STUDIES ON NEOTROPICAL FAUNA AND ENVIRONMENT, 2016
http://dx.doi.org/10.1080/01650521.2016.1258107

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Food quality affects wing-form, demographic traits and number of yeast-like symbionts (YLS) in the planthopper vector, *Delphacodes kuscheli* (Hemiptera: Delphacidae)

María E. Brentassi[a,b], Arnaldo Maciá[a,b], Daniela de la Fuente [a], María E. Cano [a] and Ana M. Marino de Remes Lenicov[a,c]

[a]División Entomología, Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de Paseo del Bosque S/N La Plata (UNLP), La Plata, Argentina; [b]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina; [c]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

ARTICLE HISTORY
Received 24 June 2016
Accepted 3 November 2016

KEYWORDS
Yeast-like symbionts; macroptery; brachyptery; oat; food quality

ABSTRACT

Delphacodes kuscheli, the main vector of maize Mal de Río Cuarto disease in Argentina, was used to analyze the effect of food quality on wing-form, demographic traits and number of yeast-like symbionts (YLS). First instar nymphs were caged on the leaf apex or on the ligular zone of oat plants, characterized by having low and high nutritional value respectively, and followed in their development to adulthood. Food quality affected the proportion of wing morphs, with an increased number of brachypterous individuals on the higher food quality zone. Macropterous and brachypterous females from the ligular zone had higher survivorship, shorter development time and higher number of YLS. For males, development time and number of YLS did not differ significantly in individuals fed from both zones, while body and wing length were superior in individuals fed on the ligular zone. Results suggest that females allocate the resources from feeding on a richer zone to reduce the pre-reproductive period and to increase the number of YLS, and males acquire a larger size while maintaining development time and YLS number. This is the first study quantifying the variation of number of YLS according to food quality in a Neotropical planthopper pest.

RESUMEN

Delphacodes kuscheli, el principal vector del Mal de Río Cuarto del maíz en Argentina, fue usado para analizar el efecto de la calidad del alimento sobre las formas alares, rasgos demográficos y número de yeast-like symbionts (YLS). Se acondicionaron ninfas de primer estadio en el ápice foliar o en la zona ligular de plantas de avena, caracterizadas por tener un bajo y alto valor nutricional, respectivamente, para el insecto y se siguió su desarrollo hasta el estado adulto. La calidad del alimento afectó la proporción de morfos alares, mostrando una tendencia a incrementarse el número de braquípteros en la zona de mayor calidad alimenticia. Las hembras macrópteras y braquípteras de la zona ligular tuvieron mayor supervivencia, menor tiempo de desarrollo y mayor número de YLS. En machos, el tiempo de desarrollo y el número de YLS no difirieron entre individuos alimentados sobre ambas zonas, mientras que la longitud del cuerpo y de las alas fue superior cuando se alimentaron sobre la zona ligular. Estos resultados sugieren que las hembras destinan los recursos obtenidos de la alimentación en una zona más nutritiva del hospedero para reducir el período pre-reproductivo y a incrementar el número de YLS, los machos adquieren un tamaño corporal mayor manteniendo el tiempo de desarrollo y el número de YLS. Este es el primer estudio que cuantifica la variación en el número de YLS de acuerdo a la calidad de alimento en un delfácido plaga en la región Neotropical.

Introduction

Studies of dispersal polymorphism in insects have played a pivotal role in advancing the understanding of population dynamics, life history evolution, and the physiological basis of adaptation, with wing polymorphism being the most commonly studied (Zera & Denno 1997). Planthoppers are sap-feeding hemipterans that include several agricultural pests around the world and among them the family Delphacidae is the largest and most studied (Denno & Roderick 1990). Wing dimorphism in delphacids involves fully winged individuals capable of flight (macropters) and flightless adults with their wings reduced to varying degrees (brachypters) (Denno

CONTACT María E. Brentassi eubrenta@fcnym.unlp.edu.ar; eubrenta@hotmail.com

et al. 1985) and it results from a combination of both genetic and environmental variation (Roff 1986; Matsumura 1996). Among the environmental cues that influence wing form, crowding, host plant condition, temperature and photoperiod have been mentioned (Denno et al. 1985; Denno & Roderick 1990; Denno 1994). In many species, the production of macropters is density-dependent and is intensified by limited or nutritionally inadequate food (Denno 1994). In these species, macropters are able to effectively escape deteriorating habitats and colonize better ones elsewhere (Denno et al. 1991). In macropterous morphs, the energy used to develop wings and wing muscles and to fuel flight is not available for reproductive investment and a trade-off may arise between flight capability and reproduction (Denno et al. 1989; Zera & Denno 1997).

Food quality has an important effect on the ecology of these pests. Planthoppers, as well as other auchenorrhynchans, establish mutualistic symbiosis with microorganisms in order to compensate the scarce nutritional value of their diets (phloem or xylem sap) (Moran 1998; Sandstrom & Moran 1999). Delphacids have bacterial and eukaryotic endosymbionts, with the obligate endosymbionts known as “yeast-like symbionts” (YLS) (Fungi: Class Pyrenomycetes, family Clavicipitaceae) being the most widely studied (Noda et al. 1995; Hou et al. 2013; Cao et al. 2015). The YLS are located in mycetocytes formed by fat body cells in the abdomen or in intracellular spaces in the fat bodies (Noda et al. 1995; Xet-Mull et al. 2004) and are vertically transmitted by transovarial infection (Cheng & Hou 2001; Michalik et al. 2009). The YLS are involved in supplying hosts with essential amino acids (Chen et al. 1981), play a role in nitrogen metabolism (Sasaki et al. 1996) and supply the main source of sterol (24-methylenecholesterol), a precursor of ecdysone (Noda & Saito 1979). In addition, YLS are involved in embryonic development and yolk synthesis (Lee & Hou 1987) and may be linked to variation in host plant resistance to planthoppers (Ferrater et al. 2013) as well as resistance to pesticides (Zhang et al. 2008). To date the population dynamic of YLS throughout the life cycle of planthoppers has been studied in two important rice pests of Asia: *Laodelphax striatellus* (Fallén) (Noda 1974; Noda & Saito 1979) and *Nilaparvata lugens* (Stål) (Chen et al. 1981). In Argentina, the population dynamic of YLS in the planthopper *Delphacodes kuscheli* Fennah has been recently studied (Liljesthröm et al. 2016).

Delphacodes kuscheli Fennah (Hemiptera: Delphacidae), a species whose generic status is being revised, is a multivoltine species widely distributed in Argentina (32–35° S) that feeds on phloem sap of several cultivated and wild gramineous plants (Remes Lenicov & Virla 1999; Brentassi & Remes Lenicov 2007; Remes Lenicov & Paradell 2012). This planthopper is the most important vector of the Mal de Río Cuarto virus (MRCV), a fijivirus (Reoviridae) that seriously affects maize (*Zea mays* L.) production in northern and central provinces of Argentina (Remes Lenicov et al. 1985; Lenardón et al. 1987; March et al. 1997; Giménez Pecci et al. 2012). MRCV has also been reported in Uruguay (Ornaghi et al. 1999). Virus transmission occurs in spring when macropterous adults disperse to feed on juvenile maize plants (a non-preferential host). This is due to the senescence of winter grasses which generally serve as reservoirs of MRCV and which are preferential hosts for *D. kuscheli*, such as oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Tesón et al. 1986; Remes Lenicov et al. 1991; Ornaghi et al. 1993). In natural populations *D. kuscheli* shows wing dimorphism, with macropterous and brachypterous individuals, the former being the predominant morph (Ornaghi et al. 1993, 2011; Remes Lenicov & Virla 1999). Brachypterous morphs have been detected in endemic zones of MRC during spring, especially on oat but also on other cereals such as wheat, barley, triticale and rye (Remes Lenicov et al. 1991; Remes Lenicov & Virla 1999; Boito & Ornaghi 2008).

Several aspects of the biology of *D. kuscheli*, such as feeding behavior (Brentassi & Maldonado 2002, Brentassi & Remes Lenicov 2005, 2007), interactions with parasitoids (Virla 2004) and fungus pathogens (Toledo et al. 2007, 2011) and population ecology (Grilli & Gorla 1997, 2002; Trumper & Garat 2001; Grilli 2008; Ornaghi et al. 2011) have been studied. Regarding the effect of host plant food quality on the wing morphs the studies are scarce. *Delphacodes kuscheli* is sensitive to the nutritional quality of different parts of wheat plants, which affects the proportion of wing morphs (Brentassi et al. 2014b). On the other hand, YLS which improve the feeding of planthoppers have been detected in the abdomen of nymphs and adults and in the primary oocyte of *D. kuscheli* (Brentassi et al. 2014a).

Increased attention to YLS is needed due to their relationship with host insect nutritional requirements (Chen 2009), so understanding more about their biology would contribute to the development of more sustainable pest control approaches (Cao et al. 2015).

To date, variation of the number of YLS in sap-sucking insects in response to different food quality sites of their host plants has not been reported. In order to better understand the biology of the MRCV vector and the aspects that could influence its population dynamics, we examined the effect of food quality on wing-form in *D. kuscheli*, as well as demographic traits, and quantified the number of obligate endosymbionts (YLS) in individuals fed on different food quality sites. Furthermore, the relationship between YLS number and body size was analyzed.

Materials and methods

Insects and plants

The founding insects (nymphs and adults) were collected in 2012 on oat fields in Río Cuarto (Córdoba, Argentina), an endemic area of MRCV. They were carried to the laboratory at the Entomology Division (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata) and reared on oat (*Avena sativa* L., Poaceae) plants at 24 ± 1°C, 50–55% RH and a L16:D8 photoperiod.

For the experiments we selected oat, a preferential host and the main overwintering host of *D. kuscheli*. Plants were grown from seeds in a 100 ml plastic pot filled with fertile soil under controlled conditions in an environmental chamber (Percival Scientific Inc., Perry, Iowa, USA) and irrigated when necessary. We used plants with two expanded leaves and a third non-expanded one.

Demographic traits in relation to food quality

We selected two zones of oat plants with extreme values of the selectivity index for *D. kuscheli* according to Brentassi et al. (2014b). The first (selected) was the ligular zone of the second leaf which includes: the ligule, the upper 0.5 cm of the leaf sheath and the basal 0.5 cm of the leaf blade. The second (avoided) zone was the apex of the second leaf blade (3 cm approximately). The two zones are assumed to have different nutritional values, with the ligular zone having a higher nutritional quality due to the presence of an intercalary meristem and hence a higher nitrogen content (Langer 1979; Mattson 1980; Rademacher & Nelson 2001).

Insects were randomly assigned to small cages on oat plants. On each plant the two zones were enclosed by means of a cylindrical plastic cage 3 cm in height and 1.5 cm in diameter, with both extremes covered with cotton and a lateral opening covered with a fine mesh as in Brentassi et al. (2014b) (Figure 1). In each cage (considered a replicate) a single 0–1-day-old first instar nymph was introduced for feeding and development. To prevent a decrease of plant quality due to caging and feeding, the cage and the insect were placed every two days on a new plant on the corresponding zone. This procedure was repeated until the death of the insect. Individual insects were checked daily until the emergence of adults. Adults were classified according to sex and wing morph and the proportion of each sex and morph were calculated. We measured the following demographic traits: nymphal survivorship, development time, proportion of sex and wing morphs, body size (length and weight) and wing length.

Nymphal survivorship was calculated as number of adults/initial number of first instar nymphs and development time as number of days from egg hatching to molt to the adult stage.

Total body length from the tip of the head (vertex) up to the extreme of the abdomen as well as the wing length (from the axillary angle to the wing tip) were recorded under binocular lens and expressed in mm. Adults were individually weighed on a four-digit balance (Acculab Sartorius Group, Göttingen, Germany) to the nearest 0.0001 g.

YLS in relation to food quality

The numbers of YLS in individuals of both sexes and wing morphs fed on different food quality plant zones were quantified. The number of YLS/individual was calculated following the procedure of Noda (1974) and Undeen and Vávra (1997). Each individual was macerated with a steel micropestle in an Eppendorf tube (2.5 ml) to which 0.5 ml of sterile water was added. Homogenization was carried out with manual agitation and the content of the tube was filtered with a fine mesh before use. Counts were made in a hemocytometer (Neubauer chamber, BOECO Germany). The YLS present in the 25 large squares of each central grid of the chamber were counted and then averaged. We calculated: symbionts/ml = 10^4 × number of symbionts in the count.

Relationships between number of YLS (taken as dependent variable) and weight and body length (as independent variables) were analyzed in females.

Data analysis

Number of individuals was compared across sexes, wing morphs and plant zone with contingency tables using χ^2 as the statistic to assess mutual independence of factors (Zar 2010).

To compare percentages of surviving individuals (pooled sexes) from the ligular zone with those from the leaf apex, we performed a test for differences between two proportions, with the following formula (Zar 2010):

$$Z = p_{\text{ligular zone}} - p_{\text{leaf apex}} / \sqrt{(PQ)/n_{\text{ligular zone}} + (PQ)/n_{\text{leaf apex}}}$$

Figure 1. Experimental setup for the feeding experiment of *Delphacodes kuscheli* on oat plants: (A) stage of two expanded leaves, and details of (B) enclosures in the ligular zone and (C) on the apex of leaves.

where

$p_{\text{ligular zone}} = n_{\text{ligular zone}}/N_{\text{ligular zone}}$, $p_{\text{leaf ápex}} = n_{\text{leaf apex}}/N_{\text{leaf apex}}$, $P = (n_{\text{ligular zone}} + n_{\text{leaf apex}})/(N_{\text{ligular zone}} + N_{\text{leaf apex}})$, $Q = 1 - P$.

n = number of individuals reaching adulthood and N = number of individuals at the start of the experiment.

The same formula was applied to compare percentages of wing morphs (pooled sexes) in each zone. In this case: n = number of individuals of one morph (brachypterous or macropterous) and N = total number of adults.

All variables were tested for homogeneity of variances using Levene's test, and normality was checked with Shapiro–Wilk's test ($p > 0.05$). In females, homoscedascity was corroborated for all responses ($p > 0.05$). The variables weight and YLS number rendered normal, but wing length and development time showed significant deviation from normality. Therefore, both variables were log-transformed to meet assumptions for subsequent testing. In males, development time, wing length and weight showed significant deviation from normality and homogeneity of variances, thus we used a log transformation for these variables thereinafter.

In our analysis, we considered wing morphs as categorical effects rather than a response to experimental manipulations because it is well known that parameters of traits measured differ depending on wing morph as well as on sex in planthoppers (Denno & Roderick 1990) and consequently both subpopulations were not analyzed jointly. In males, few individuals (n = 6) were brachypterous and some cells during data analysis had zero variances. Accordingly, data from brachypterous males were excluded and the effect of wing morph was removed for this sex.

Two experiments were run in the same conditions of temperature, humidity, photoperiod and quality of plants, but in different years (2013 and 2014, during November and December in each year) to achieve acceptable insect sample sizes, and a comparison of means for each variable for year was done with paired t-tests. When no differences were detected between means for a particular variable, data from both years were pooled for subsequent analysis and considered as a single set. Differences between means across years were significant only for weight in both sexes. As a double check for this across-years difference in weight, a parametric ANOVA with year, plant zone and female wing morph as main effects plus double and triple interactions was performed on females' data, in order to test possible significant interactions between main classification factors. The same analysis was performed for males, but since only one wing morph was incorporated to the model, a one-way ANOVA was run with plant zone as the source of variation. Again, year was the only significant factor affecting weight of females and males. Thus, weight was analyzed separately for each year with two univariate, parametric bifactorial ANOVAs with plant zone and wing morph as main effects plus the interaction between them in females. For males, mean weights in each plant zone were compared by t-tests by years.

A multivariate analysis of variance (MANOVA) was applied to females' data to examine the relationship between development time, body length, wing length and YLS number, and two categorical predictor variables. The latter were plant zone (ligular and leaf apex) and wing morph (macropters, brachypters) (fixed effects). MANOVA was chosen because multiple responses were measured and significant correlations between them were corroborated (Quinn & Keough 2002). Wilk's lambda was the statistic used to examine the significance of main factors (wing morph and plant zone) and their interaction, under of the null hypothesis of no differences between group centroids. After rejection, further analyses consisted in pairwise contrasts between mean responses applying Hotelling-Lawley T^2 statistic, because we compared means of responses in the ligular zona vs. the leaf apex, and in macropters vs. brachypters. Also, pairwise comparisons of individual response variables were compared by Bonferroni tests in order to control for the experiment wise error rate that could be inflated by multiple comparisons (Scheiner 2001). A MANOVA was applied to males' data with plant zone as a single effect, followed by Bonferroni tests to compare mean responses in development time, body and wing length, and YLS number of macropterous individuals.

Relationships between number of YLS (taken as dependent variable) and weight and body length (as independent variables) in females were explored with simple linear regression analysis (Zar 2010). Data were discriminated by wing morph, since macropterous and brachypterous individuals can have a distinct relationship between some variables owing to the difference in wing length between both groups.

Results

Demographic traits

Interdependence of sexes, wing morphs and plant zone

The analysis of contingency tables based on frequencies of macropterous and brachypterous individuals from both sexes and both plant zones showed that these factors were not mutually independent ($\chi^2 = 128.97$, 4 df, $p < 0.05$). Further analysis looking for partial independence showed that the effect of sex was independent of plant zone ($\chi^2 = 0.581$, 1 df, $p > 0.05$), and that wing morph was dependent upon plant zone ($\chi^2 = 5.26$, 1 df, $p < 0.05$) (Figure 2). The wing morph was dependent on sex ($\chi^2 = 8.06$, 1 df, $p < 0.05$), where in females the proportion of macropterous (51%) was almost equal to the proportion of brachypterous morphs (49%), while in males macropterous individuals (83%) were more prevalent than brachypterous (16%).

Survivorship

The percentage of survivors of *D. kuscheli* on the ligular zone (90%, n = 45, $N_{\text{ligular zone}} = 50$) was significantly superior to the percentage of survivors on the leaf apex (58.3%, n = 35, $N_{\text{leaf apex}} = 60$) (Z = 3.72, 108 df, $p = 0.0003$).

Wing morphs

The percentage of brachypterous individuals on the ligular zone (44%, n = 20, $N_{\text{ligular zone}} = 45$) was significantly superior to that on the leaf apex (20%, n = 7, $N_{\text{leaf ápex}} = 35$) (Z = 2.29, 78 df, $p = 0.024$). On both zones brachypterous females prevailed over brachypterous males (leaf apex: six females and one male; ligular zone: 15 females and five males).

Development time, body length, wing length and YLS number

In females, MANOVA showed that time spent as nymphs, body and wing length and number of symbionts per individual depends on whether females developed on the leaf apex or in the ligular zone and whether they were macropterous or brachypterous (Table 1).

The plant zone effect, summarized in Table 2, showed that the development time was significantly longer on the leaf apex (= 24.24 days, SE = 0.80,

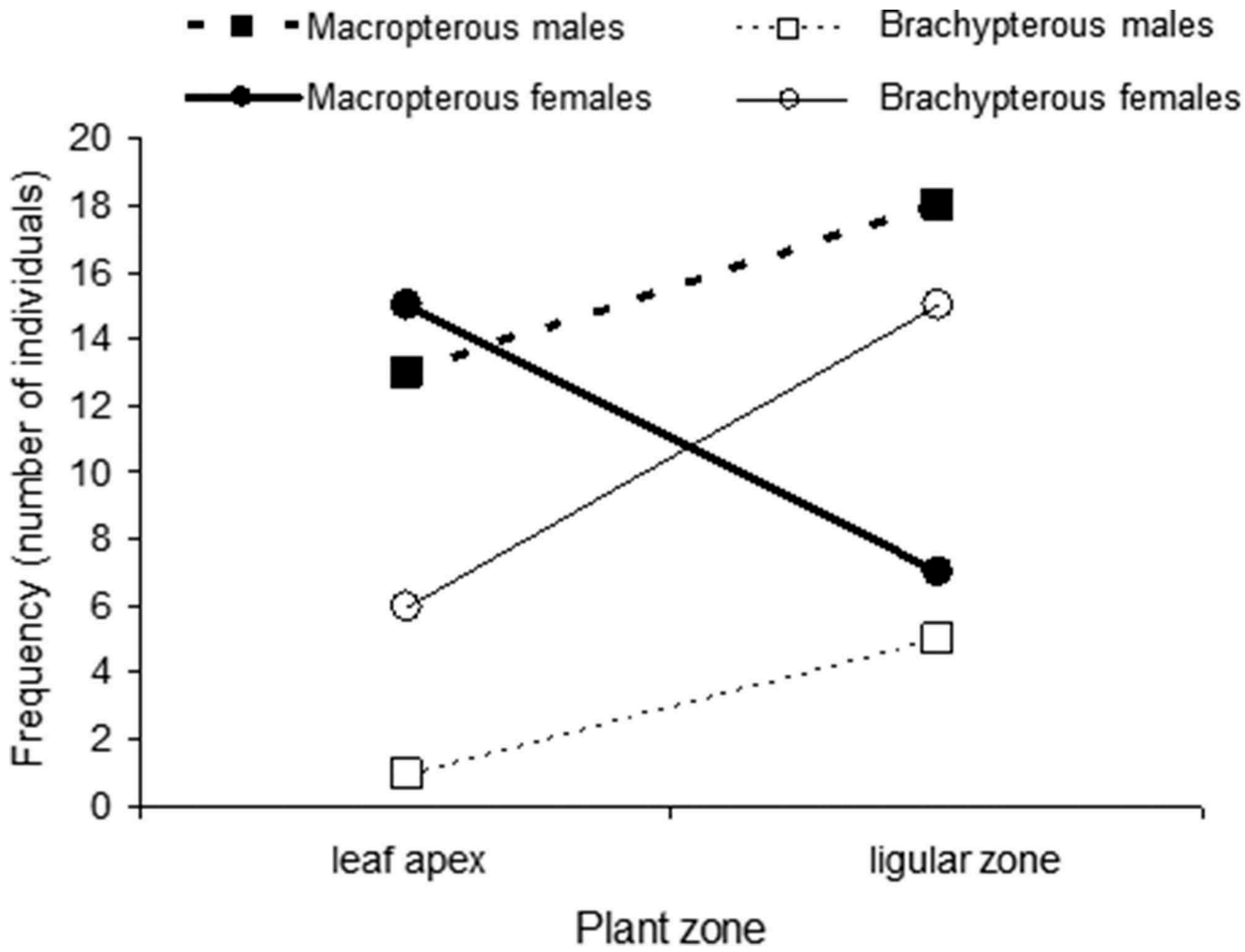

Figure 2. Frequencies of macropterous and brachypterous individuals of *Delphacodes kuscheli* of both sexes after development on the ligular zone or the leaf apex of oat plants in the laboratory.

Table 1. MANOVA results for effects involving plant zone (leaf apex and ligular zone) and wing morph (macropterous and brachypterous) on development time, wing length, body length and YLS number of *Delphacodes kuscheli.*

Sex	Effect	Wilks Λ value	F	Effect df	Error df	*p*
Female	Plant zone (A)	0.747	2.968	4	35	0.033
	Wing morph (B)	0.059	138.375	4	35	<0.001
	A × B	0.749	2.937	4	35	0.034
Male	Plant zone	0.667	3.119	4	25	0.033

Table 2. Pairwise comparisons with contrasts between effects involving response variables of female *Delphacodes kuscheli* confined on the oat leaf apex or on the ligular zone.

Response variable	Hotelling T^2 value	F	Effect df	Error df	*p*
Development time	0.171	6.494	1	38	0.015
Body length	0.065	2.466	1	38	0.125
Wing length	0.006	0.222	1	38	0.640
YLS number	0.106	4.014	1	38	0.052

n = 21) than on the ligular zone (= 21.38 days, SE = 0.41, n = 21). There were no significant differences between the body length of females feed from the leaf apex (= 2.83 mm, SE = 0.06, n = 21) and those feed from the ligular zone (= 2.99 mm, SE = 0.05, n = 21). Body length of brachypterous females from the ligular zone (= 3.05 mm) was longer than that from the leaf apex (= 2.81 mm) although not statistically significant (t = 1.87, 11 df, *p* = 0.088). The wing length of females from the ligular zone (= 2.07 mm, SE = 0.14, n = 21) did not differ significantly from the wing length of females from the leaf apex (= 2.48 mm, SE = 0.12, n = 21).

The number of YLS per individual was higher in females fed on the ligular zone (= 334 107 cells, SE = 27 135, n = 21) than in females fed on the leaf apex (= 210 000 cells, SE = 27 833, n = 21). Although marginal, this difference was statistically non-significant.

The wing morph effect, summarized in Table 3, showed that the development time was significantly longer in macropterous (= 23.68 days, SE = 0.82, n = 22) than in brachypterous females (= 21.85 days,

Table 3. Pairwise comparisons with contrasts between effects involving response variables of macropterous and brachypterous females of *Delphacodes kuscheli.*

Response variable	Hotelling T^2 value	F	Effect df	Error df	*p*
Development time	5.488	208.549	1	38	< 0.001
Body length	0.022	0.848	1	38	0.363
YLS number	0.300	11.391	1	38	0.002

Table 4. ANOVA results for effects involving plant zone (leaf apex and ligular zone) and wing morph (macropterous and brachypterous) on weight of female *Delphacodes kuscheli* in 2013 and 2014.

Year	Effect	MS	df	F	*p*
2013	Plant zone (A)	1.76×10^{-10}	1	0.00053	0.982
	Wing morph (B)	6.36×10^{-8}	1	0.192	0.668
	A × B	2.04×10^{-7}	1	0.615	0.446
	Error	3.31×10^{-7}	14		
2014	Plant zone (A)	8.80×10^{-7}	1	2.7319	0.113
	Wing morph (B)	2.25×10^{-7}	1	0.6975	0.413
	A × B	1.31×10^{-8}	1	0.0406	0.842
	Error	3.22×10^{-7}	21		

SE = 0.46, n = 20). The difference between the body length in macropterous (= 2.85 mm, SE = 0.05, n = 22) and brachypterous (= 2.98 mm, SE = 0.06, n = 20) females was not significant. The average of YLS number per brachypterous female (= 354,250 cells, SE = 25 913, n = 20) was significantly higher than in macropterous females (= 197,329 cells, SE = 24 759, n = 22).

Bonferroni tests showed that females from the ligular zone had a shorter preimaginal development, and the lowest value was registered for brachypterous morph (Figure 3(*A*)). Body lengths (Figure 3(*B*)) and wing lengths (Figure 3(*C*) were similar in females from both plant zones and brachypterous females from the ligular zone had highest mean numbers of YLS/ind (Figure 3(*D*)).

In macropterous males, there was a significant effect of plant zone on development time, body length, wing length, and YLS number. Bonferroni tests showed that body and wing length were longer in individuals from the ligular zone than from the leaf apex (Figure 3(F, G)). There were non-significant differences between mean development time and mean YLS number in both groups ($p > 0.05$) (Figure 3(E, H)).

Weight

Weight was analyzed by year due to the fact that this response varied between the experiments carried out during 2013 and 2014.

In females, in both years, the effect of plant zone and wing morph on weight was not significant, neither the plant zone by wing morph interaction (Table 4). Different trends were observed in 2013 and 2014. In 2014, mean weight of females from the ligular zone was higher than females from the leaf apex, and brachypterous individuals were heavier than macropterous; this trend was not observed the previous year.

In males, in both years, there were non-significant differences between mean weights of individuals from the leaf apex and from the ligular zone (2013: leaf apex = 0.48 mg; ligular zone = 0.533 mg ($t = 0.573$, 6 df, $p = 0.588$); 2014: leaf apex = 0.480 mg; ligular zone = 0.533 mg ($t = 1.677$, 20 df, $p = 0.109$).

YLS and body size

Relationship between response variables

In females of both morphs a significant positive relation was observed between the number of YLS and the body length and weight. These relationships can be expressed as follows: Macropterous females: YLS = −826854 + 359663 body length, $r^2_{adj} = 0.56$, $p < 0.001$; YLS = −37994 + 2×10^8 weight, $r^2_{adj} = 0.46$, $p < 0.001$. Brachypterous females: YLS = −465847 + 275200 body length, $r^2_{adj} = 0.37$, $p = 0.002$; YLS = 223861 + 8×10^7 weight, $r^2_{adj} = 0.28$, $p = 0.009$.

Discussion

Demographics traits in relation to food quality

We found that feeding of *D. kuscheli* from first to fifth nymphal instar on the ligular zone of oat resulted in a higher survivorship, a shorter duration of development time and increased the percentage of brachypterous individuals. In addition, females tended to be larger and males exhibited a significant increase in their body size. These results indicate that plant food quality increase the fitness of *D. kuscheli*. Similar results were reported by Brentassi et al. (2014b) for *D. kuscheli* feeding on wheat plants. It has been widely reported that planthoppers had higher fitness on nitrogen-enriched host plants. Lu et al. (2004) showed that the delphacid *N. lugens* increased its reproductive potential on fertilized rice plants, and similarly Wang et al. (2006) found that *Peregrinus maidis* (Ashmead) growing on plants with high nitrogen concentrations had a significantly shorter development time, higher immature survival rate and adult longevity, and an increase of intrinsic rate of population growth. Thus, we demonstrate that some fitness traits are related to

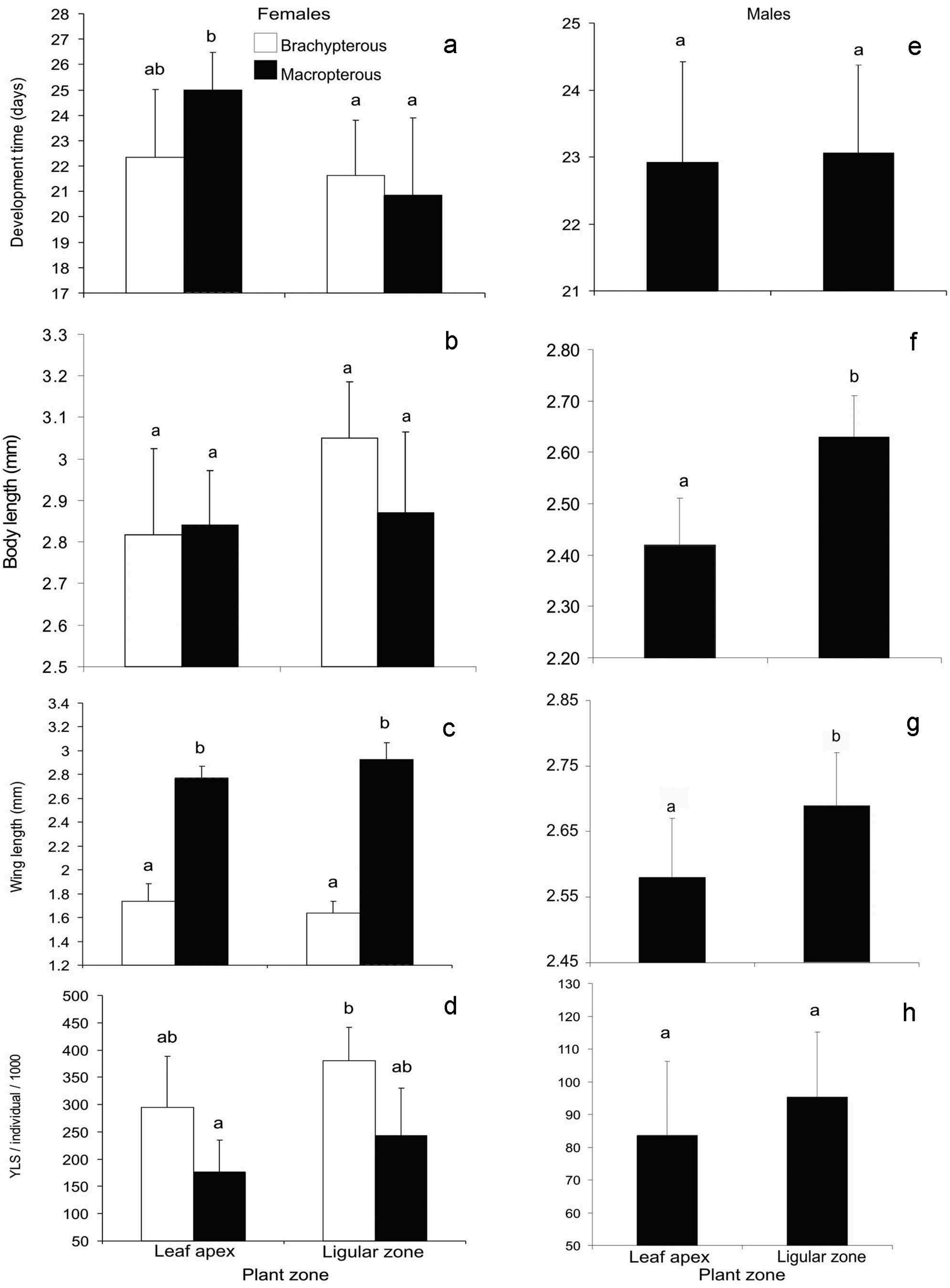

Figure 3. Comparison of mean (+ 95% CI) development time, body length, wing length, and number of yeast-like symbionts between brachypterous and macropterous females (A to D) and males (E to H) of *Delphacodes kuscheli*, fed on the leaf apex or the ligular zone of oat. Means sharing the same letter are not significantly different after Bonferroni test.

within-plant variability besides among-plants variability in resource quality.

Regarding wing polymorphism, we found that food quality affects the proportion of wing morphs in *D. kuscheli* as well. In natural populations macropters are the predominant morph; however, during spring when *D. kuscheli* population increases, brachypters (females and males) were mainly detected on oat and other winter cereals and it was suggested that food quality, particularly the nitrogen content, could favor this wing morph in *D. kuscheli* (Remes Lenicov et al. 1991; Boito & Ornaghi 2008; Ornaghi et al. 2011; Brentassi et al. 2014b). Denno and Roderick (1990) mentioned that during spring, in multivoltine species belonging to the genus *Delphacodes*, there is a correlation between the populations of adults and maximum levels of nitrogen in their host and also suggested that food quality can affect the proportion of wing morphs. Zhang (1983) reported that nutritional factors such as total amounts of nitrogen and free amino acids in the leaf sheaths where the young nymphs of *N. lugens* predominantly feed were of primary importance as factors that influence wing morph. Denno et al. (1985) argued that in *Prokelisia* species, as in most delphacids, production of macropterous individuals is influenced by inadequate nutrition due to nitrogen deficiency, age, senescence or wilting of the host plant.

Macropterous and brachypterous morphs in planthoppers show different demographic traits (Zera & Denno 1997; Rioja et al. 2010). We found that the developmental time was significantly longer in macropterous than in brachypterous females of *D. kuscheli*, suggesting that reproductive activities might be delayed in the former. Brachypterous females reproduce earlier in life (shorter pre-oviposition period) and are generally more fecund than macropterous females (Kisimoto 1965; Mochida 1973; Denno et al. 1989). In the delphacid *P. maidis*, for example, brachypterous females presented a shorter preoviposition period, a longer oviposition period and deposited on average a higher number of eggs than macropterous females (Rioja et al. 2010).

In our experiments, brachypterous males were obtained in a low percentage (one from the leaf apex and five from the ligular zone) so we were not able to test for statistical differences. Despite this, there seems to be a trend for the development of more brachypterous males on the ligular zone than in the leaf apex. Denno and Roderick (1990) stated that the advantage of brachyptery in males is less clear than in females. There is no evidence for a delay in gonadal development in macropterous males as there is in females (Mochida 1973). However, brachypterous males of *N. lugens* outcompete macropterous males for mates under certain circumstances (Ichikawa 1982). In *Prokelisia marginata* (Van Duzee), brachypterous males outlive macropterous ones (Roderick 1987). Thus, it was suggested that brachyptery is advantageous in both sexes as long as conditions remain favorable for development and mates are available (Denno et al. 1989; Zera & Denno 1997).

No differences were observed between body lengths of females feeding in both zones of oat plants; however, we observed the trend that bodies of brachypterous females were longer than those of macropterous females and that bodies of brachypterous females fed on the ligular zone were longer than those of females fed on the leaf apex. Also, we found that the weight of females fed on the ligular zone was significantly higher than that of females from the leaf apex and that brachypterous individuals were heavier than macropterous in 2014. We suspect that the difference recorded in the weight between years could be related to the genetics of the insect population or to subtle environmental differences during rearing. Denno and McCloud (1985) argued that increased body size of the delphacid *P. marginata* was directly related to a better resource quality, and a positive relationship between body size and fecundity was reported. In fact, the delphacid *Dicranotropis hamata* (Boheman) showed higher fecundity when fed on plants with higher food quality (Cook & Denno 1994).

YLS and food quality

The higher number of YLS in females than males and in brachypterous than macropterous morphs in both sexes agrees with the findings of Noda (1974) and Noda and Saito (1979) in *L. striatellus* and of Chen et al. (1981) in *N. lugens*. Noda (1974) argued that the higher number of YLS in females was related with the transovarial transmission of the YLS to the next generation. Interestingly, our findings showed that in females of both morphs a significant positive relation was observed between the number of YLS and the body size (body length and weight). Denno and McCloud (1985) mentioned that body size was in positive relation with fecundity in *P. marginata* and Xu et al. (2009) reported that in *L. striatellus* a positive correlation between daily fecundity and number of YLS occurs. According to these findings, larger individuals of *D. kuscheli* with high YLS number could lead to a higher fecundity in females and consequently to increase the fitness of this pest.

It is worth discussing in depth the association between YLS, body length and fecundity in *D. kuscheli*, and applying a more complex model that could better express the relationship among them. We used here a linear regression instead, in order to explore a general trend but, as can be inferred from the low r^2 values, nonlinear relationships may exist.

Food quality

Our study showed that plant food quality affects demographic traits, wing morphs and number of YLS in *D. kuscheli*. A significant fact is the different response exhibited by both sexes when they fed on leaf zones with different food quality. While macropterous and brachypterous females fed on the higher food quality zone allocated food resources to reduce the duration of developmental time and also to increase the number of YLS, males used food resources to increase their body size. Also, a high food quality increased the proportion of brachypterous morphs. This fact could explain the presence, in natural conditions, of brachypterous individuals during spring, especially on oat in the reproductive stage of development, as reported by Remes Lenicov et al. (1991); Trumper and Garat (2001) and Boito and Ornaghi (2008). Our findings also showed that food quality increased YLS number in females, which could indicate a better reproductive success of the pest and contribute to their establishment on host plants.

The results of this study are an important step for understanding YLS–*D. kuscheli*–host plant interactions, which may contribute to comprehending the dynamics of this pest. Further studies in field conditions are necessary to thoroughly understand how host plant food quality affects the proportion of wing form in *D. kuscheli* and to evaluate the implications on the fitness and dispersion of this pest.

Acknowledgments

We thank Dr Gerardo Liljesthröm who assisted us with valuable comments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de La Plata (UNLP) [PIP-UNLP. 2014]. Daniela de la Fuente and María E. Cano were scholarship assistants from Consejo Interuniversitario Nacional, Universidad Nacional de La Plata and Facultad de Ciencias Naturales y Museo, Universidad Nacional de la Plata respectively.

ORCID

Daniela de la Fuente http://orcid.org/0000-0002-5614-7322
María E. Cano http://orcid.org/0000-0002-6101-2246

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