

## MORPHOMETRICS OF EGGS, NYMPHS, AND ADULTS OF *BACTERICERA COCKERELLI* (HEMIPTERA: TRIOZIDAE), GROWN ON TWO VARIETIES OF TOMATO UNDER GREENHOUSE CONDITIONS

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### ABSTRACT

The effects of 2 varieties of tomato, *Solanum lycopersicum* L., i.e., 'Charanda F1' and 'Rafaello', were evaluated on the morphometrics of *Bactericera cockerelli* (Sulc). Eggs, nymphs, and adults of *B. cockerelli* were collected from 2 varieties of tomato, 'Charanda F1' and 'Rafaello', under greenhouse conditions in the Colegio de Postgraduados, Campus Montecillo, Texcoco, the State of Mexico, during the periods Oct-Dec 2009 and Jan-Mar 2010. Since 2000-2001 the *B. cockerelli* cultures were maintained on tomato with no exposure to agrochemicals. Adult *B. cockerelli* were kept in individual growth chambers constructed of wooden frames covered with organza cloth, under 14:10 h L:D and temperatures ranging from 10 to 25 °C. For morphometric analysis of the eggs, the following variables were investigated: egg length (LH), egg width (AH), and pedicel length (PED). For nymphs, the variables were: body length (LC), body width (AC), and antennal length (ANT). For adults, the variables were: body length (LC), body width at thorax (ACT), wing length (LALA), and wing width (ANALA). The 2 tomato varieties were found to have differential morphometric effects on *B. cockerelli* as follows: no significant differences on egg length ( $F_{1,41} = 0.57$ ;  $P = 0.4551$ ), but egg width was differentially affected by the variety of tomato ( $F_{1,41} = 11.92$ ;  $P = 0.0013$ ). There were significant differential effects of tomato variety on nymphs: body length ( $F_{4,324} = 1199.2$ ;  $P < 0.0001$ ), body width ( $F_{4,324} = 900.72$ ;  $P = 0.0001$ ); and antennae length ( $F_{4,324} = 883.93$ ;  $P = 0.0001$ ). Body length of the adults ( $F_{1,117} = 7.11$ ;  $P = 0.0087$ ) was differentially affected by the 2 different tomato varieties. None of the plants showed any symptoms of infection by '*Candidatus Liberibacter solanacearum*', which is known to cause effects on *B. cockerelli* fitness traits. Body width and antennal length of nymphs can be recommended to differentiate all 5 nymphal instars on this pest species, which has practical implications.

Key Words: Potato psyllid, tomato psyllid 'Charanda F1', 'Rafaello', Psylloidea, morphology

### RESUMEN

Se evaluó el efecto de dos variedades de jitomate ('Charanda F1' y 'Rafaello') en la morfometría de *B. cockerelli* (Sulc). Se colectaron huevos, ninfas y adultos criados en dos variedades de jitomate 'Charanda F1' y 'Rafaello', en condiciones de invernadero en el Colegio de Postgraduados, Campus Montecillo, Texcoco, México, en el periodo Octubre-Diciembre 2009-Enero-Marzo 2010. Las condiciones generales de la cría fueron: la colonia de insectos origen se integró desde el periodo 2000-2001 sobre plantas de tomate, sin aplicación de agroquímicos; los adultos fueron mantenidos en jaulas individuales de marco de madera cubiertos con tela de organza, bajo un periodo de 14 h de luz, con un rango de temperatura de 10 a 25 °C. En el análisis de morfometría de huevos se consideraron las variables: Longitud de Huevo (LH), Ancho de Huevo (AH) y Longitud de Pedicelo (PED). En ninfas, Largo de Cuerpo (LC), Ancho de Cuerpo (AC) y Longitud de Antenas (ANT). En adultos, Longitud de Cuerpo (LC), Ancho de Cuerpo en Tórax (ACT), Longitud de Ala (LALA), Ancho de Ala (ANALA). Se encontró que el efecto de las variedades de jitomate en la morfometría, no presenta diferencias significativas sobre la Longitud de huevos ( $F_{1,41} = 0.57$ ;  $P = 0.4551$ ); aunque el Ancho de huevo sí resultó afectado por la variedad de jitomate sobre la que se criaron los insectos ( $F_{1,41} = 11.92$ ;  $P = 0.0013$ ). Los estados ninfales, para las variables Largo de Cuerpo ( $F_{4,324} = 1199.2$ ;  $P < 0.0001$ ), Ancho de Cuerpo ( $F_{4,324} = 900.72$ ;  $P < 0.0001$ ) y Largo de An-

tenas ( $F_{4, 324} = 883.93$ ;  $P < 0.0001$ ), resultaron diferenciadas en forma significativa debido a la variación de los diferentes ínstares; esta diferencia tiene implicaciones prácticas porque dichas variables podrían ser usadas como guías para identificar las cinco fases ninfales. La longitud del cuerpo del adulto ( $F_{1, 117} = 7.11$ ;  $P = 0.0087$ ), se afecta cuando *B. cockerelli* se cría sobre las variedades de jitomate mencionadas.

Palabras Clave: psylido de la papa, psylido del tomate 'Charanda F1', 'Rafaello', Psylloidea, morfología

The evolution of insect populations is closely linked with that of the species they feed upon, and this seems especially the case of some genera and species of the Psylloidea (White & Hodkinson 1985; Burckhardt & Lauterer 1997; Hodkinson 2009). The insects of the Psylloidea feed on the sap of host plants by introducing their stylets and making use of the sugars and amino acids of the plant that they cannot synthesize themselves (Thao et al. 2000; Percy 2003). *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), variously known as the tomato psyllid and potato psyllid, is an insect pest species that feeds mainly on plants of the Solanaceae family and some members from several other families of plants (Pletsch 1947; Burckhardt & Lauterer 1997; Crespo-Herrera et al. 2012). There is evidence relating to the effect of the host plants on the development and morphology of insects from diverse genera and taxonomic families, such as *Bemisia tabaci* (Berlinger 1986; Perring et al. 1993; Huang et al. 2008) and the pea aphid, *Acyrtosiphon pisum* Harris (Frantz et al. 2010). Some studies focused specifically on the effects of certain Solanaceae on the reproductive ability of *B. cockerelli* (Liu et al. 2004; Liu et al. 2006a; Yang & Liu 2009; Vargas-Madríz et al. 2011), or on its fitness (Nachappa et al. 2012).

The architecture and the content of secondary metabolites of cultivated plants are some characteristics that cause differences in the feeding and oviposition behaviors of phytophagous insects, as happens in the case of the psyllid, *Boreioglycaspis melaleuca* Moore, on *Melaleuca quinque-nervia* (Cav.) S. T. Blake (Myrtales: Myrtaceae), whose leaf nitrogen and carotene contents are factors that modify the behavior of the mentioned psyllid. Also Hodkinson et al. (2001) and Casteel et al. (2006) proved that the Mi-1.2 gene in wild tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae) affects the reproductive ability of *B. cockerelli*. This gene confers resistance in tomato against *Meloidogyne* spp., the potato aphid (*Macrosiphum euphorbiae* Thomas) and the silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring). The main effect of the Mi-1.2 gene is to cause necrotic zones to develop around the areas attacked by nematodes and repels females of *Meloidogyne* spp., thus reducing oviposition. Some reports mentioned the possibility of the existence of at least 2 'biotypes' of *B. cockerelli* in several geographical zones of the US, Mexico

and Guatemala (Jackson et al. 2009; Nelson et al. 2011), captured from diverse host plants such as tomato and chili pepper (*Capsicum* spp.; Solanales: Solanaceae) (Liu et al. 2006b; Liu et al. 2007), potato (Abdullah 2008), and eggplant (*Solanum melongena*, L.; Solanales: Solanaceae) (Yang & Liu 2009). Majority of the work done focuses on studying variation in the reproductive ability of *B. cockerelli* feeding on different host plants, but the effects of host plants the morphology of *B. cockerelli* have not been investigated. Such effects of host plants on insect morphology are fundamental in understanding morphological variation within insect species, and they are considered as evidence to determine 'biotypes' (Claridge & Den Hollander 1983). Thus, the present study is intended to give evidence on the possible effect of 2 varieties of tomato on the morphology of eggs, nymphs, and adults of *B. cockerelli* under greenhouse conditions.

#### MATERIALS AND METHODS

The experiments were conducted under greenhouse and laboratory conditions at the Colegio de Postgraduados, Campus Montecillo, Texcoco, the State of Mexico. Temperature and RH were recorded every 30 min, using electronic sensors and data logger, HOBO, model H-08-032-08 #333699, placed on the tomato plants used in the experiments. The tomato varieties used in this study were the 2 hybrids: 'Charanda F1' and 'Rafaello', which are grown frequently by tomato farmers in México because of their good agronomic behavior, i.e., very long fruiting periods (indeterminate development) and long postharvest shelf life (Tigchelaar 1986; Pérez & Castro 1999; Mendoza-De Jesús et al. 2010; Del Val et al. 2010).

#### *Bactericera cockerelli* Colonies

The 2 tomato varieties were seeded in planting trays; 20 plants from each of the 2 varieties were selected for the study. Seedlings were transplanted into polyethylene bags containing 2 kg of 50% Canadian Growing Mix 1VM and 50% Tezontle (porous volcanic rock) when the plants were 31 d old. Plants were moved to growth chambers (62 cm long × 95 cm wide × 95 cm high) framed with wooden boards and covered with plain weave mesh to exclude insects, especially *Bemisia tabaci*

Gennadius (Hemiptera: Aleyrodidae). The materials were maintained under a 14:10 h L:D with temperatures ranging from 10 to 25 °C.

No insecticides were used while rearing *B. cockerelli* on the 2 varieties of tomato. *Bactericera cockerelli* fourth and fifth instars were collected from the "Rio Grande" tomato variety grown in chambers of the Biological Control Greenhouse in the Colegio de Postgraduados and later taken to the experimental greenhouse. These insects were cultured for 7 generations on each of the 2 tomato varieties mentioned above. Subsequently these *B. cockerelli* were maintained for 4 generations in new growth chambers either on 'Charanda F1' or on 'Rafaello'; and this was done to avoid parasites or depredation of any kind.

The durations of the nymphal and adult stages of *B. cockerelli* were determined on the 2 varieties of tomato in the greenhouse. Possible presence/absence of '*Candidatus Liberibacter solanacearum*' and its effects on morphology on the different stages of insect was not measured. From each colony, 65 adults in their reproductive stage were taken randomly to guarantee a cohort of 100 eggs on 1 d in each growth chamber. Three chambers were set up for each tomato variety, with 5 plants per chamber, and 65 adults were introduced per chamber. After 24 h, the adults were taken out and the number of eggs laid was recorded. This was later adjusted to 100 eggs. Hatching and nymphal growth were registered daily until they transformed into adults. Genitalia of *B. cockerelli* was used to identify the species.

To identify *B. cockerelli* abdomens and genitalia were removed from 5 adult males following protocols of Crawford (1910) and Crawford (1914). The abdomens were placed for 1 to 2 min in 10% potassium hydroxide to eliminate abdominal fat. The abdomens were cleaned by placing them in hot (80 °C) distilled water for 15 min, and 1% acetic acid was used to rinse off the potassium hydroxide from each specimen. The abdomens were then placed in 70% alcohol, and then in 100% ethanol for 1 min to finish cleaning the cuticle. During the cleaning process alcohol was changed with an insulin syringe 3 times to eliminate any plant debris. After removing the abdomens from alcohol, they were placed in xylene to dealcoholize and clear them; xylene was changed twice. Then each abdominal specimens was carefully placed on a slide, using small cardboard pieces to avoid crushing the abdomens. Canadian balsam was used as the mounting medium because it has the appropriate refraction index for viewing by stereo microscope. Genitalia were later observed in the stereo microscope and specimens under study was confirmed as *B. cockerelli* based on the keys described by Crawford (1910 & 1914) and (Tuthill 1945).

Photographs of the taxonomic characters of each developmental stages were taken based

on the descriptions by Lehman (1930), Tuthill (1945), Marín-Jarillo et al. (1995), Burckhardt & Lauterer (1997), and Abdullah (2008).

#### Morphometry of Eggs, Nymphs, and Adults of the *B. cockerelli*

Eggs, nymphs, and adults of *B. cockerelli* grown on 2 varieties of tomato, 'Rafaello' and 'Charanda' were collected in 2009-2010. The eggs and nymphs were collected from the leaves of the host plants using entomological forceps and placed in jars with 70% ethanol. The egg, nymph and adults samples were dried and placed on slides in order to photograph them with a Carl Zeiss Tessovar photomicroscope connected to a Pixera digital camera. Care was taken to standardize the age of the insects used. Eggs were used regardless of whether they were fertile or infertile. The metric ruler was also photographed to calibrate the Image Tool (Wilcox et al. 2002) measuring software. The photographs were digitized in JPEG format.

The taxonomic differentiation of *B. cockerelli* adults was done using taxonomic keys described by (Crawford 1910a, 1910b, 1911a, 1911b, 1914). In the morphometric analysis of the eggs, the following variables were considered: egg length (LH), egg width (AH), and pedicel length (PED). The measured variables in the case of the nymphs were: body length (LC), body width (AC), and antennae length (ANT). The nymphs were classified according to instars, from instar 1 to 5, to immediately carry out the measurements of the mentioned variables.

In the case of morphometry of adults, the following variables were considered: body length (LC), body width at the thorax (ACT), wing length (LALA), and wing width (ANALA). Measurements of 30 adult specimens were made. Data on measurements of various life stages were analyzed using one-way analysis of variance (ANOVA, SAS Institute Inc. 2005). The effect of the factors: 'variety' and 'sex', and their possible interaction on the morphometric measured variables were assessed. The significance of the considered factors was tested through the application of the GLM procedure in SAS (SAS 2005) with alpha = 0.05. The Tukey test was used ( $P = 0.05$ ) to separate means of measurements involving the different instars; and the  $F$  test was used to assess the interaction between sex and tomato variety. The possible significance of the differences in the means of the morphological measurements due to the 'variety' and 'sex' factors were tested by Student's normalized  $t$  test, at the same significance level.

## RESULTS

In 2009, the incubation period for the egg stage was 7 days on 'Charanda F1' and 8 days on 'Rafa-

ello' (Table 1). The durations of the nymphal stage were observed to overlap with mean values of 32 d on 'Charanda F1' and 31 d on 'Rafaello'. The adult stage was 44 days on 'Charanda F1' and 45 days on "Rafaello". Thus, the duration of the complete cycle (egg-adult) was 63 days on 'Charanda F1' and 68 days on 'Rafaello'. In 2010, the incubation period for the egg stage was 13 days on 'Charanda F1' and 10 days on 'Rafaello'. The durations of the nymphal stage were again found to overlap with mean values of 42 days on 'Charanda F1' and 41 days on 'Rafaello'. The adult stage was 41 days on 'Charanda F1' and 42 days on 'Rafaello'. Therefore the duration of the complete cycle (egg-adult) was 69 days for 'Charanda F1' and 70 days for 'Rafaello' (Table 1).

#### Egg Morphometry

The morphometric measurements of *B. cockerelli* eggs showed numerical differences when obtained from the 2 different tomato varieties (Table 2). The egg length and width variables were greater on 'Rafaello', while pedicel length was greater on 'Charanda'. Statistical comparison of the means of the morphological variables of *B. cockerelli* eggs indicated significant differences only in egg width (Table 2).

#### Effect of the Tomato Variety on Morphometry of Nymphal Instars

Neither of the 2 tomato varieties showed a significant differential effect on the means of the pooled measurements of morphological traits of the 5 *B. cockerelli* nymphs (Table 3). Nevertheless mean body length was greater on 'Rafaello', while the body width and antennal length were greater on 'Charanda'. However the 2 tomato varieties showed significant differential effects on the morphometry of individual nymphal instars (Table 4). Thus the interaction of the variety with the body length of *B. cockerelli* was significant,

and the main factor in this interaction was the instar. Only the instar had a significant effect on the body width of the nymphs. Antennal length showed significant variation caused by the instar-variety interaction, and instar was the main factor in determining this effect. Tomato variety had no influence on antennal size of *B. cockerelli* nymphs (Table 4).

Body length of nymphs (Table 5) showed significant differences among instars, with the exception of instars 2 and 3, whose body lengths overlap. But, body width and antennal length were significantly different among all of the 5 instars. These results have much practical value because body width and antennal length of nymphs can be recommended to differentiate nymphal instars on this pest species.

#### Adult Morphometry

The morphometry of adult specimens of *B. cockerelli* varied due to the differential effects of the 2 tomato varieties, sex of the insect, and the interaction between these factors (Table 6). The occurrence of a statistically significant interaction between variety and sex indicated a differential effect on the antennal length and anterior wing width morphometric variables when the same sex was evaluated on the 2 tomato varieties. Clearly *B. cockerelli* females displayed morphometric differences when they developed on one or the other tomato variety; and the same was true for males.

No significant differences were observed in body length between the sexes of the adults. There were no significant differences observed in thorax width (Table 7). Antennal length was affected by the tomato variety, sex of the insect, and their interaction (Table 6). The anterior wing length was affected only by the insect's sex, while anterior wing width was affected by the tomato variety, sex of the insect and their interaction (Table 6). Statistical comparison of the means of body length, antennal length, and anterior wing width

TABLE 1. DURATION OF THE NYMPH AND ADULT STAGES OF *BACTERICERA COCKERELLI* REARED ON 2 TOMATO VARIETIES UNDER GREENHOUSE CONDITIONS IN MONTECILLO STATE OF MEXICO, OCT-DEC 2009 AND JAN-MAR 2010

STAGES	'CHARANDA F1'	'RAFAELLO'	STAGES	'CHARANDA F1'	'RAFAELLO'
	(days)	(days)		(days)	(days)
Duration of life stage in 2009			Duration of life stage in 2010		
Egg	7	8	Egg	13	10
Nymph 1	11	9	Nymph 1	19	18
Nymph 2	13	11	Nymph 2	16	16
Nymph 3	16	16	Nymph 3	16	15
Nymph 4	17	15	Nymph 4	17	15
Nymph 5	19	20	Nymph 5	17	19
Adult	44	45	Adult	41	42
Egg-Adult	63	68	Egg-Adult	69	70

Note: The time of development from the egg to adult is not the sum of the different stages because do not necessarily at the end of one stage indicate the star of the next stage.



TABLE 2. COMPARISON OF MEAN MORPHOMETRIC MEASUREMENTS ( $\mu\text{M} \pm 1\text{SD}$ ) OF *BACTERICERA COCKERELLI* EGGS OF ADULTS REARED ON 2 TOMATO VARIETIES IN MONTECILLO STATE OF MEXICO, 2009-2010.

Variable	'CHARANDA F1' Mean	'RAFAELLO' Mean	t	P
Egg Length	285.97 $\pm$ 14.92	296.87 $\pm$ 38.04	-1.25	0.22 <sup>NS</sup>
Egg Width	129.18 $\pm$ 6.65	140.18 $\pm$ 15.53	-3.04	0.005 <sup>**</sup>
Pedicle Length	184.03 $\pm$ 23.36	175.24 $\pm$ 21.09	1.29	0.20 <sup>NS</sup>

<sup>NS</sup> = Not significant ( $P \leq 0.05$ ); <sup>\*\*</sup> significant ( $P \leq 0.01$ ).

variables obtained on tomato varieties showed differential effects of tomato varieties and sexual gender (Table 7). Sexual gender differentially affected anterior wing length and anterior wing width variables on the tomato varieties.

Comparison of the mean values of the morphometric variables of *B. cockerelli* adults indicated that the 'Charanda' variety induced greater body length, antennae length, anterior wing length and width, while the Raffaello variety resulted in greater body width at the thorax and anterior wing width (Table 7).

#### DISCUSSION

The durations of the egg and the first instar in 2009 were similar to those reported by Yang et al. (2009), who observed a value of 6 days for the same variable on eggplant and bell pepper, however the durations of the other instars were very different. The same tendency occurred in 2010. A possible explanation for this phenomenon could be the different range of temperature used in this work (21 °) in contrast with the range used by Yang et al. (2009) (26.7 °) and the different plant hosts used in both studies. Great differences in durations within the life cycle of the *B. cockerelli* reported by different authors indicates the need for more careful research on this species under the specific conditions of different tomato producing areas. In Mexico tomato production occurs in many areas with different climatic conditions; and precise knowledge of the life cycle in producing areas is needed to make decisions about controlling this insect pest. Our results of the egg incubation period coincide with those reported by

Lehman (1930), Pack (1930), Knowlton & Janes (1931), List (1939), Pletsch (1947) and Abdullah (2008), but they do not coincide with respect to the duration of the nymph, adult, and the total biological cycle. The differences could be due to different plant varieties and environmental conditions used in the different studies. Nachappa et al. (2012) recently reported some effects of '*Candidatus Liberibacter solanacearum*' (a.k.a. '*Ca. L. psyllaurosus*') on the fitness of *Bactericera cockerelli*, but not on the morphology. In this study we did not consider the possible effect of this bacterium on the morphometry of the insect. Yet, as an anonymous reviewer suggested, the presence of this bacterium has the potential to cause what appear to be cultivar effects resulting from pathogen-induced changes in phloem pressure, nutritional status, etc. However in our study none of the plants showed symptoms of '*Candidatus Liberibacter solanacearum*' infection. Therefore we do not think that any of the observed effects were caused by the influence of bacteria.

#### Egg Morphometry

There was no significant difference in lengths of the eggs ( $F_{1,41} = 0.57$ ;  $P = 0.4551$ ) obtained from the 2 different tomato varieties (Table 2), but egg width was affected by the tomato variety on which the insects were cultured ( $F_{1,41} = 11.92$ ;  $P = 0.0013$ ). In this regard Morales & Cermeli (2001) point out that the preference in oviposition of an insect on a host plant is influenced by its age and the environmental conditions where the plant developed. Future studies should investigate the reason for differences in eggs morphometrics in-

TABLE 3. MEAN VALUES ( $\mu\text{M} \pm 1\text{SD}$ ) OF MORPHOMETRIC VARIABLES OF *BACTERICERA COCKERELLI* NYMPHS (INSTARS 1 TO 5) GROWN ON 2 TOMATO VARIETIES IN MONTECILLO STATE OF MEXICO, 2009-2010. MEASUREMENT DATA FROM ALL 5 INSTARS WERE POOLED.

Variable	CHARANDA F1	RAFAELLO	t	P
	Mean	Mean		
Body Length	1273.75 $\pm$ 423.11	1294.00 $\pm$ 415.89	-0.44	0.66 <sup>NS</sup>
Body Width	842.76 $\pm$ 313.53	836.41 $\pm$ 298.07	0.19	0.85 <sup>NS</sup>
Antennae Length	209.38 $\pm$ 95.06	208.28 $\pm$ 80.87	0.11	0.91 <sup>NS</sup>

<sup>NS</sup> = Not significant ( $P \leq 0.05$ ),  $n = 165$  specimens.

TABLE 4. VARIATION FACTORS AND SIGNIFICANCE ON THE MORPHOMETRY OF *BACTERICERA COCKERELLI* NYMPHS GROWN ON 2 TOMATO VARIETIES IN MONTECILLO STATE OF MEXICO, 2009-2010.

Source	Degrees of freedom	Mean squares
Body length		
Instar	4	13499749.38**
Variety	1	36808.62 <sup>NS</sup>
Instar*variety	4	28028.38*
Body width		
Instar	4	7215007.08**
Variety	1	1651.16 <sup>NS</sup>
Instar*variety	4	6797.31 <sup>NS</sup>
Antennae length		
Instar	4	593524.51**
Variety	1	99.38 <sup>NS</sup>
Instar*variety	4	4634.35**

<sup>NS</sup>= Not significant ( $P \leq 0.05$ ); \*, \*\* Significant with ( $P \leq 0.05$ ) and highly significant with ( $P \leq 0.01$ ).

duced by 'Rafaello' and 'Charanda F1'. Possibly differences in phytochemicals produced by these varieties are responsible for this response of the insect. Orozco-Cardenas et al (2001) and Orozco-Cárdenas & Ryan (1999) reported high production of  $H_2O_2$  in tomato plants after the attack of some aphids, and that this might provide an early defense in being able to cause an often drastic reduction in insect feeding (Harborne 1993).

#### Nymphal Morphometry

The nymphal instar of *B. cockerelli* is one of the least studied developmental stages in the American continent, whereas exhaustive studies on the nymphal stages of the Psylloidea superfamily have been conducted in Europe (White & Hodkinson 1985; Ossiannilsson 1992). Body length ( $F_{4,324} = 1199.2$ ;  $P < 0.0001$ ), body width ( $F_{4,324} = 900.72$ ;

TABLE 5. DIFFERENTIATION OF MEAN MORPHOMETRIC VARIABLES ( $\mu$  M) OF NYMPHAL INSTARS OF *BACTERICERA COCKERELLI* GROWN ON 2 TOMATO VARIETIES IN MONTECILLO STATE OF MEXICO, 2009-2010.

Instar	Body length	Body width	Antennae length
1	652.22 d	390.05 e	92.71 e
2	1135.92 c	709.47 d	159.71 d
3	1184.54 c	769.74 c	181.18 c
4	1681.47 b	1145.66 b	287.04 b
5	1763.17 a	1187.58 a	323.61 a

Means with the same letter in the same column are not statistically different, Tukey ( $P < 0.05$ ).

TABLE 6. FACTORS OF VARIATION AND SIGNIFICANCE ON THE MORPHOMETRY OF *BACTERICERA COCKERELLI* ADULTS REARED ON 2 TOMATO VARIETIES IN MONTECILLO STATE OF MEXICO, 2009-2010.

Source of variation	Degrees of freedom	Mean squares and significance
Body length		
Variety	1	859536.13**
Sex	1	20358.07 <sup>NS</sup>
Variety*sex	1	8595.36 <sup>NS</sup>
Body width		
Variety	1	318.60 <sup>NS</sup>
Sex	1	2787.54 <sup>NS</sup>
Variety*sex	1	387.95 <sup>NS</sup>
Antennae length		
Variety	1	121260.12**
Sex	1	74570.69*
Variety*sex	1	121789.77**
Forewing length		
Variety	1	5627.59 <sup>NS</sup>
Sex	1	301567.74**
Variety*sex	1	22741.19 <sup>NS</sup>
Forewing width		
Variety	1	35009.00**
Sex	1	64688.65**
Variety*sex	1	15816.40*

\*, \*\* =  $F$  test significance ( $P \leq 0.05$  and  $P \leq 0.01$ ); <sup>NS</sup> = not significant

$P < 0.0001$ ), and antennae length ( $F_{4,324} = 883.93$ ;  $P < 0.0001$ ) variables were significantly differentiated for each *B. cockerelli* nymphal instar. These distinctions have practical implications, because they can be used to differentiate the 5 nymphal instars of *B. cockerelli*. Instar classification can be adequately accomplished by body length, except in the cases of instars 2 and 3, which overlap in body length. However all 5 instars can be reliably differentiated by body width and antennal length. This is of a much practical value for any researcher who needs to sort the nymphal instars of this triozid insect.

#### Adult Morphometry

Mean body length of *B. cockerelli* adults reared on 'Rafaello' tomato differs from that of those reared on 'Charanda' tomato ( $F_{1,117} = 7.11$ ;  $P = 0.0087$ ). However sexual gender per se did not affect body length ( $F_{1,117} = 0.09$ ;  $P = 0.7603$ ). This seems strange given that it is known that the females have a greater body size than the males (Plestch 1947; Burckhardt & Lauterer 1997). Possible such a difference could be detected by analyzing a very large sample. Body width at the tho-

TABLE 7. EFFECT OF TOMATO VARIETIES AND SEXUAL GENDER OF *BACTERICERA COCKERELLI* ADULTS ON THEIR MORPHOMETRY IN MONTECILLO STATE OF MEXICO, 2009-2010.

TRAIT	Charanda F1		Rafaello		<i>t</i>	<i>P</i>	Female		Male		<i>t</i>	<i>P</i>
	<i>n</i>	Mean	<i>n</i>	Mean			<i>n</i>	Mean	<i>n</i>	Mean		
LC	60	1656.1	60	1486.8	3.3	0.001**	60	1558.5	60	1584.5	-0.5	0.62 <sup>NS</sup>
ACT	57	542.8	58	546.1	-0.4	0.71 <sup>NS</sup>	57	549.4	58	539.6	1.1	0.28 <sup>NS</sup>
LA	60	694.04	48	626.6	2.5	0.01**	48	639.6	60	683.6	-1.6	0.10 <sup>NS</sup>
LALA	60	2118.0	58	2104.2	-3.2	0.001**	58	2162.7	60	2061.4	4.6	<0.0001**
ANALA	60	780.9	59	815.2	-3.2	0.001**	59	821.3	60	774.9	4.5	<0.0001**

LC = Body length; ACT = Body width at the thorax; LA = Antennae length; LALA = Anterior wing length; ANALA = Anterior wing width. \*, \*\* significance of *t* test ( $P \leq 0.05$  and  $P \leq 0.01$ ); <sup>NS</sup> = not significant.

rax is not affected by the tomato variety ( $F_{1,112} = 0.13$ ;  $P = 0.7145$ ), nor by the sexual gender of the insect ( $F_{1,112} = 1.18$ ;  $P = 0.2803$ ). Antennal length of the adults of *B. cockerelli* is affected by tomato variety ( $F_{1,105} = 7.29$ ;  $P = 0.0081$ ) and by sexual gender ( $F_{1,105} = 4.49$ ;  $P = 0.0365$ ). It is known that the males have larger antennae than females, perhaps because of mating behaviors. Related to this, an adult male-attractive pheromonal compound of *B. cockerelli* had been detected recently (Guedot et al. 2010). This is difference of antennal size between males and females is well documented for *Cacopsylla bidens* (Sulc) (Soroker et al. 2004) and *Diaphorina citri* Kuwayama (Onagbola et al. 2008). Anterior wing length of *B. cockerelli* showed no variations induced by either of the 2 tomato varieties ( $F_{1,115} = 0.40$ ;  $P = 0.5301$ ), but there were highly significant differences due to the sexual gender of the insect ( $F_{1,115} = 21.25$ ;  $P < 0.0001$ ). This coincides with multiple results that indicate a greater wing size in females than in males (Knowlton & James 1931; Plestch 1947; Soroker et al. 2004; Liu et al. 2007). Anterior wing width was differentiated both by variety ( $F_{1,116} = 12.34$ ;  $P = 0.0006$ ) and the sex of the insect ( $F_{1,116} = 22.79$ ;  $P < 0.0001$ ). Yang & Liu (2009) presented developmental parameters of *B. cockerelli* on eggplant and chili pepper plants, and they obtained lower reproduction ratios in chili pepper, although the generation time was longer in this host plant. These researchers asserted worked that differences in its reproductive ability of *B. cockerelli* are a function of the host plant. This could cause the appearance of biotypes in *B. cockerelli*. It is important to point out that Yang & Liu (2009) did not provide a rigorous taxonomic identification of this insect. Moreover, their study lacked a morphometric analysis to relate the effect of the host plant to the insect's morphology. The morphometric analysis proposed in the present paper enhances the data shown in the life tables for *B. cockerelli*, and it supplies precise information on the taxonomic identification of the studied insect. In many published scientific works the latter point is usually taken for granted, however specific conditions could cause chang-

es in the table of life. The plants in our study did not show any symptoms 'Candidatus Liberibacter solanacearum' infection, and we do not think that any of the observed effects were caused by the influence of bacteria.

#### CONCLUSIONS

The tomato varieties, 'Rafaello' and 'Charanda', had a differential effect on *B. cockerelli* egg width, but not on egg length or egg pedicel length. Also, we were able to differentiate all 5 nymphal instars by body width and antennal length. Antennal length of nymphs has the potential to support the development of practical methodologies for identifying nymphal instars of *B. cockerelli* and of phylogenetically related insects.

Effects of the 'Rafaello' and 'Charanda' tomato varieties were identified on the morphometric variables of adults of *B. cockerelli*, i.e., body length, antennal length, and wing width. Sexual gender of insect influenced antennal length, wing length, and wing width. Additional research is needed to elucidate the precise way in which the host plants affect the morphometry of the different development stages of *B. cockerelli*. It is necessary to evaluate the effects of number of additional solanaceous crop species on the population growth of *B. cockerelli*, and to relate life table parameters to the variation in morphometric dimensions.

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