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# Effect of the oviposition period and age of the females of *Dalbulus maidis* (Hemiptera: Cicadellidae) in the emergence of egg parasitoids

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## Abstract

Little is known about the effect of adult leafhopper age and its oviposition period on the emergence of egg parasitoids. The objective of this study was to determine the emergence rate of egg parasitoids that attack eggs of the corn leafhopper, *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae), when the adult corn leafhopper differs in age and is subject to different lengths of time for the oviposition process. A total of 4 treatments compared adult females of *D. maidis* of 2 different ages, young (2-wk-old) and mature (8-wk-old), as well as 2 periods of oviposition of different lengths (3 and 6 d). The adult parasitoids emerging from the eggs of *D. maidis* were *Anagrus virlai* Triapitsyn and *Anagrus columbi* Perkins (both Hymenoptera: Mymaridae). When analyzing the interactions of leafhopper age at oviposition, it was found that the age of the leafhopper females (2-wk-old vs 8-wk-old) had a different effect on the number of oviposited eggs for the 2 oviposition periods. On the other hand, in the emergence of parasitoids, there were no significant differences between age and oviposition period, but the emergence of parasitoids was related to the number of eggs oviposited by *D. maidis*.

Key Words: corn leafhopper; parasitism; Hymenoptera

## Resumen

Se conoce poco sobre el efecto de la edad de la chicharrita adulta y el período de oviposición por la chicharrita en la emergencia de parasitoides de huevo. El objetivo del presente estudio fue determinar la tasa de emergencia en los parasitoides de huevos que atacan a la chicharrita del maíz *Dalbulus maidis* (DeLong) (Hemiptera: Cicadellidae) cuando la chicharrita adulta difiere en edad y está sujeta a diferente longitud de tiempo durante el proceso de oviposición. Se realizaron un total de 4 tratamientos, en donde se compararon hembras de *D. maidis* de 2 edades diferentes, jóvenes (2 semanas de edad) y maduras (8 semanas de edad), así como dos períodos de oviposición con diferente longitud de tiempo (3 y 6 d). Los parasitoides adultos que emergieron de los huevos de *D. maidis* fueron *Anagrus virlai* Triapitsyn y *Anagrus columbi* Perkins (Hymenoptera: Mymaridae). Al analizar la interacción entre los cuatro tratamientos, se encontró que la edad de las hembras (2 semanas de edad contra 8 semanas de edad) tiene un efecto diferente sobre el número de huevos ovipositados para cada uno de los 2 períodos de oviposición. Por otro lado, en la emergencia de parasitoides, no hubo diferencias entre la relación edad de la chicharrita y periodo de oviposición, pero la emergencia de parasitoides esta relacionada con el numero de huevos ovipositados por *D. maidis*.

Palabras Claves: chicharrita del maíz; parasitismo; Hymenoptera

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In hemipteran insects, oviposition may occur on the stem of the plant, over the surface of the leaf, or on the middle vein (Thompson 1978; Heady et al. 1985). This is the case of the corn leafhopper, *Dalbulus maidis* (De Long) (Hemiptera: Cicadellidae), which is a pest throughout Latin America because it transmits 3 important diseases of maize (Nault & Madden 1985; Nault 1990). These are maize rayado fino virus, maize bushy stunt phytoplasma, and corn stunt spiroplasma (Nault & Ammar 1989; Nault 1990). *Dalbulus maidis* has a high reproductive capacity with the ability to oviposit approximately 15 to 37 eggs per d (Pitre 1970; Moya-Raygoza 2016), which attracts egg parasitoids of the genus *Anagrus* sp. (Hymenoptera). A greater number of eggs laid on a plant increases damage to the plant by adult leafhoppers (Larsen et al. 1992; Singh & Rana 1992). The processes of oviposition and feeding by leafhoppers increases the emissions of volatiles produced by the plant, thus attracting parasitoids (Erb et al. 2010).

The parasitoid wasps *Anagrus columbi* Perkins and *Anagrus virlai* Triapitsyn (both Hymenoptera: Mymaridae) (Triapitsyn 1997) are found abundantly in maize fields (Moya-Raygoza & Becerra-Chiron 2014; Moya-Raygoza et al. 2014). They are solitary and have short life cycles (Meyerdirk & Moratorio 1987; Corbet & Rosenheim 1996), take approximately 17 d to develop, and the average life expectancy of adults is 5 d, whereas few adults survive more than 12 d (Virla 2001). Although they reproduce easily (Waage & Ming 1984), their emergence is related to the characteristics of the host egg, which are given by the female that lays the eggs (Moratorio 1987; Remes Lenicov & Virla 1993; Hanks et al. 1995), and the quantity of available eggs (Moya-Raygoza 2016; Segoli 2016). Selection of a host egg of a certain quality may give reproductive or physiological advantages to the parasitoid, and increase the probability of successfully emerging from the egg in which it develops (Vinson 1976; Van Alphen et al. 1986). For example, it has been shown

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that parasitoids have a greater preference for eggs laid by younger rather than older adults (Moya-Raygoza 2016). On the other hand, an increased length of oviposition period would increase the number of eggs available for the parasitoids and an inverse parasitism rate.

In *D. maidis* it has been observed that the number of eggs laid varies with the age of the adult (Pitre 1970; Madden et al. 1986; Moya-Raygoza 2016). The age of the female that lays the host egg directly affects the interaction with parasitoids of the genus *Anagrus*, influencing the development, parasitism rate, and emergence of the parasitoid (Ehler & Van Den Bosch 1974; Moreno et al. 2009; Zhang et al. 2013). Even though age and quality of the host egg have been well studied, there is no information on the interaction between the age of the adult female and the length of oviposition by leafhoppers, and their effect on the emergence of egg parasitoids. Therefore, the objective of this study is to show how parasitism affects parasitoid insect emergence from *D. maidis* eggs laid by young (2-wk-old) and mature (8-wk-old) leafhoppers, at 2 different periods of oviposition (3 and 6 d). The results of this study may be applied in insect pest control programs using *D. maidis* as a model for the development of egg parasitoids for field use.

Materials and Methods

The study was conducted during the rainy season in Aug 2016 in maize fields located in Zapopan, Jalisco, Mexico, located at 20.733333°N, 103.500000°W, and 1,662 masl. *Dalbulus maidis* adults were collected from maize grown in the Zapopan area and reared under lab conditions to perform the experiments. The emergence of *D. maidis* egg parasitoids was evaluated by 4 treatments: (1) Young-3 (2-wk-old leafhoppers with 3 d oviposition period); (2) Young-6 (2-wk-old leafhoppers with 6 d oviposition period); (3) Mature-3 (8-wk old leafhoppers with 3 d oviposition period); and (4) Mature-6 (8-wk-old leafhoppers with 6 d oviposition period).

OVIPOSITION OF *DALBULUS MAIDIS*

Sixty pots containing two 6-leaf stage maize plants were used for this study. A leaf cage (2.3 cm × 4.2 cm × 5.5 cm) containing 10 adult females of *D. maidis* was placed on 1 of the leaves of each plant. In each pair of pots, the 4 treatments were conducted; in the first pot, 1 maize plant had the Young-3 treatment, and the other maize plant had the Young-6 treatment. In the second pot, 1 maize plant had the Mature-3 treatment, and the other plant had the Mature-6 treatment. Each treatment was replicated on 30 maize plants.

The pots were placed in the greenhouse where they received ambient daylight and were watered daily, and the temperature in the greenhouse was maintained between 25 and 27 °C.

Exposure of *Dalbulus maidis* Eggs in Maize Field

After the oviposition period, all adult female leafhoppers were removed, and the pots containing leaves with healthy eggs (sentinel eggs) were transported immediately to the field. The sentinel plants were placed close to the maize field margins. The pots were placed in pairs (containing all 4 treatments) with about 5 m distance between each pair of pots. The pots with the sentinel plants received water every other d to reduce plant stress, and remained by the maize field for 5 d to allow exposure to naturally occurring egg parasitoids.

Emergence of Parasitoids

After the exposure to parasitoids in the maize field, the sentinel plants were transported to the laboratory, where the number of *D. mai-*

*dis* eggs were counted on each exposed leaf. At this point, egg filaments were evident because these filaments are produced 72 h after oviposition (Heady & Nault 1984). Leaves containing the parasitized eggs were cut and placed individually on moist absorbent paper in a Petri dish, and wrapped with transparent plastic. This was done to maintain the viability of the leaf, and to avoid the escape of the parasitoids (Moya-Raygoza et al. 2012; Moya-Raygoza & Triapitsyn 2015). The dishes were transferred to a rearing room with controlled conditions of 25 °C, 50% relative humidity, and a photoperiod of 12:12 h (L:D) where they remained for 45 d, allowing enough time for the appearance of adult parasitoids (Virla et al. 2009; Moya-Raygoza & Becerra-Chiron 2014). Dishes were checked every third d using a stereoscope (Stemi DV4, Carl Zeiss, Oberkochen, Germany). All parasitoids that emerged were deposited in 2 mL vials and preserved with 95% ethanol for identification.

Identification of Parasitoids

All parasitoids emerged from *D. maidis* eggs from the 4 treatments were identified with the help of taxonomic keys (Chappini et al. 1996; Triapitsyn 1997, 2015; Pinto 2006), and their identification was confirmed by Sergei V. Triapitsyn (University of California, Riverside, California, USA).

DATA ANALYSIS

The number of eggs laid were evaluated using Generalized Linear Models, where the age of the adult female *D. maidis* (young 2-wk-old, and mature 8-wk-old), and 2 periods of oviposition (3 and 6 d) were used as the fixed factors. The emergence of parasitoids was evaluated through Generalized Linear Models with distribution of quasipoisson type errors; the age of the leafhoppers and the oviposition time were used as fixed variables, and the egg abundance as covariates. Data were analyzed with the R Core Team software (2019).

Results

Two species of parasitoids of the genus *Anagrus* were present in the field during the study. Of the 4 treatments that were carried out (Young-3, Young-6, Mature-3, and Mature-6), a total of 381 adult egg parasitoids were obtained during the study; 363 belong to the species *A. virlai*, of which 208 were females and 155 males, and 18 of *A. columbi*, where we obtained 8 females and 10 males (Table 1).

EGGS OVIPOSITED BY *DALBULUS MAIDIS*

There was an interaction between the age of the female and the oviposition period ( $F_{1,116} = 23.838$ ;  $P = 0.0005$ ). It was observed that in 3

**Table 1.** Total number of females and males adult parasitoids emerging from *Dalbulus maidis* eggs during the rainy season 2016 in the 4 treatment combinations. Young (2-wk-old) and mature (8-wk-old) adults allowed to oviposit for 3 or 6 d.

Treatments	Emergед parasitoids			
	<i>Anagrus virlai</i>		<i>Anagrus columbi</i>	
	Female	Male	Female	Male
Young-3	69	56	4	0
Young-6	46	41	1	0
Mature-3	7	4	0	0
Mature-6	86	54	3	10
	208	155	8	10
Total parasitoids per species	363		18	

d oviposition, fewer eggs were laid by mature females (8-wk-old) than by young females (2-wk-old). However, this correlation was reversed when the period of oviposition was increased to 6 d, with mature females laying more eggs with an average of 5 eggs per d (Fig. 1).

#### Adult Parasitoids Emerged from *Dalbulus maidis* Eggs

The age of the leafhoppers ( $F_{1,116} = 1.08$ ;  $P = 0.743$ ) and the time of oviposition ( $F_{1,116} = 2.558$ ;  $P = 0.113$ ) did not show significant differences; parasitoids emerged from eggs laid by both young and mature leafhoppers, and the oviposition period was 3 or 6 d. However, the number of parasitoids emerging was related to the number of eggs oviposited by the female *D. maidis* ( $F_{1,116} = 1.221$ ;  $P < 0.0005$ ) (Fig. 2).

## Discussion

It has been observed that the age of the host is an important factor for parasitism, as well as for the number of eggs laid for emergence of parasitoids (Schmidt 1994; Virla 2001; Amalin et al. 2005; Zhou et al. 2014; Moya-Raygoza 2016). The previous effects are related to the physiological characteristics of the female host (Moratorio 1987; Remes Lenicov & Virla 1993; Hanks et al. 1995) and the oviposition period before the exposure of the eggs to the parasitoid.

Like Pitre (1967), we observed that the age of the female *D. maidis*, as well as the oviposition period length, affected the number of eggs laid. Young females laid more eggs in 3 d than did the mature ones, whereas the mature leafhoppers laid more eggs in a 6 d period compared to the young ones. Madden et al. (1986) mentioned that adult leafhoppers have a resting time in egg laying; it is likely that the young leafhoppers in our experiment used the 3 d oviposition period to lay a large number of eggs to ensure the development of nymphs and to increase their population. This idea is supported by the work done by Moya-Raygoza and Garcia-Medina (2010), where they mention that during an oviposition period of 3 d the female *D. maidis* laid a large number of eggs.

We confirmed that the number of eggs laid by *D. maidis* affects the number of emerging egg parasitoids, such as *A. virilai* and *A. columbi*, found within maize fields during the rainy season. Virla et al. (2009) and Moya-Raygoza et al. (2012, 2014) reported similar findings in the field when observing parasitism by these micro wasps of eggs of this leafhopper. Moya-Raygoza (2016) obtained the parasitoid *A. virilai* from eggs of *D. maidis*, showing that this wasp responds positively to higher densities of host eggs.

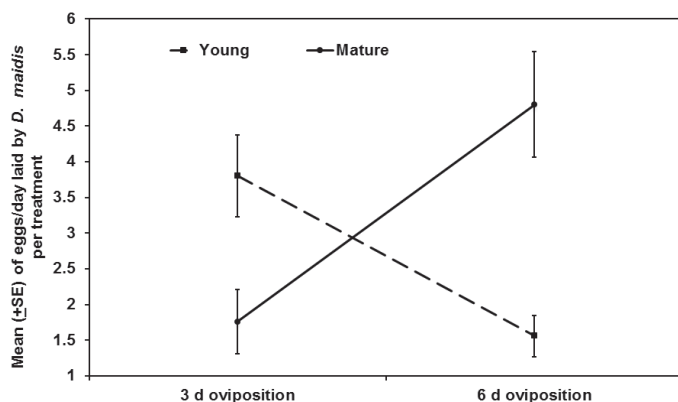


Fig. 1. Average ( $\pm$  SE) number of eggs laid per d by *Dalbulus maidis* in treatments (Young-3, Young-6, Mature-3, and Mature-6). The circles represent the mature (8-wk-old) leafhoppers, whereas the squares represent the young (2-wk-old) leafhoppers.

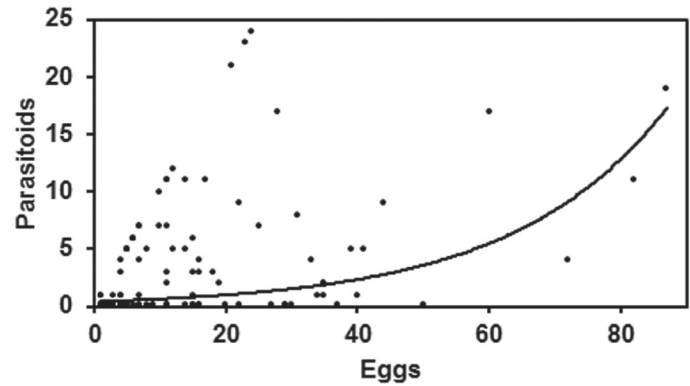


Fig. 2. Emergence of adult parasitoids relative to number of eggs laid by *Dalbulus maidis* females.

Our results confirm that the age of the adult leafhopper and the period of oviposition in combination do not constitute an important factor for the emergence of parasitoids of the 2 *Anagrus* species. Since parasitism was observed in eggs laid in both 3 and 6 d oviposition periods, and parasitoids emerged from eggs laid by young and mature leafhoppers, we concur with Iwasa et al. (1984), who mentioned that this parasitoid oviposits in almost all hosts in which the survival of the progeny is guaranteed, including the specialists who have ranges of size or age. This is mentioned by Krugner et al. (2009), where he worked with the parasitoid *Anagrus epos* Girault (Hymenoptera: Mymaridae), which is a natural enemy of the leafhopper *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) and parasitic eggs of any age, where in all the exposed treatments he obtained parasitoids of the genus *Anagrus* spp. from the eggs of *D. maidis*. The relationship between the number of available host eggs and the number of parasitoids emerged was worth noting, because the parasitoids' priority is to find a place to develop. The female parasitoid tries at all times to maximize total reproductive success by laying eggs from maturity to death (Iwasa et al. 1984).

Several studies have been carried out on the quality of the host egg, where the age of the egg is an important factor for the development of the parasitoid, and where it is demonstrated that there is a greater preference towards young eggs rather than long-lived ones. This may be due to the quality of the available resources for the progeny of the parasitoids (Zhou et al. 2014). Hanks et al. (1995) mentioned that the parasitoid *Avetianella longoi* Siscaro (Hymenoptera: Encyrtidae) prefer to parasitize younger eggs of *Phoracantha semipunctata* Fabricius (Coleoptera: Cerambycidae), because there appears to be higher parasitism than in older eggs; however, the emergence of parasitoids occurred from most of the parasitized eggs. A similar case was reported by Miura and Kobayashi (1998), where the mature eggs that had been laid by *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) were less parasitized, with preferential parasitism by *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) on younger eggs.

Irvin and Hoddle (2005) obtained wasps of the genus *Gonatocerus* sp. (Hymenoptera: Mymaridae) from *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) eggs up to 10 d old. However, new eggs may be more attractive to parasitoids, because Leibe et al. (1979) and Pizzol et al. (2012) observed higher parasitism by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) in *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae) eggs that had been oviposited only 1 or 2 d earlier. In previous studies, the age of the adult host was not documented.

Currently there are few studies that show the relationship between the length of oviposition and the age of the adult host insect;



therefore, this work provides important information, giving a broader view of the factors that may influence the quality of the egg, therefore affecting the emergence and development of the parasitoid. This information could be used in the implementation of biological control programs involving the establishment and rearing of natural enemies. In conclusion, the emergence of *A. virlai* and *A. columbi* parasitoids from eggs laid by leafhoppers of different ages (2- and 8-wk-old) and eggs laid over 2 different oviposition periods (3 and 6 d) could be very important and informative to establishing biological control programs. Both the age of the adult host insect and the length of oviposition are shown to have an effect on the quantity and quality of the eggs available for *A. virlai* and *A. columbi*.

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