ECOSYSTEMS

Influence of the site of oviposition on the level of egg parasitism in the corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae)

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Abstract: The corn leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae), transmits three important plant pathogens that adversely affect corn crop and ranges from the USA to Argentina. The vector has a rich natural enemy complex that generates high levels of parasitism, but its populations are persistent and prevalent. We characterized the oviposition sites of *D. maidis* on young corn plants in order to verify the hypothesis that the vector has an oviposition strategy for mitigating parasitism. Oviposition locations on plants were assessed in the laboratory and eggs within corn plants were exposed to natural parasitism in a cornfield. Eggs were located mostly laid in the unfolded leaves and were attacked by five parasitoid species. Parasitism was significantly affected by the class of leaf and the position of the egg in the leaf. *Anagrus virlai* Triapitsyn was the most abundant parasitoid species, which emerged significantly higher in the basal blade than other species. Our results suggest that leafhoppers minimize egg parasitism by laying their eggs within concealed locations on the plant.

Key words: *Anagrus virlai*, Cicadellidae, corn leafhopper, egg parasitoids, enemy-free space, oviposition.

INTRODUCTION

In tropical and subtropical America, the corn leafhopper *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) is the cause of enormous damage to maize (*Zea mays* L.) since it efficiently transmits three important plant pathogens that adversely affect this crop: Corn stunt spiroplasma (*Spiroplasma kunkelii* Whitcomb), Maize bushy stunt phytoplasma (*Candidatus* Phytoplasma asteris) and Maize rayado fino virus (Marafivirus) (Laguna & Gimenez Pecci 2012). In these regions, *S. kunkelii* is the most dominant pathogen affecting maize. The highest infection rates have been found in Central America, Peru, Brazil and Argentina, with 100% of the crop plants affected in many maize fields (Oliveira et al. 1998).

Dalbulus maidis presents a broad distribution throughout the USA and Argentina (Triplehorn & Nault 1985). It is a specific herbivore, feeding and reproducing only on plants of the genus *Zea* (maize and teosintes) (Bellota et al. 2013) and, while it prefers lower elevations, it can be found in a wide range from sea level to 3200 m in the Peruvian Andes (Nault 1990). Pitre (1967) indicated that the eggs are laid embedded into the plant tissues mostly along the midrib on the upper side of more fully developed leaves; Heady et al. (1985) stated that most eggs are laid in the midrib of the basal half of maize leaves; and recently Bellota et al. (2013) established that at least 99% of eggs were inserted into the midrib. These data suggest that the corn leafhopper displays an egg-laying behavior that is practically invariable. However, it is unknown

if this pattern is extended throughout the entire geographical distribution area.

The corn leafhopper has a rich parasitoid complex, and they can be divided into two guilds: those that attack eggs and those that affect the nymphs and adults. *Dalbulus maidis* has 18 known egg parasitoid species (16 of which occur in Argentina); these are members of the Chalcidoidea (Hymenoptera), belonging to Mymaridae, Trichogrammatidae, Aphelinidae, and Eulophidae (Luft Albarracin et al. 2017). Mortality caused by egg parasitoids is a key factor in several Auchenorrhyncha populations (Denno & Roderick 1990, Böll & Herrmann 2004). Previous studies of the impact of egg parasitoids on *D. maidis* demonstrated high levels of parasitism, with mean values exceeding 50% (Luft Albarracin et al. 2017). However, vector populations in maize fields present an elevated density (Bushing & Burton 1974, Luft Albarracin et al. 2008, Meneses et al. 2016).

The importance of an enemy free-space to insect herbivores is widely recognized (Atsatt 1981, Jeffries & Lawton 1984, Raymond et al. 2016). Likewise, there is a correlation between oviposition preference and offspring performance, with other phytophagous insects and Auchenorrhyncha in general (Cook & Denno 1994). Specifically for invertebrate herbivores, the habitat patch is represented by the host plant (Vanbergen et al. 2007), which can therefore avoid parasitism through a preference for oviposition sites in which parasitoids are rare or absent (Quicke 1997, Williams et al. 2012). Host use varies during the life of conspecific herbivorous insects (Jaenike 1990). Moreover, preferences for oviposition within a host plant could differ according to age and plant size, given variation in the chemical and physical properties of the plant tissues (Thompson 1988, Williams et al. 2001).

The different sectors of the leaves on the plant could be considered a "patch" and invertebrate herbivores therefore make decisions regarding which resource patch to visit and how long to stay there for oviposition. These are central issues for foraging animals since they affect the rate at which they encounter resources and thus their reproductive success. In this way, for parasitoids, encountering a host is directly related to the fitness of an individual, and decisions leading to patch and host selection are made under strong selective pressure (Castelo et al. 2003, Williams et al. 2012).

Dalbulus maidis may present a strategy that allows it to escape from the selective pressure of egg parasitoids by placing a proportion of its eggs in concealed sites. In this case, the vector oviposits in different parts of the plant, but parasitoids are less likely to locate eggs deposited in certain sites and therefore the proportion of eggs affected by parasitoids should differ in different sectors of the plant. From a biological control perspective, different host location strategies may have strong effects in shaping levels of parasitism (Peri et al. 2011).

Knowledge of the interactions between an insect pest and its host-plant and parasitoids is essential for the development of effective management strategies. The aims of this study are therefore to characterize the oviposition sites of *D. maidis* in young maize plants and to verify the hypothesis that this leafhopper presents an escape strategy for mitigating parasitoid attacks.

MATERIALS AND METHODS

Origin of the *Dalbulus maidis* specimens and laboratory rearing

A *D. maidis* colony was established using individuals collected during the summer in Los Nogales, Tucumán, Argentina (S 26° 42', W 65°

13', elevation 588 m). The vector was placed in aluminum breeding cages (50 x 50 x 50 cm) with the lateral and upper sides covered with nylon mesh (organdy type) for ventilation. Potted maize plants (each pot of 6.3 dm^3) were placed inside as a food source and for oviposition. The maize variety "Leales 25 plus" (INTA 2016) was used both for maintenance of the corn leafhopper colony and for all of the assays. The colony was reared in a greenhouse in San Miguel de Tucumán (S 26° 48', W 65° 14', elevation 500 m) under the following conditions that reflect the natural field conditions for the leafhopper (23 to 30 °C, natural photoperiod and no humidity control).

Location of eggs and its relationship with the plant architecture

In order to avoid corn leafhopper eggs misidentification, because there are other leafhoppers species using corn as host plant (Luft Albarracin et al. 2008), we infested plants into the laboratory and after that exposed them as sentinel eggs following the method described by Luft Albarracin et al. (2017). So, to obtain eggs and characterize the location of the eggs within the plant architecture, potted maize plants in vegetative stage were used (V$_3$ and V$_4$ phenological stages with three and four unfolded leaves, respectively). In the laboratory, each plant was individually exposed for 24 h to 4-6 females of *D. maidis* in cages (30 x 30 x 50 cm) covered with nylon mesh. The plants were then checked for eggs and the location of oviposition recorded. This procedure of exposition was repeated 18 times. This assay was conducted at 25 ± 4 °C, 70-80% RH and natural photoperiod. The location of eggs on the plants was considered as follows: 1) between leaves: fully unfolded leaves (with leaf collar) and those with no visible collar forming the whorl; 2) within leaves: leaf sheath or blade; and

3) within the leaf blade: basal and apical region, including eggs laid in the midrib or those in the blade (Fig. 1).

Natural egg parasitism

The potted plants containing eggs of *D. maidis* were exposed to parasitization in a maize field at the site "El Manantial" (Tucumán Province, Argentina: S 26° 49' 50.2'', W 65° 16' 59.4'', elevation 495 m). These sentinel eggs were placed inside the maize field at no more than 3 m from the edge of the field. After eight days of field exposure, plants were transferred to the laboratory. The different parts were cut from each plant, labeled and placed in Petri dishes with wet tissue paper on the bottom and covered with a polyethylene film to avoid desiccation of eggs and plant tissues and to contain any emerging nymphs and/or wasps. The eggs were checked daily to ensure host plant quality until the emergence of all nymphs and/or adult wasps, in cases where the eggs were parasitized. The parasitoid specimens were preserved in 70% ethanol and later slide-mounted in Canada balsam following established standard practices (Noyes 1982). The parasitoids were identified using available specific keys (Triapitsyn 2015, Viggiani 1981). Voucher specimens were deposited in the entomological collection of the Instituto Fundación "Miguel Lillo", San Miguel de Tucumán, Argentina (IMLA).

Statistical analysis

Firstly, we show a general description of the number of eggs laid in the three different parts of the leaves (sheath, blade, or midrib), discriminated by plant class (V$_3$, V$_4$). Secondly, we used generalized linear mixed models (GLMM) (Bolker et al. 2008, Zuur et al. 2009) to determine the effects of leaf class and position in the leaf on the number of eggs, parasitism and proportion of emergence of parasitoids. We
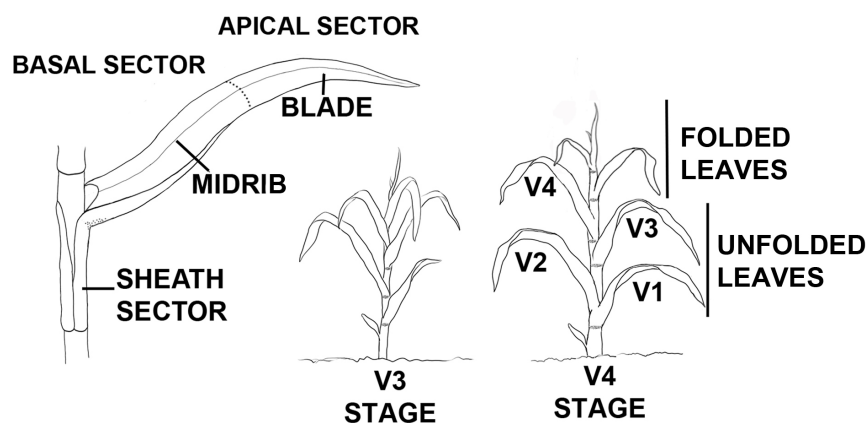
Figure 1. Schematic representation of parts of the maize plant considered in the analysis of egg location. V3-V4: plants with three or four fully unfolded leaves, respectively.

refer to parasitism as the number of eggs with parasitism over the number of eggs, taking values between 0 and 1. The fixed effects were leaf class (folded and unfolded leaves) and position in the leaf (sheath, basal or apical blade, basal or apical midrib). Eggs were not detected in the sheath of folded leaves; therefore, the level sheath was removed from all of the analyses (lack of variation leads to overestimation of the effects of the interaction between the factors position in the leaf and leaf class). The random effects were plant (experimental individual) and plant class (V_3 and V_4). These random effects were considered to allow random variation of the intercept between plant classes (V_3 and V_4), and random variation of the intercept between plants within each plant class (plant nested in plant class, see *Location of eggs and its relationship with the plant architecture* in Material and Methods).

We modeled the variation in number of eggs, parasitism, and proportion of emergence of parasitoids, according to the requirements of the error structure in each case. To model variation in the number of eggs, we used a zero-inflated mixed model with negative binomial error structure and log link function. To model variation in parasitism, we used a mixed model with Gaussian error structure and identity link function. We considered a second model with

the same above structure but in which the number of exposed eggs was considered as an offset, because the available number of eggs affects the probability of parasitism. To model the variation in wasp emergence, the available data corresponded to V4 plants with unfolded leaves. Therefore, in this case the only fixed effect considered was the position of the eggs in the leaf. Since *Anagrus virlai* Triapitsyn was the dominant parasitoid species (74% of the emergence represented), we considered a model with emergence of *A. virlai* as a response variable, and the proportion of emergence of other species as an offset variable. We also constructed the inverse model, in which the emergence of other wasp species was the response variable and the emergence of *A. virlai* was considered as an offset. Comparisons between groups were done with Tukey-HSD tests. All analyses and graphs were conducted in R 3.5.1 (R Development Core Team 2018), including the specific packages ggplot2 (Wickham 2016), glmmTMB (Brooks et al. 2017) and sjPlot (Lüdecke 2018). The database and R script are available upon request.

RESULTS

Oviposition sites of *Dalbulus maidis*

We obtained 187 *D. maidis* eggs in the V_3 plants; 20 (10.7%) located in the sheaths, 53 (28.3%) on

the midrib and 114 (61.0%) in the leaf blades. A total of 495 eggs were found in the V$_4$ plants; 46 (9.3%) located in the sheath, 198 (40.0%) in the midrib and 251 (50.7%) in the leaf blade. Eggs laid in the blades represented the highest proportion in the V3 plants (Table I).

When the location of eggs in different sectors of the plant was considered, more eggs were found to have been laid in the apical blade of V$_3$ plants (mean 1.02), followed by the basal midrib sector (mean 0.65), both in unfolded leaves. However, in the V$_4$ plants, eggs were mostly laid in the basal midrib sector (mean 2.47), followed by the apical blade sector (mean 2.13), also in unfolded leaves (Table II).

The number of eggs was significantly higher in unfolded leaves, and this effect was significantly positive in the basal sector and of higher magnitude in the midrib. The number of eggs was significantly affected by both leaf class and position, and by the interaction between leaf class and position (Fig. 2a; Supplementary Material - Table SI).

Parasitoid attacks

From the field-exposed eggs, we obtained 194 adult parasitoids belonging to five species, the Mymaridae *Anagrus virlai* (144 specimens = 74.2%), *A. flaveolus* Waterhouse (24 specimens = 12.4%) and *A. nigriventris* Girault (4 specimens = 2.1%), and the Trichogrammatidae *Paracentrobia tapajosae* Viggiani (15 specimens = 7.7%) and *Pseudoligosita longifrangiata* (Viggiani) (7 specimens = 3.6%).

As with the number of eggs, parasitism was significantly affected by leaf class, position on the leaf, and the interaction between leaf class and position (Fig. 2b; Table SII). During the experimental test (see Materials and Methods), parasitoids had the same opportunities to attack eggs laid throughout the plant, however results showed the highest parasitism on unfolded

leaves, though this effect was significantly negative for those eggs laid on the apical midrib (Fig. 2b). This indicates that the incidence of parasitism in the apical midrib was lower than expected (Fig. 2b). The parasitism was lower than expected in the basal midrib when we considered the number of exposed eggs as an offset (Fig. 2b; Table SIII).

In the unfolded leaves of V$_4$ plants, we found that the proportion of parasitoid emergence was positively and significantly affected by the position in the leaf (Fig. 2c; Table SIV). The apical midrib showed the significantly lowest emergence. However, all positions in the leaf had a negative significant effect on emergence when we considered the parasitism as an offset in the model (Fig. 2c; Table SV). The magnitude of this effect was 1.5 times higher in the apical midrib than in the basal blade. This result reveals that emergence differs among different positions in the leaf.

The emergence of *A. virlai*, the most abundant egg parasitoid, was significantly affected by its position in the leaf (Fig. 2d; Table SVI). The emergence of *A. virlai* was significantly lower in the apical midrib (mean 0.018). However, the model revealed that emergence in *A. virlai* was significantly higher in the basal blade (mean 0.115) when we considered the emergence of other wasp species as an offset in the model (Fig. 2d; Table SVII). Taking into account the number of eggs as an offset in the model, the magnitude of effects was similar to the model without the number of eggs as an offset.

The proportion of emergence of other wasp species was zero in the basal blade, where *A. virlai* was dominant. The emergence of other wasp species was significantly affected by the position of the egg in the leaf (Fig. 2e; Table SVIII). This effect was significant only for the basal blade when the emergence of *A. virlai* was considered as an offset in the model (Fig. 2e;

Table I. Location of *Dalbulus maidis* eggs in different parts of the maize leaves, data of total numbers (n), percentage (%) mean and standard deviation (SD) are presented. V_3 – V_4: plants with three or four fully unfolded leaves, respectively. Significant differences between plant classes within each leaf sector location are indicated by an asterisk; significant differences between leaf sectors within each plant class location are indicated by a different letter (Tukey HSD test; $p < 0.05$). Note that percentages indicated within brackets are added within each plant class or total and therefore they must be read by file.

	Sheath		Midrib		Blades	
	n (%)	Mean eggs/ sample ± SD	n (%)	Mean eggs/ sample ± SD	n (%)	Mean eggs/sample ± SD
V_3	20 (10.7)	0.19 ± 0.6 a	53 (28.3)	0.25 ± 0.7 a *	114 (61.0)	0.53 ± 1.1 b
V_4	46 (9.3)	0.28 ± 1.2 a	198 (40.0)	0.61 ± 2.5 ab *	251 (50.7)	0.77 ± 1.8 b
Total	66 (9.7)	0.24 ± 0.9	251 (36.8)	0.46 ± 1.9	365 (53.5)	0.68 ± 1.6

Table SIX). Thus, variation in the emergence of the different wasp species did not depend on the distribution of eggs in the leaf. These results suggest the spatial segregation and/or higher competing abilities of *A. virlai* wasps.

DISCUSSION

The results of the present study support the hypothesis that *D. maidis* employs strategies to escape the egg parasitoids. It mitigates the risk of oviposition (as mortality due to egg parasitoids) by laying eggs in specific places within the corn plant, like in the apical blade or in the basal midrib of unfolded leaves in V3 and V4 plants, respectively. Sites with the highest number of eggs are expected to have the highest level of parasitism but it was not true for those laid in the basal midrib of V4 plants. This could constitute a "bet-hedging" strategy, which may promote adaptation to high levels of parasitism. Moreover, competition or segregation processes among wasp species may contribute to offering windows of opportunity for improved performance of the *D. maidis* escape strategy, in order to fight against high levels of parasitism.

In general, herbivorous insects oviposit where offspring performance is high; however,

in several species like the planthoppers (Delphacidae, a related group of Cicadellidae), factors such as host plant abundance and the presence of natural enemies may influence the relationship between oviposition preference and offspring performance (Cook & Denno 1994). They may avoid parasitism by preferentially selecting oviposition sites where the risk of parasitism may be lower: "enemy-free spaces" where vulnerability to one or more natural enemy species is reduced or eliminated (Quicke 1997, Jeffries & Lawton 1984, Williams et al. 2012). The choice of oviposition site may also result from selective pressures exerted by natural enemies; for example, *Anagrus atomus* (L.) selectively oviposits in the eggs of *Javesella pellucida* (F.) (Delphacidae), deposited in leaf blades (53% of 1377 eggs parasitized) rather than those inserted into stems (0% of 17189 eggs parasitized) (Denno 1994).

The ability of parasitoids to locate and attack their hosts is a result of successive behavioral steps regulated by physical, chemical and biochemical factors (Vinson 1985, Colazza et al. 2004, 2014). For parasitoids that affect Cicadellidae eggs, there are factors that present particular challenges to host selection: a) the eggs, small in size, are endophytic and relatively

Table II. Descriptive statistics for the *Dalbulus maidis* dataset, according to the maize plant class, the leaf class and the position on the leaf. Data on "eggs" refer to the mean proportion of eggs/sector/plant; data on "parasitoids" refer to the proportion of emerged adult parasitoids; data on "parasitism" refer to the proportion of attacked eggs. Note: standard deviation is given within parentheses and the highest proportions are marked in bold. Significant mean differences between plant classes within each leaf class and sector location are indicated by an asterisk; significant differences between leaf class and sector location within each plant class are indicated by a different letter (Tukey HSD test; $p < 0.05$).

Measurement	Response variable	Leaf class	V_3					V_4				
			apical blade	basal blade	apical midrib	basal midrib	Sheath	apical blade	basal blade	apical midrib	basal midrib	sheath
n	Eggs	folded	54	54	54	54	54	90	90	90	90	90
		unfolded	54	54	54	54	54	72	72	72	72	72
Proportion of positive values		folded	0.24 (0.43)	0.06 (0.23)	0.02 (0.14)	0.06 (0.23)	0 (0)	0.12 (0.33)	0.02 (0.15)	0 (0)	0.01 (0.11)	0 (0)
		unfolded	0.54 (0.50)	0.37 (0.49)	0.11 (0.32)	0.35 (0.48)	0.20 (0.41)	0.65 (0.48)	0.44 (0.50)	0.11 (0.32)	0.43 (0.50)	0.18 (0.39)
	Parasitoids	folded	0.07 (0.26)	0.06 (0.23)	0 (0)	0 (0)	0 (0)	0.08 (0.27)	0 (0)	0 (0)	0 (0)	0 (0)
		unfolded	0.44 (0.50)	0.30 (0.46)	0.07 (0.26)	0.30 (0.46)	0.19 (0.39)	0.51 (0.50)	0.38 (0.49)	0.10 (0.30)	0.38 (0.49)	0.11 (0.32)
Mean	Eggs	folded	0.43a* (0.90)	0.09 (0.45)	0.04 (0.27)	0.11a (0.50)	0 (0)	0.19a* (0.56)	0.03a (0.23)	0 (0)	0.02a (0.21)	0 (0)
		unfolded	**1.02**b (1.41)	0.57 (1.04)	0.19 (0.65)	0.65b* (1.01)	0.37 (0.83)	2.13b (2.91)	1.08b (1.85)	0.25 (0.88)	**2.47**b* (4.79)	0.64 (1.68)
	Parasitoids	folded	0.15a (0.60)	0.09 (0.45)	0 (0)	0a (0)	0 (0)	0.11a (0.44)	0a (0)	0 (0)	0a (0)	0 (0)
		unfolded	**0.83**b (1.36)	0.46 (0.91)	0.07 (0.26)	0.48b* (0.82)	0.33 (0.80)	1.36b (2.08)	0.74b (1.29)	0.19 (0.66)	**1.63**b* (3.84)	0.29 (0.98)
	Parasitism	folded	0.07a (0.26)	0.06a (0.23)	0 (0)	0a (0)	0 (0)	0.07a (0.25)	0a (0)	0 (0)	0a (0)	0 (0)
		unfolded	**0.42**b (0.49)	0.29b (0.45)	0.07 (0.26)	0.27b (0.43)	0.18 (0.38)	**0.41**b (0.44)	0.32b (0.44)	0.09 (0.27)	0.27b (0.39)	0.07 (0.20)

hidden from the antagonists; b) they are available in the host plant for a short time and the quality of the eggs as a resource decreases with time. It has been shown that successful parasitism of eggs by various egg parasitoids declines as the host ages, and that well-developed eggs produce almost no parasitoid offspring (Cronin & Strong 1994, Al-Wahaibi & Walker 2000). Increasing plant architectural complexity can reduce parasitoid foraging efficiency and thus the suppression of herbivores (Andow & Prokrym 1990, Williams et al. 2012). It could therefore affect insect abundance through changes in parasitoid foraging behavior (Guiti Prado & Frank 2013). In several studies, host-finding success in parasitoids was found to be higher in simple structured plants than in those with complex structures (Andow & Prokrym 1990, Lukianchuk & Smith 1997, Gingras et al. 2003, Williams et al. 2012).

In our study, we recorded that certain parts of the plants that hold a high number of eggs

were not as parasitized as expected. Cronin & Strong (1994) analyzed the relationship between Auchenorrhyncha species and its parasitoids and stated that there is a preponderance of cases of spatial density independence (74%); such density-independent relationships are characterized by high variance in the parasitism rate per host patch, which is considered to be a powerfully stabilizing force. Finally, these authors affirm that as long as the spatial distribution of parasitism is clumped (heterogeneously), a proportion of the population will be relatively

free of attack, producing population stability and persistence.

For females of the specialized corn leafhopper, considering that maize is a seasonal crop in which almost all of the host plants present the same stage of development (a homogeneous environment), heterogeneity in the "corn leafhopper-egg parasitoid" relationship would be mainly produced by the different parts of the plant. The way in which host eggs are exploited after they are found will affect the impact of the parasitoid on the host population (Collins & Grafius 1986) and therefore oviposition behavior
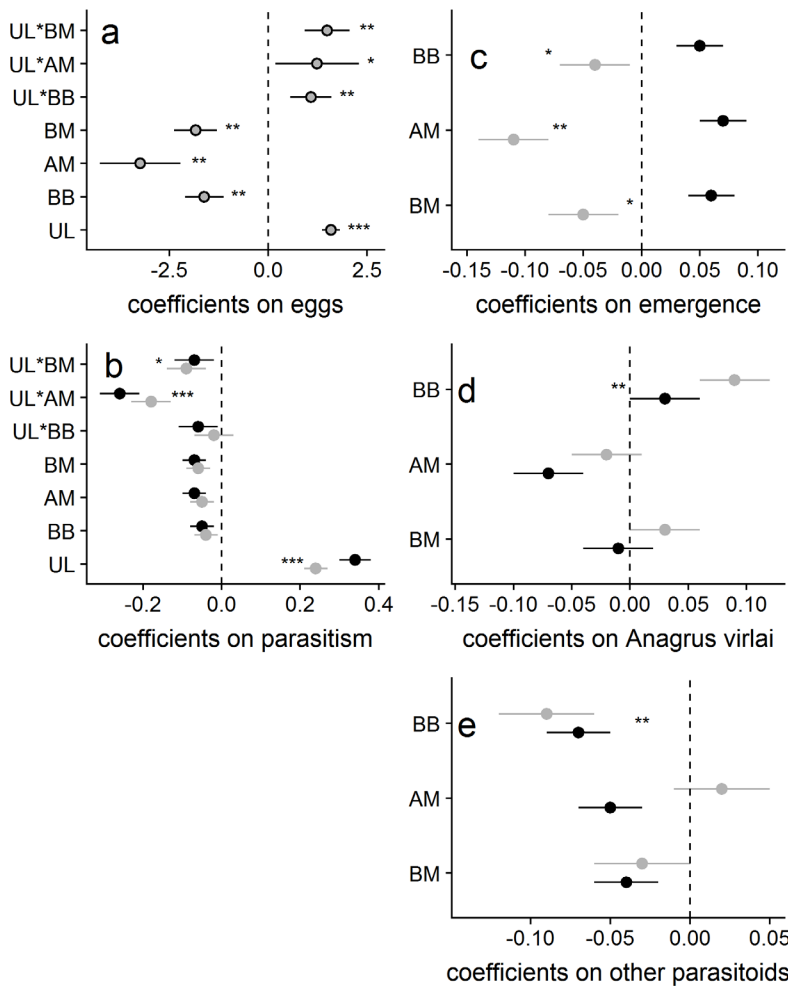

Figure 2. a Effect estimates for a zero-inflated negative binomial model of the number of *Dalbulus maidis* eggs. b Effect estimates for a Gaussian model on parasitism (black points) accounting for the number of exposed *Dalbulus maidis* eggs (gray points). c Effect estimates for a Gaussian model on the proportion of emergence of parasitoids (black points) taking into account the number of parasitoids of *Dalbulus maidis* (as offset) (gray points) in unfolded leaves of V4 plants. d Effect estimates for a Gaussian model on the proportion of emergence of *Anagrus virlai* (black points) accounting for the proportion of emergence of other wasp species (gray points) in unfolded leaves of V4 plants. e Effect estimates for a Gaussian model on the proportion of emergence of other wasp species (black points) accounting for the proportion of emergence of *Anagrus virlai* (gray points) in unfolded leaves of V4 plants. BM: Basal midrib; AM: Apical midrib; BB: Basal blade; UL: unfolded leaves. Points depict the coefficient value; the whisker depicts the standard error. Significant differences are indicated by asterisks (* p < 0.05; ** p < 0.01; *** p < 0.001). Detailed statistics are shown in Supplementary material.

is very important (Al-Wahaibi & Walker 2000, Agboka et al. 2003). Most species of *Anagrus* Haliday move and oviposit from one egg of *Nilaparvata lugens* (Stål) to the next (Denno 1994); however, females of *Anagrus delicatus* Dozier deposit only a small fraction of their eggs in a clutch of *Prokelisia marginata* (Van Duzze) before dispersing (Cronin & Strong 1990). Like *A. delicatus*, *A. virlai* oviposits in a few eggs that are in very close proximity, and then searches for more eggs in another site of the plant (Virla E.G. & Luft Albarracin E., unpublished data), so the existence of an important aggregation of eggs in a particular sector of the plant (i.e., the apical blade of unfolded leaves in V3 plants or the basal sector of the midrib in V4 plants) increased the opportunity of a partial escape from parasitism.

Rates of emergence of the corn leafhopper egg parasitoid varied among different positions in the leaf; the apical midrib showed the lowest emergence. In the literature, there is no evidence of the potential effect of density-independent factors (such as insolation or dehydration) increasing the mortality of larvae of endophytic egg parasitoids when they are located in exposed sectors of plants. In contrast, mortality caused by natural enemies (i.e. predators) has been extensively explored by several authors, especially in planthoppers (Cook & Perfect 1989, Denno & Roderick 1990).

Our results suggest that the emergence of the mymarid *A. virlai* was affected by the position of the egg in the leaf. Moreover, on comparison of the emergence rates of all species of parasitoids, the level of emergence of *A. virlai* was higher in the basal blade, whereas for other wasp species this was zero. Interspecific competition among developing larvae of different species could occur in the field. Chiappini et al. (2015) demonstrated that *A. virlai* (identified as *A. incarnatus*) is a facultative gregarious parasitoid

in larger Cicadellidae eggs while, in smaller eggs, only a single individual develops per egg. This behavior seems to be determined by a competition between the larvae that hatch from the eggs, often supernumerary within a single host, rather than the result of decision-making of the ovipositing female.

Dalbulus maidis has an important egg parasitoid complex (Luft Albarracin et al. 2017), but the level of specificity of these species is unknown in depth. *Anagrus virlai* is widely distributed in South America and has a wide range of hosts (mainly Cicadellidae and Delphacidae) (Triapitsyn et al. 2018). In this sense, Triapitsyn et al. (2018) suggest that the interaction between *A. virlai* and their widespread and main host, *D. maidis*, might constitute a co-evolutionary process (i.e., reciprocally affected fitness). Our results support this premise, requiring a deeper research.

Regarding corn leafhopper endophytic oviposition, the results allow us to state that most eggs were located within the blade tissues of the leaves. We therefore have evidence that the oviposition behavior of studied population differs slightly from that described in previous studies carried out with North American populations. When we analyzed the data at a deeper level [i.e. folded vs. unfolded leaves and distribution in different sectors (blade vs. midrib and position with respect to the stem: apical vs. distal)], the unfolded leaves hosted more eggs, with a higher number located in the basal portion of the leaves, particularly in the midrib. Previous studies demonstrated that *D. maidis* eggs are embedded mostly along the midrib (Pitre 1967, Heady et al. 1985, Bellota et al. 2013). The fact that, in plants with lower phenological development (V3), a higher proportion of eggs were found in the blades could be due to the reduced supply of well-developed midribs. The possibility that the observed differences

may be due to over exposure of plants to the vector was discounted because we used lower densities and shorter exposure times [Pitre (1967) exposed 5 females during 12 days; Heady et al. (1985) exposed plants to 20 females for 48 h, Bellota et al. (2013) used 10 females for 60 h]. The used maize germplasm was discounted as a factor because Bellota et al. (2013) and others demonstrated that *D. maidis* behavior is similar in landraces and in maize hybrids. The observed differences may be attributed to differential behavior: the studied population, from the southernmost distribution of the species, displayed behavior that differed from that of the North American populations. A previous study showed that *D. maidis* in Mexico consists of at least two genetically distinct sub-populations (Medina et al. 2012), and that some populations from the northeastern and central-southern regions of Brazil differ morphologically, with one appearing to be genetically isolated (Oliveira et al. 2007). The next step should be to compare the oviposition behavior of a Mexican and an Argentinean population and to verify whether the vector presents a similar escape strategy in its center of origin.

This work contributes to a better understanding of egg parasitoids and their relation to the population growth of the corn leafhopper, the effects on vector ecology and plant diseases, since they can act to prevent the hatching of eggs thus disrupting the epidemiological cycle (Ballal 2013). In addition, this information should be of value for developing of biological control programs.

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SUPPLEMENTARY MATERIAL

Table SI. Summary of the generalized linear mixed model of the effects of leaf class (folded, unfolded) and leaf sector (basal blade, apical blade, basal midrib, apical midrib) on the number of eggs oviposited by *Dalbulus maidis* on experimental corn plants. The model accounted for random variation between plants within each plant class (plant nested in plant class). se: standard error; ICC: intra-class correlation.

Table SII. Summary of the generalized linear mixed model of the effects of leaf class (folded, unfolded) and leaf sector (basal blade, apical blade, basal midrib, apical midrib) on the number of parasitoids of *Dalbulus maidis* on experimental corn plants. The model accounted for random variation between plants within each plant class (plant nested in plant class). se: standard error; ICC: intra-class correlation.

Table SIII. Summary of the generalized linear mixed model of the effects of leaf class (folded, unfolded) and leaf sector (basal blade, apical blade, basal midrib, apical midrib) on the number of parasitoids taking into account the number of eggs of *Dalbulus maidis* (offset) on experimental corn plants. The model accounted for random variation between plants within each plant class (plant nested in plant class). se: standard error; ICC: intra-class correlation.

Table SIV. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical

blade, basal midrib, apical midrib) in V4 plants on the emergence of parasitoids on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation.

Table SV. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical blade, basal midrib, apical midrib) in V4 plants on the emergence of parasitoids taking into account the number of parasitoids of *Dalbulus maidis* (offset) on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation.

Table SVI. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical blade, basal midrib, apical midrib) in V4 plants on the emergence of *Anagrus virlai* parasitoids on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation

Table SVII. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical blade, basal midrib, apical midrib) in V4 plants on the emergence of *Anagrus virlai* parasitoids taking into account the emergence of other parasitoids (offset) on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation.

Table SVIII. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical blade, basal midrib, apical midrib) in V4 plants on the emergence of other parasitoids (not *Anagrus virlai*) on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation.

Table SIX. Summary of the generalized linear mixed model of the effects of leaf sector (basal blade, apical blade, basal midrib, apical midrib) in V4 plants on the emergence of other parasitoid species taking into account the emergence of *Anagrus virlai* parasitoids (offset) on experimental corn plants. The model accounted for random variation between plants. se: standard error; ICC: intra-class correlation.

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