteria. The population from El Refugio, Durango, in open field chili, in number of insects obtained was limited, which decreased the probability of taking those carriers of the bacteria.

The rest of the samples negative for *Ca.* L. solanacearum were those from greenhouses in the towns of San Juan Tianguismanalco, Puebla and Héctor Márquez, Poanas, Durango, in chili and tomato, respectively, concluding that these populations have not been in contact with individuals or hostesses carrying the bacteria.

## Conclusions

The morphometrics presented of the 28 populations of *B. cockerelli* analyzed represents a major advance in the characterization of this important vector in the main areas producing Solanaceae in Mexico, in addition to having determined the presence of *Ca.* L. solanacearum in the populations of *B. cockerelli* from thirteen states of Mexico. Being all the populations of *B. cockerelli* identified as Central haplotype.

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