HABITAT, BODY SIZE AND REPRODUCTION OF THE LEAFHOPPER, *DALBULUS ELIMATUS* (HEMIPTERA: CICADELLIDAE), DURING THE WINTER DRY SEASON

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Abstract

Although the Mexican corn leafhopper *Dalbulus elimatus* (Ball) is an important vector of maize pathogens, little is known about its biology in natural conditions, particularly about its overwintering habitats. Winter populations are a source of infestation of crops and are responsible for efficient distribution of offspring in the new habitat that in this case is maize cultivated in the rainy season (Jun-Oct). This study was conducted in the habitats were *D. elimatus* adults have green food available during the Mexican winter (dry) season (Nov-May) in central Mexico, such as on senescent maize, green wild grasses that grow on the edges of maize fields, and volunteer maize and maize seedlings. *Dalbulus elimatus* adults were found in Nov on senescent maize, in Dec on green wild grasses, and in Apr on volunteer maize. These results suggest that adults can overwinter locally. Moreover, a sudden appearance of *D. elimatus* adults occurred in Jul when maize was planted, at the onset of the rainy season. These immigrant adults are mainly females that exhibit larger body size (wing length and head width) and carry higher numbers of mature eggs than females collected during the winter season.

Key Words: Local overwintering, migration, maize, body size, scenescent maize, wild grasses

Resumen

A pesar que la chicharrita Mexicana del maíz *Dalbulus elimatus* (Ball) es un importante vector de patógenos al maíz, poco se sabe sobre su biología en condiciones naturales, particularmente sobre sus hábitats donde pasa el invierno (estación seca). Las poblaciones de los insectos que invernan son fuente de infestación en cultivos y son responsables de una eficiente propagación de sus descendientes en el nuevo hábitat, que en este caso es maíz cultivado en la estación lluviosa (Jun a Oct). Esta investigación fue realizada en los hábitats donde los adultos de *D. elimatus* podrían estar presentes durante el invierno o estación seca de México, que ocurre de Nov a May. Dichos hábitats fueron maíz en senectud, pastos verdes que crecen en los márgenes del cultivo de maíz, maíz voluntario que crece de las semillas originadas al momento de la cosecha y nuevo maíz sembrado al inicio de la estación lluviosa. Los adultos de *D. elimatus* fueron encontrados en Nov sobre maíz en senectud, en Dic sobre los pastos verdes y en Abr sobre el maíz voluntario. Estos resultados sugieren que los adultos de *D. elimatus* pueden pasar el invierno localmente. Además, una repentina aparición de adultos de *D. elimatus* ocurrió en Jul, cuando el nuevo maíz fue sembrado, al comienzo de la estación lluviosa. Estos adultos inmigrantes fueron casi en su totalidad hembras, que mostraron un tamaño de cuerpo grande y un gran número de huevos maduros, cuando se les comparó con hembras colectadas durante la estación seca.

Little is known about where and how overwintering leafhopper vectors pass the dry season; and this is the case for the Mexican corn leafhopper, *Dalbulus elimatus* (Ball) (Hemiptera: Cicadellidae). *Dalbulus elimatus* is an important vector of 3 maize (*Zea mays* L. ssp. *mays*) plant pathogens. It efficiently transmits *Maize rayado fino virus* (MRFV), maize bushy stunt phytoplasma (*Candidatus* Phytoplasma asteris) and corn stunt spiroplasma (*Spiroplasma kunkelii* Whitcomb) (Nault 1980; Nault 1990; Ebbert et al. 2001).

Dalbulus elimatus is distributed in the Neartic region, i.e., in the southern of United States and throughout Mexico. This leafhopper evolved in central Mexico and prefers to live at elevations higher than 1,000 m asl (Barnes 1954; Triple-

horn & Nault 1985; Nault 1990; Moya-Raygoza 2002). The Mexican corn leafhopper feeds and reproduces on *Zea* (maize and teosintes) its main host plants (Nault & Madden 1985). Also, Barnes (1954) demonstrated that *D. elimatus* not only feeds and reproduces on *Zea* but also on barley *Hordeum vulgare* L., wheat *Triticum aestivum* L. and the wild grasses *Bromus laciniatus* Beal, whereas most of the wild grasses can be used for feeding.

Dalbulus elimatus feeds and reproduces on maize during the rainy season (Jun to Oct) (Barnes 1954). But little is known about where the adults are located throughout the dry season. In Mexico the dry season typically occurs from Nov to May and is characterized by low rainfall, reduced temperatures and short day-lengths (Larsen et al. 1992). In the dry season maize which had been planted at the onset of the rainy season dries out, and wild perennial grasses grow in Dec, Jan and Feb. Later in Apr volunteer maize appears within the stover and stubble of the harvested maize. Finally in Jun or Jul the rainy season starts and new maize crops are planted.

In this study we searched for the overwintering sites of *D. elimatus* and determined body size (wing length and head width) and reproductive status (number of mature eggs) of the females collected. Therefore, the objectives were: 1) to survey *D. elimatus* adults on senescent maize, green grasses, volunteer maize and new maize, 2) to compare wing length and head width between *D. elimatus* females collected during the dry season on senescent maize, green grasses, volunteer maize and new maize, and 3) to investigate whether *D. elimatus* that colonize the new maize crop are composed mainly of females, which may carry high numbers of mature eggs. Females are important because they are responsible for colonizing new maize. This study contributes to understanding of overwintering biology of an important but little studied maize leafhopper vector.

Material and Methods

Dalbulus elimatus on Senescent Maize, Wild Grasses and New Maize (2004-2005)

The study was conducted in Zapopan, Jalisco state, because this region represents the most significant agronomic habitat for maize cultivation in Mexico at high elevations (>1,000 m asl). Most maize in Mexico (≈6,100,000 ha) is grown at high elevations where it usually is cultivated only during the rainy season and where no maize is grown during the dry season. In Zapopan the lowest temperatures reach -2 °C during the dry season. *Dalbulus elimatus* adults were collected at the Zapopan site (20°44'N, 103°30'W, 1,662 m asl) on green senescent maize with 12-14 leaves on 10 Nov 2004; on green wild grasses [*Chloris gayana* Kunth, *Cynodon dactylon* (L.), and *Paspalum* sp.] that grew at the edges of harvested maize fields on 24 Dec 2004; and on new maize on 27 Jul 2005, at the onset of the rainy season. These sampling dates were selected because host resources for *D. elimatus* adults were available in the field at that time. Plants were sampled on each sample date by a 39.3 cm diameter sweep net for 20 min (approximately 1400 sweeps) to capture *D. elimatus* adults. Collected adults were preserved in 70% ethanol. The numbers of *D. elimatus* adults collected on senescent maize, green grasses and new maize were determined. Ten females were randomly selected from each of these 3 sample categories, and their wing lengths and head widths were determined with a stereoscope microscope (Stemi DV4, ZEISS). Wing length was determined as the overall length of the right forewing, and head width was determined as the distance between the compound eyes. Each female was dissected to count the number of mature eggs. Mature eggs were elongated, whitish and approximately double the size of eggs still being formed.

Dalbulus elimatus on Volunteer and New Maize (2009)

This second part of the study was performed because throughout 2004-2005 in the Zapopan region, we observed that volunteer maize germinated from seeds left in the fields after the harvest. These plants could host *D. elimatus* adults. Consequently, *D. elimatus* adults were sought out and collected from volunteer maize at the Zapopan site on 24 Apr 2009. The volunteer maize had 6-8 leaves. Also, at the Zapopan site, *D. elimatus* adults were collected on 15 Jul on new maize, which had been planted on 24 Jun, and on which the first leaves appeared on 5 Jul. In this second part of the study, we sampled the adults on the new maize crop, because of the importance of immigrants that arrive on it. We used the same adult sampling methodology as in the first part of the study. Thus the numbers of *D. elimatus* adults collected on volunteer and on new maize were determined. Ten females were randomly selected from volunteer maize and another 10 from new maize in Zapopan location to determine their wing lengths and head widths. Each female was dissected to count the number of mature eggs. The number of selected females, and method of determining wing lengths and head widths in the first and second parts of the study were identical, and they had already been employed for characterizing *Dalbulus maidis* (Delong & Wolcott) overwintering populations (Moya-Raygoza et al. 2005; Moya-Raygoza et al. 2007 (a); Moya-Raygoza et al. 2007 (b)). *Dalbulus elimatus* adults were identified using the keys of Triplehorn & Nault (1985). Finally, no males were included in the determinations of wing length and head width because of the low numbers of males collected during the dry season.

Comparison between females and males by each sample date was performed with a chi square goodness of fit test. Also a Kruskal-Wallis test was used to compare the number of mature eggs of different categories of females. A one-way ANOVA test was performed to determine differences between female wing length and female head width. All statistical analyses were performed using SPSS software (SPSS 11.5 for Windows, SPSS, Chicago, Illinois).

Results

Dalbulus elimatus adults were collected on senescent maize, green wild grasses and volunteer maize throughout the dry season. Upon the onset of the rainy season and after the new maize crop had been planted, *D. elimatus* adults suddenly appeared and were collected from the new maize. Females and males were collected in similar percentages on senescent maize and green wild grasses during the dry season in 2004, whereas on volunteer maize (2009), and on new maize in 2005 and 2009 more females than males were found (Table 1).

Females collected in 2004-2005 on senescent maize on 10 Nov, on green grasses on 24 Dec and on new maize on 27 Jul, and females collected in 2009 on volunteer maize on 24 Apr and on new maize on 15 Jul displayed differences in body size in terms of wing length (F = 16.91; df = 4, 45; P = 0.001) (Fig. 1) and head width (F = 12.68; df = 4, 45; P = 0.001) (Fig. 2). The females with the smaller body size were collected on green grasses in the first part of the study (2004-2005); and females with the largest body size were collected on new maize in the same period (2004-2005). Also in the second part of the study (2009) females with the largest body size were again collected on new maize. Females that colonized the new maize also had high numbers of mature eggs (H = 4.31; df = 2; P = 0.11). Females with mature eggs were collected on volunteer maize during Apr 2009, on new maize during Jul 2005, and again on new maize during Jul 2009 (Fig. 3). Females collected in Jul on new maize had an average of 4 mature eggs, whereas females collected on volunteer maize had less than 2 mature eggs; and no eggs were found in females collected from senescent maize and green grasses.

Discussion

Results of this study demonstrated that *D. elimatus* adults can overwinter locally at high elevations. During the dry season we found *D. elimatus* adults on senescent maize (Nov), green wild grasses (Dec) and volunteer maize (Apr) at more than 1,000 m asl at Zapopan, Jalisco, Mexico. Throughout the dry season, *D. elimatus* adults were deprived of maize, their main host plant, for several mo. Larsen et al. (1992) found that corn leafhopper *D. maidis* females, a sister species that uses maize as primary host plant, can survive for long period (2 mo) on moist sand under winter conditions, and we think that *D. elimatus* females do the same, and that they can live locally for long periods without maize or other feeding host.

Dalbulus elimatus adults were collected at the edges of maize fields in Zapopan on wild green grasses [*Chloris gayana* Kunth, *Cynodon dactylon* (L.), and *Paspalum* sp.], once the maize had become senescent and its tissues had become dry. The grasses were used by *D. elimatus* adults for feeding or shelter, but not as hosts for reproduction (Barnes 1954). Although the *D. elimatus* population was sampled throughout the entire dry season, no adults were found after these wild grasses dried out in Feb (Moya-Raygoza 2002). *Dalbulus elimatus* females collected on green grasses were smaller than those on maize. Small body size in delphacid planthoppers is associated with habitat persistence and low dispersal (Denno & McCloud 1985; Denno 1994). In our case, persistence is made possible by the green grasses, and volunteer maize throughout the winter. Furthermore, small females that were collected on the green grasses proved able to survive the low winter temperatures. Larsen et al. (1993) found under laboratory conditions that *D. elimatus* adults tolerated temperatures of -5 °C, i.e., the low temperatures that occur at high elevations in Zapopan; and Larsen et al. (1993) suggested that adults overwinter in protected habitats at high elevations. In this study we report on the overwintering habitats of *D. elimatus*.

Table 1. Numbers of females and males of *Dalbulus elimatus* in standard samples in different habitats at Zapopan, Jalisco, Mexico.

Habitat	Collection date	Females	Males	x^2	P
Senescent maize	10 Nov 2004	11	11	0.00	1.00
Green grasses	24 Dec 2004	49	60	1.11	0.29
Volunteer maize	24 Apr 2009	12	4	4.00	0.04 (*)
New maize	27 Jul 2005	15	1	12.25	0.001 (*)
New maize	15 Jul 2009	16	1	13.23	0.001 (*)

*= significantly different.

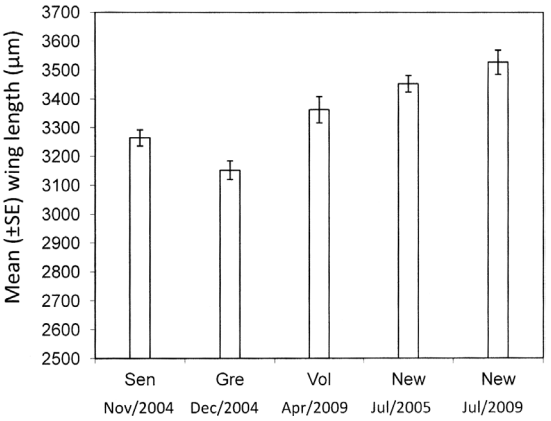

Fig. 1. Wing lengths of *Dalbulus elimatus* females (mean ± SE) collected during the dry season and on new maize at the onset of the rainy season in the Zapopan region in central Mexico. Sen = Senescent maize, Gre = Green grasses, Vol = Volunteer maize, New = New maize.

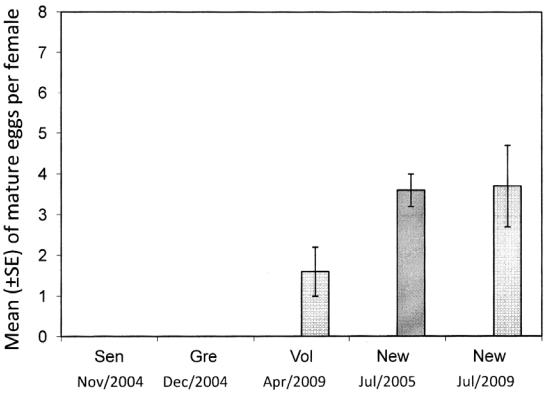

Fig. 3. Mature eggs of *Dalbulus elimatus* females (n = 10) collected during the dry season and on new maize at the onset of the rainy season in the Zapopan region in central Mexico. Sen = Senescent maize, Gre = Green grasses, Vol = Volunteer maize, New = New maize. Females from senescent maize and green grasses had zero mature eggs.

Furthermore in this same overwintering habitat of Zapopan, we are reporting for first time the presence of *D. elimatus* adults on volunteer maize with 6-8 leaves in Apr, 2 mo after the wild grasses had dried out. These results agree with those of Summers et al. (2004), who that found volunteer maize was an important resource for *D. maidis* survival during the winter in California´s San Joaquin Valley.

Dalbulus elimatus adults collected on green grasses in Dec and volunteer maize in Apr present a different body size than *D. elimatus* adults that appear on new maize in Jul, suggesting that those appearing in Jul are immigrants. Leafhoppers

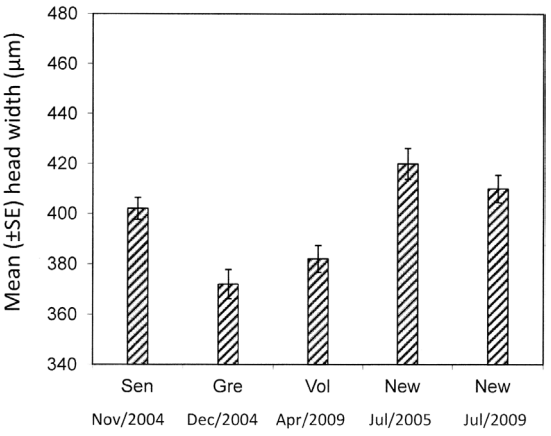

Fig. 2. Head widths of *Dalbulus elimatus* females (mean ± SE) collected during the dry season and on new maize at the onset of the rainy season in the Zapopan region in central Mexico. Sen = Senescent maize, Gre = Green grasses, Vol = Volunteer maize, New = New maize.

that reach the adult stage do not increase in size (Gullan & Cranston 2000). Therefore leafhoppers adults on volunteer maize are not the immigrants onto the maize crop in Jul, based on difference in adults body size. Previously, body size has been used as tool to determine if *D. maidis* adults that colonize new maize in Jul are immigrants (Moya-Raygoza et al. 2007a). Immigrant *D. elimatus* adults collected on new maize in Jul 2005 and Jul 2009 showed the largest body sizes. A larger body size is important, because selection favors large body size in migrant species (Roff 1991). Also, these females had a high number of mature eggs. A high rate of reproduction by immigrants is the main reason for the success of migrant insects, since this ensures more efficient distribution of offspring in the new environment (Holland et al. 2006). The new environment in this case is maize planted at the onset of the rainy season. Also, immigrant *D. elimatus* adults were mainly females. Drake and Chapman (1965) considered that the excess of females of the aster leafhopper *Macrosteles quadrilineatus* Forbes, indicated the presence of immigrants. Similar results were reported for the potato leafhopper *Empoasca fabae* (Harris), i.e., first-generation immigrants collected on alfalfa were female-biased, which supports the hypothesis that females comprise the majority of immigrants (Emmen et al. 2004).

The immigrant hypothesis for *D. elimatus* also is supported because recently (Palomera et al. 2012) found genetic variation among *D. maidis* populations to be low or absent by analysis of their Internal Transcribed Space Region 2 (ITS2) and cytochrome oxidase subunit, dalCOI, suggesting high gene flow among populations. The same lack of or low genetic variation among *D. elimatus* populations may also prevail. Immi-

grant adults could come from regions where barley, wheat, maize or other Poaceae are cultivated, and where *D. elimatus* adults have been collected at the end of the dry season. For example, *D. elimatus* populations are found at Sayula (19°52'N, 103°33'W, 1,366 m asl, and 96 km from Zapopan) throughout the dry season on maize, barley and sorghum *Sorghum bicolor* (L.) Maench (Poacea) crops grown with irrigation. *Dalbulus elimatus* displays migratory behavior and can take advantage of seasonal winds that arrive with intertropical convergence events that occur in central Mexico when the rainy season starts (Taylor et al. 1993). Consequently *D. elimatus* adults could be transported by the winds from Sayula or other regions where adults are present to Zapopan site or other high elevation sites where maize is cultivated during the wet season.

*Dalbulus elimatu*s and *D. maidis* have evolved to exploit the ephemeral annual maize habitat during the rainy season (Nault 1990). Both leafhopper species, which have maize as their main host plant, show faster development and produce more progeny compared to other *Dalbulus* congeners (Nault & Madden 1985). Consequently, immigrant adults are very important because they reproduce and exploit maize in space and time to produce a second generation with a high number of individuals.

In conclusion, the results reported here suggest that *D. elimatus* overwinter as active adults in different habitats, and that the large migrant females carrying a high number of mature eggs colonize the new maize crop. The latter females are responsible for the colonization of maize seedlings planted at the onset of the rainy season.

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