Egg Parasitoids of *Dalbulus maidis* (Hemiptera: Cicadellidae) Within Maize Agroecosystems and in the Edge Zones of Maize Fields, and on Maize Varieties During the Wet Season in Mexico

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Abstract

Little is known about *Dalbulus maidis* (DeLong) egg parasitoids within maize fields, in the edge zones that surround these fields, and the parasitism on *D. maidis* eggs oviposited on different maize varieties. The objectives of the present study were first to understand which egg parasitoid species attack *D. maidis* eggs within maize fields and in the surrounding edge zones, and second, to compare parasitism on two maize varieties (land race Ancho-pozolero and hybrid Tigre-Asgrow) during the maize-growing wet season. We used maize plants with sentinel eggs to attract the egg parasitoids in two consecutive wet seasons, in 2015 and 2016. In 2015, *Anagrus* sp. (Hymenoptera: Mymaridae) and *Paracentrobia* sp. (Hymenoptera: Trichogrammatidae) parasitized *D. maidis* eggs within the maize field and on its edges. However, much more parasitism was seen within the maize agroecosystem than in the maize edge zones. In 2016, two Mymaridae species, *Anagrus columbi* Perkins and *Anagrus* sp, and two Trichogrammatidae genera, *Pseudoligosita* sp. and *Paracentrobia* sp., attacked the *D. maidis* eggs laid on maize hybrids but not those oviposited on the maize land race. Our findings indicate that parasitism of corn leafhopper eggs differs with agroecosystem location and maize varieties.

Key words: Mymaridae, Trichogrammatidae, biological control, crop protection, Hymenoptera

The corn leafhopper *Dalbulus maidis* (DeLong) is one of the most important insect pests in the Americas (Nault 1990), because nymphs and adults of this specialist pest of maize are efficient vectors of plant pathogens of maize (Nault 1980). Maize in tropical America is generally cultivated annually, taking advantage of rainfall during the wet season, and it is in this season that the corn leafhopper population reaches its highest numbers (Moya-Raygoza et al. 2007). Therefore, it would stand to reason that the corn leafhopper and its egg parasitoids should be adapted to survive and reproduce in this seasonal maize habitat. In seasonal crops, edge zones surrounding the fields are important because the flowering plants growing in these zones are a source of nectar and pollen that help to maintain the parasitoid population, particularly when the crop is absent (English-Loeb et al. 2003).

At the onset of the rainy/wet season, corn leafhopper adults colonize maize seedlings and develop two consecutive generations, reaching a great abundance within the maize field during the second generation (Todd et al. 1991). Nymphs and adults of *D. maidis* are parasitized by (Hymenoptera: Dryinidae) (Moya-Raygoza and Trujillo-Arriaga 1993), (Diptera: Pipunculidae) (Virla et al. 2009a), and (Strepsiptera: Halictophagidae) species (Kathirithamby and Moya-Raygoza 2000). However, most of the parasitism during the wet season occurs by hymenopterans that attack the eggs of the corn leafhopper, reaching a parasitism level of 90% in some maize fields (Moya-Raygoza et al. 2014). Hymenopterans are therefore very important for the biological control of *D. maidis*. These wasps are composed of a community of Mymaridae and Trichogrammatidae species (Virla et al. 2009b; Moya-Raygoza et al. 2012, 2014), and one species of Aphelinidae and one species of Eulophidae (Luft Albarracin et al. 2017). In contrast, *D. maidis* adults are scarce in grasses that inhabit the maize edge zones during the wet season (Pinedo-Escatél and Moya-Raygoza 2018), and no study has investigated the presence of hymenopterans that attack corn leafhopper eggs in maize edge grasses during this season.

At the onset of the winter season, cultivated maize plants start to dry out, and *D. maidis* adults begin to disperse locally or perform long migrations (Oliveira et al. 2013). In central Mexico, corn leafhopper adults disperse temporarily from November to January to green grasses that inhabit the maize field edges (Pinedo-Escatél and Moya-Raygoza 2015) or move to volunteer maize, uncultivated...
maize found during winter in several countries of the Americas (Summers et al. 2004, Carloni et al. 2013, Oliveira et al. 2013). Edge grasses and volunteer maize are overwinter habitats for *D. maidis* egg parasitoids such as Mymaridae and Trichogrammatidae species (Moya-Raygoza and Becerra-Chiron 2014).

Mymaridae and Trichogrammatidae are mobile, and readily adapt to different habitats (Antolin and Strong 1987, Fournier and Boivin 2000, Gurr et al. 2011). The adults of these wasp parasitoids are attracted to honeydew, a rich source of sugars produced by leafhoppers when feeding, and to pollen and nectar, produced by flowering plants that inhabit the edge zones of maize fields. They can also be attracted by volatiles produced by the plants when damaged by the leafhopper, with these volatiles acting as indirect plant defenses. In the case of *D. maidis* adults, the damage is produced when feeding and during oviposition (Larsen et al. 1992). Indirect defenses produced via volatiles in maize when damaged by piercing-sucking insects have been little investigated (Erb et al. 2010). On the other hand, in chewing insects, Tamiru et al. (2011) found that when damage occurs in land race maize versus hybrid maize, more parasitoids are attracted to the land race than to the hybrid maize. They suggest that indirect defenses have been reduced in hybrid maize during crop breeding. So far, no studies have investigated whether adult egg parasitoids prefer to attack corn leaffopper eggs on maize edge grasses or within the maize agroecosystem, during the wet season, when both choices are available. Moreover, little is known about how egg parasitoids respond to different varieties of field maize.

This is the first study comparing egg parasitoids of *D. maidis* in maize edge zones versus inside maize fields during the maize-growing wet season. In addition, we compared adult egg parasitoid emergence in a maize land race with that in a maize hybrid within the maize field. The first objective of the present study was to determine which parasitoid species attack *D. maidis* eggs in maize fields and maize edge zones, along with the rate of adult parasitoids emerged. This investigation was carried out during the wet season of 2015. The second objective was to compare the numbers of adult parasitoids emerged in a maize land race and a maize hybrid, and this objective was carried out during the wet season of 2016.

**Materials and Methods**

The present study was performed in the Zapopan region in Jalisco, Mexico. This region was selected because it is representative of the environmental conditions and the maize crop practices conducted in Mexico. It is located at 20°44' N, 103° 30' W and an elevation of 1,662 m above sea level. Most of the maize in Mexico and tropical America is cultivated during the wet season using rainfall. In Mexico, the wet rainy season starts in June and ends in October. This study was conducted during the 2015 and 2016 wet, maize-growing seasons. First, in 2015, egg parasitoids of *D. maidis* were collected in a maize agroecosystem and its edge zones. Second, in 2016, parasitism of *D. maidis* by egg parasitoids was compared between two different maize varieties, with both varieties placed within the maize agroecosystem.

**Egg Parasitoids of *D. maidis* in Maize Fields and on Maize Edge Zones During the Maize-Growing Wet Season of 2015**

This first experiment was conducted to characterize egg parasitism in field conditions using laboratory-reared *D. maidis* females from Zapopan. Two-week-old females were allowed to oviposit on potted maize plants of a land race variety called Ancho-pozolero. All plants were at the three to five leaf stage. For an oviposition period of 72 h, 10 females were confined in a single-leaf cage, containing one leaf of live maize. Maize plants and insects were maintained in a rearing room at 25 ± 2°C, 50% relative humidity, and a photoperiod of 12:12 (L:D). After the oviposition period, females were removed, and plants with eggs (sentinel eggs) were transported to the field. There, they were distributed randomly in the maize field and maize edge zone. The edge zone was 1 m from the maize field and the variety planted in the maize field was the hybrid UdeG-2006. Maize plants with sentinel eggs are an efficient method for investigating egg parasitoids and parasitism (Virla et al. 2009b; Moya-Raygoza et al. 2012, 2014; Moya-Raygoza and Triapitsyn 2015). Thirty pots were placed within the maize field and 30 pots were placed in the maize edge zones. This configuration was repeated for a total of four replicates; one per month starting in July and ending in October, for a total sample of 120 pots in the maize edge zones and 120 pots within the maize field.

The grass species growing in the maize edge zones were *Eragrostis pilosa*, *Brachiaria plantaginea*, *Cynodon dactylon*, and *Eleusine indica*. The maize plants with sentinel eggs were placed within the maize field or edge grasses, where they remained for 5 d to allow exposure to egg parasitoids. After 5 d, the plants with sentinel eggs were returned to the laboratory, where the number of *D. maidis* eggs per leaf was counted. Once the eggs were counted, each leaf, with the attached egg mass, was cut from the plant and placed in a Petri dish lined with wet paper to maintain moisture for the leaf and egg. In addition, each Petri dish was covered with plastic film, which prevented the escape of emerged adult egg parasitoids. Petri dishes were maintained in the rearing room at our laboratory at the same conditions used for oviposition period for 45 d until all adults emerged. Throughout the 45 d, the petri dishes were checked every other day, using a ZEISS (DV4) microscope, and any adult parasitoids that had emerged were collected. Emerging adult parasitoids were stored in 95% ethanol for identification.

**Comparison of *D. maidis* Egg Parasitoids in Two Maize Varieties, Land Race and Hybrid, During the Maize-Growing Wet Season of 2016**

This second experiment was conducted within the maize field only, because the first experiment had confirmed that this habitat presented the highest levels of egg parasitoids. The experiment was performed in September, using laboratory-reared *D. maidis* females from Zapopan. The two varieties compared were a land race called Ancho-pozolero and a maize hybrid called Tigre-Asgrow, both at the three to five leaf stage. To obtain sentinel eggs, we used the same methodology as that described above for the first experiment, including the same number of *D. maidis* females, oviposition period, and rearing room conditions. A total of 60 pots were prepared; 30 with the maize land race and 30 with the maize hybrid. For each of the two maize varieties considered, the 30 pots with maize plants carrying sentinel eggs were distributed, in pairs and randomly, throughout the maize field. In the maize field was planted the hybrid UdeG-2006. Field exposure of sentinel eggs in the field was as described for experiment 1. *Dalbulus maidis* eggs were exposed once. The number of oviposited eggs per leaf was determined for each maize variety, and emerged adult egg parasitoids were collected and stored in ethanol for identification.

**Identification of Adult Egg Parasitoids Emerging From *D. maidis* Eggs**

The identification of adult parasitoids that emerged in the two experiments was performed using the taxonomic keys published by
Pinto (2006) and Triapitsyn (1997, 2015). Identification was subsequently confirmed by Serguei V. Triapitsyn (University of California, Riverside, USA).

### Data Analysis

The numbers of adult egg parasitoids that emerged during the 2015 and 2016 experiments were compared by $\chi^2$ test. In the first experiment (2015), the number of sentinel eggs exposed on edges vs. maize field was compared by Wilcoxon test. Also, in the 2015 experiment, we conducted a $\chi^2$ test to compare the number of adult parasitoids emerged from the maize edges with those emerged from within the maize field. In the second experiment (2016), a Wilcoxon test was conducted to compare the number of eggs laid on the maize land race versus the maize hybrid. The number of eggs was compared to corroborate an equal number of sentinel eggs in each variety were exposed. Moreover, a $\chi^2$ test was performed to compare the number of adult egg parasitoids emerged from each of the maize varieties in this second experiment. All statistical tests were conducted using the software R (R Core Team 2017).

### Results

Several species of adult egg parasitoids emerged from *Dalbulus maidis* eggs laid on maize leaves during the wet seasons of 2015 and 2016 in the different treatments; maize edges, within the maize field, and on sentinel plants of two varieties. The number of adult egg parasitoids among the 2015 and 2016 experiments was different ($\chi^2 = 135.58$, df = 2, $P = 0.01$), with very few parasitoids on the maize edges compared with within the maize field (Table 1). The emerged adult egg parasitoids included the hymenopteran wasps *Anagrus* sp. and *Anagrus columbi* Perkins (both Mymaridae) and *Paracentrobia* sp. and *Pseudoligosita* sp. (both Trichogrammatidae). However, *Anagrus* sp. and *Paracentrobia* sp. were the most common and abundant parasitoids (Table 1).

#### Egg Parasitoids of *Dalbulus maidis* Within Maize Fields and in Maize Edge Zones During the Maize-Growing Wet Season of 2015

The corn leafhopper females oviposited similar numbers of eggs on the maize land race Ancho-pozolero used in edge zones and within a maize field ($W = 3833.00$, $P = 0.11$; Fig. 1). Therefore, parasitoids living on the maize edges and within the maize field had similar numbers of *Dalbulus maidis* eggs available to parasitize.

The number of adult egg parasitoids emerged from sentinel *Dalbulus maidis* eggs was different between maize plants placed in edge zones and maize plants placed within the maize field ($\chi^2 = 39.34$, df = 1, $P = 0.01$), with more adult parasitoids emerging from the sentinel plants placed within the maize field (Fig. 2). The species of adult egg parasitoids that emerged were *Anagrus* sp. and *Paracentrobia* sp. in both treatments.

#### Table 1. Total number of adult egg parasitoids that emerged from *Dalbulus maidis* sentinel eggs placed in maize edge zones and within maize fields during 2015, and sentinel eggs on two varieties of maize placed within maize fields during 2016

| Maize plants with sentinel eggs                            | Paracentrobia sp. | Anagrus columbi | Anagrus sp. | Pseudoligosita sp. | # of exposed eggs |
|-------------------------------------------------------------|-------------------|-----------------|-------------|-------------------|------------------|
| Maize edge zone (land race maize) 2015, $n = 120$           | 1                 | 0               | 1           | 0                 | 361              |
| Into maize field (land race maize) 2015, $n = 120$          | 7                 | 0               | 37          | 0                 | 527              |
| Into maize field (land race maize) 2016, $n = 30$           | 0                 | 0               | 0           | 0                 | 287              |
| Into maize field (maize hybrid) 2016, $n = 30$              | 3                 | 3               | 7           | 2                 | 341              |
| Total number of egg parasitoids                             | 11                | 3               | 45          | 2                 |                  |

$n = $ total number of maize plants with sentinel eggs. # = total number of *Dalbulus maidis* eggs exposed per treatment.

#### Egg Parasitoids of *Dalbulus maidis* on Maize Land Race Versus Maize Hybrid Within the Maize Field During the Wet Season of 2016

*Dalbulus maidis* females laid similar numbers of eggs on the land race Ancho-pozolero and maize hybrid Tigre-Asgrow ($W = 190.50$, $P = 0.71$; Fig. 3). These maize plants with sentinel eggs were placed in pairs within the maize field and had the same opportunity of being attacked by adult egg parasitoids.
Different numbers of adult egg parasitoids emerged from *D. maidis* eggs laid on the maize land race versus eggs laid on the maize hybrid ($\chi^2 = 15.00$, df = 1, $P = 0.01$; Fig. 4). No adult egg parasitoids emerged from the *D. maidis* eggs oviposited on the maize land race. In contrast, *Anagrus* sp., *A. columbi*, *Pseudoligosita* sp., and *Paracentrobia* sp. emerged from the maize hybrid Tigre-Asgrow.

**Discussion**

Seasonal maize habitats are very common in tropical America, but little is known about the egg parasitoids that attack corn leafhoppers within maize fields and in their edge zones during the wet (growing) season. It has been previously reported that parasitoids attack the eggs (Moya-Raygoza and Becerra-Chiron 2014), nymphs, and adults (Becerra-Chiron et al. 2017) of the corn leafhopper in edge habitats throughout the winter, when the maize crop is absent. In the present study, we found that once maize is planted during the wet season, egg parasitoids attacking *D. maidis* eggs are most abundant in the maize field and almost absent affecting the abundance of egg parasitoids on the edges, because it is possible that parasitoid species are abundant affecting eggs of another leafhopper species in the edges. Within the maize agroecosystem, maize plants provide an unlimited food resource for *D. maidis*, which through high fecundity and great abundance becomes a major pest species (Madden et al. 1986, Nault 1990). Perhaps volatile, honeydew or both contribute in the attraction of egg parasitoid adults into the maize field. Little is known about volatile that attract adult egg parasitoids in maize when this plant is damaged by piercing-sucking insects (Erb et al. 2010). In rice, another globally important cereal, the planthopper *Nilaparvata lugens* (Stål)(Hemiptera: Delphaciidae), a piercing-sucking insect, induces 20 different volatile compounds that attract *Anagrus nilaparvatae* Pang and Wang (Lou et al. 2005a). In addition, Lou et al. (2005b) found that jasmonic acid induces volatile emissions that attract *A. nilaparvatae* to parasitize *N. lugens* eggs. In maize and rice plants, piercing-sucking insects appear to induce the emission of jasmonic acid, salicylic acid, and/or ethylene as indirect defenses (Qi et al. 2018), but the quality and quantity of such indirect defenses still needs to be determined for the maize-*D. maidis* interaction. Moreover, *D. maidis* adults produce honeydew as excrement (Larsen et al. 1992), and this honeydew is used as a food resource by egg parasitoid adults. Egg parasitoid adults can obtain energy for locomotion, survival, and reproduction through honeydew produced by leafhoppers (Tena et al. 2013). On the other hand, the grasses (Poaceae) that inhabit the maize edges, such as *Eragrostis pilosa*, *Brachiaria plantaginea*, *Cynodon dactylon*, and *Eleusine indica*, might have minimal attraction to egg parasitoids because they lack nectar, which would serve to attract egg parasitoid adults during the wet season.

Within maize fields, the most common parasitoids were *Anagrus* sp. and *Paracentrobia* sp. when *D. maidis* oviposited on the maize land race. Once we determined that egg parasitoids are abundant in this habitat, we evaluated parasitism through the emergence rate of adult parasitoids within the maize field in a treatment that allowed the parasitoids a choice between sentinel eggs on the land race of maize or a nearby pot with the sentinel eggs on a maize hybrid. Under these conditions, we found that egg parasitoid adults only emerged from the maize hybrid but not from the maize land race. We do not know if any factor avoid or limit the emergence of egg parasitoid adults in the different maize varieties. Probably lignin prevents the normal emergence of adult parasitoids. Lignin is a structural compound of cell wall that confers rigidity and it is found in high content in Bt corn (Saxena and Stotzky 2001). However, because egg parasitoid adults emerged from the maize land race in the 2015 experiment is unlikely that difference in lignin is a key factor in the absence of adult parasitoid emergence in the 2016 experiment. We suggest that the maize hybrid Tigre-Asgrow and maize land race produce volatiles when damage is produced by the corn leafhopper, but the hybrid probably emit some source of volatiles that attracts all of the parasitoids to it and not to the maize land race. Volatiles serve to attract parasitoid females to plants with leafhopper eggs and provide the females with information to find the leafhopper eggs (Chiappini et al. 2012). The parasitoid species that emerged from *D. maidis* eggs laid on the maize hybrid were *Anagrus* sp., *A. columbi*, *Pseudoligosita* sp., and *Paracentrobia* sp. These results are in accordance with those of Gaillard et al. (2018), who state that artificial selection has maintained direct defenses against specialist herbivores. These results relate to *D. maidis* since the corn leafhopper is a specialist herbivore pest in maize plants (Nault 1990). However, Tamiru et al. (2011) found different results in the chewing stem borer moth, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), which also damages maize varieties. They suggested that indirect plant defenses may be lost during crop breeding, since parasitoid attraction was absent in maize hybrid varieties, whereas in maize landraces the egg parasitoid *Trichogramma bournieri* Pintureau &
Barbault (Trichogrammatidae) and the larval parasitoid Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae) were attracted.

We found Mymaridae and Trichogrammatidae egg parasitoids within the seasonal maize habitat, where they form a complex or community of species that together can attain a 90% rate of parasitism of D. maidis eggs in Mexican maize crops (Moya-Raygoza et al. 2014). High levels of D. maidis egg parasitism by Mymaridae and Trichogrammatidae species have already been reported in countries from the Americas such as Argentina (Virla et al. 2013), Nicaragua (Gladstone et al. 1994), and Brazil (Oliveira and Lopes 2000). Interestingly, in rice cultivated in Asia, a community of egg parasitoids formed by Mymaridae and Trichogrammatidae species has also been reported to attack hoppers. In one rice agroecosystem, a 90% rate of parasitism of N. lugens and Nephotettix spp. leafhoppers by wasp species belonging to these two families was reported (Sann et al. 2018). Both of these pests transmit viruses, such as rice tungro virus, grassy stunt virus, and ragged stunt virus, in tropical rice planted continually in a tropical system. All these results suggest that egg parasitoids are important biological control agents against leafhoppers, which are vectors of plant pathogens affecting maize and rice in the Americas and Asia.

In conclusion, during the wet season, adult egg parasitoids of D. maidis preferentially actually attack the vector within maize agroecosystems rather than in edge zones. In addition, within maize fields, these adult egg parasitoids parasitize D. maidis eggs laid on the maize hybrid and not on the maize land race. Maize hybrids may therefore be beneficial for the attraction of egg parasitoids. Also, maize varieties with odors that attract egg parasitoids might be new strategy in agricultural systems for the control of corn leafhoppers in the Americas. Future studies need to be conducted to determine the volatile blend responsible for the attraction of adult egg parasitoids that attack corn leafhoppers.

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