

Male leafhopper's choice behavior in response to different maize and conspecific cues

Comportamiento de elección de la chicharra macho en respuesta a diferentes señales de maíz y de su misma especie

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Abstract

The corn leafhopper, *Dalbulus maidis* (Delong and Wolcott) (Hemiptera: Cicadellidae), is a significant pest of maize (*Zea mays* L.) across the Americas, particularly noted for its role in transmitting various phytopathogens. Although direct damage from sap ingestion is minimal, the economic impact of these pathogens has been severe, resulting in substantial yield losses. Despite the pest's agricultural importance, little research has focused on its olfactory selection and the potential influence of volatile cues from host plants or conspecifics. This study investigated whether male *D. maidis* preferentially chose volatiles emitted from maize plants, conspecifics, or a combination of both. Behavioral observations carried out in a four-armed olfactometer revealed that male leafhoppers spent significantly more time in the arm containing the scent of both sexes than the arm with the scent of other males, although responses to female scents were not significantly different across treatments. We also did not find differences regarding the number of choices for the scented arm and the visit duration in response to different stimuli from plants. The results indicate a generally neutral choice towards the tested volatiles, suggesting that *D. maidis* males may rely more on other sensory inputs for host selection. This research lays the foundation for future studies on the chemical ecology of *D. maidis*, with implications for developing innovative pest management strategies.

Keywords: Olfactometer, chemical ecology, pests, odors.

Resumen

La chicharrita del maíz, *Dalbulus maidis* (Delong y Wolcott) (Hemiptera: Cicadellidae), es una plaga significativa del maíz (*Zea mays* L.) en las Américas, especialmente por su papel en la transmisión de diversos fitopatógenos. Aunque el daño directo por la ingestión de savia es mínimo, el impacto económico de estos patógenos ha sido severo, resultando en pérdidas sustanciales en la producción del maíz. A pesar de la importancia agrícola de esta plaga, se ha investigado poco sobre su elección olfatoria y la influencia potencial de las señales volátiles de las plantas hospederas o de conespecíficos. Este estudio investigó si los machos de *D. maidis* eligen preferentemente los volátiles emitidos por las plantas de maíz, los conespecíficos o una combinación de ambos. Las observaciones conductuales realizadas en un olfatómetro de cuatro brazos revelaron que las chicharritas pasaron significativamente más tiempo en el brazo que contenía el aroma de ambos sexos que en el brazo con el aroma de otros machos, aunque las respuestas a los aromas de las hembras no fueron significativamente diferentes entre los tratamientos. Tampoco encontramos diferencias en la selección del campo aromático ni en la duración de las visitas en respuesta a diferentes estímulos aromáticos de las plantas. Los resultados indican una elección generalmente neutral hacia los volátiles probados, lo que sugiere que los machos de *D. maidis* podrían depender más de otros estímulos sensoriales para la selección de la planta hospedera. Esta investigación sienta las bases para futuros estudios sobre la ecología química de *D. maidis*, con implicaciones para el desarrollo de estrategias innovadoras de manejo de plagas.

Palabras clave: Olfatómetro, ecología química, plagas, aromas.

Introduction

The corn leafhopper *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae) is a major pest of maize (*Zea mays* L.) in the Americas, ranging from Southern United States to Argentina (Nault, 1990). This cicadellid has rarely been reported as a causal agent of direct damage to corn (e.g., ingestion and removal of sap) (Virla *et al.*, 2021), but its importance lies in its ability to efficiently transmit different phytopathogens, which can be found either alone or in combination, such as those that cause corn stunt disease (maize bushy stunt phytoplasma [MBSP] and corn stunt Spiroplasma [CSS]) and the maize rayado fino virus (MRFV) (Moya-Raygoza, 2020; de Oliveira and Frizzas, 2021). Corn production has been severely affected by these pathogens during the 19th and 20th century, with damages ranging from 40% to 100% yield loss in different countries (Jones and Medina, 2020). Several pathogens transmitted by *Dalbulus* spp. have been reported in some states (e.g., Puebla and Veracruz; Pérez-López *et al.*, 2016; Aguilera *et al.*, 2019) of Mexico, which is one of the eight main corn-producing countries in the world (Erenstein *et al.*, 2022) and has also been considered as the center of diversity and speciation of the genus *Dalbulus* (Pérez-López *et al.*, 2016).

Choice and orientation behavior in insects often involves a combination of sensory inputs, including olfaction, vision, and sounds (Drosopoulos and Claridge 2006). For example, the tea green leafhopper *Empoasca vitis* Gölthe (Hemiptera: Cicadellidae), one of the most significant pests found in regions where tea is cultivated, uses both olfaction and vision to locate its host plants (Zhang *et al.*, 2018). The fact that many cicadellid species are attracted to colors (Todd *et al.*, 1990a; Rodríguez-Saona *et al.*, 2012; Bian *et al.*, 2020; Tan *et al.*, 2023) is indicative of the importance of vision while searching for a host plant. However, volatile signals play a crucial role for numerous insect groups, including Hemiptera (Bernasconi *et al.*, 1998), and many phytophagous species depend significantly on their sense of smell to locate food, sites of oviposition or hibernation, and potential mates (Hansson, 1999; Meiners, 2015).

Despite the considerable losses that *D. maidis* can cause in maize production, there has been little attention to its olfactory orientation and the role that volatiles from its host plant or its conspecifics play in it (Coll-Aráoz *et al.*, 2019; Hill *et al.*, 2023). Previous studies have shown that *D. maidis* can respond positively to maize volatiles (hexane extracts made from corn leaves) when in synergy with an appropriate visual stimulus (i.e., green light) (Todd *et al.*, 1990b). In addition, recent studies have reported that female *Dalbulus maidis* prefer the scents

emitted by specific maize hybrids (Coll-Aráoz *et al.*, 2019). A subsequent study further determined that males and females induce varying amounts of maize volatiles (Hill *et al.*, 2023), suggesting that male *Dalbulus maidis* might also respond to volatiles from different sources. Moreover, courtship behavior has been described for both males and females, and it has been proposed that acoustic and chemical signals could play an important role in the courtship phase (Ramírez-Romero *et al.*, 2014). In the case of *D. maidis*, although nymphs and adults can be found aggregated on the whorl and leaves of corn (Nault, 1990), to our knowledge it has not been investigated whether this aggregation behavior or isolation of conspecifics is related to some form of aromatic communication.

The present study aims to determine whether male *D. maidis* preferentially orient towards aromatic signals from its host plant, conspecifics, or the "host plant-conspecific" complex. This research was designed to provide insights into the chemical ecology of *D. maidis* and to lay the groundwork for developing innovative management strategies, such as the potential use of pheromones, for monitoring and controlling this agricultural pest.

Materials and Methods

Biological material

***Dalbulus maidis*.** A colony of *D. maidis* was established with individuals collected in the municipality of El Grullo, Jalisco, Mexico (19°50' latitude and 104°16' longitude). Leafhoppers collected in the same location have been subsequently added to the colony once a year. This colony was maintained in wooden boxes (47.3 cm high × 29.7 cm long × 29.7 cm wide) with four healthy maize plants, which were monitored once a week and changed when necessary (e.g., dead or decayed plants) (Ramírez-Romero *et al.*, 2014). The colony was maintained in a greenhouse at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), University of Guadalajara.

Plants. Maize plants of the "Tuxpeño" variety were used to maintain the individuals. Seeds were supplied by Centro Internacional de Mejoramiento del Maíz y el Trigo (CIMMYT) and by Dr. Moisés Morales Rivera (University of Guadalajara, Mexico). Three seeds were sown per pot (10 cm high × 12 cm diameter) in Nutrigarden® soil substrate. Plants were maintained in a greenhouse, watered three times a week and used for colony rearing and experiments when they had four to six leaves (i.e., vegetative stage).

Corn leafhopper cohorts. To obtain individuals of similar ages, cohorts were formed weekly by introducing adult

individuals (≈ 200) from the general colony into a wooden cage with four pots. The adults were left inside the cage for 72h under rearing conditions ($25 \pm 5^\circ\text{C}$, $50\% \pm 30\%$ RH and a 12:12 [Light:Darkness] photoperiod). After the oviposition period, the adults were removed, and the cage was monitored every day to determine the day of adult emergence.

Virgin females. To obtain virgin females, cohorts were monitored daily to detect and separate third-fourth instar nymphs, which were individually placed in a circular "clip-cage" type compartment (1.8 cm high \times 1.5 cm diameter) fixed on a corn leaf for the isolated nymph to feed (details in Ramirez-Romero *et al.*, 2014). The clip-cages were checked daily to record the day of adult emergence. Once emerged, the individual was sexed, and the female leafhoppers grouped into rectangular plastic cages (5 cm high \times 4 cm long \times 2 cm wide). These cages were attached to another corn leaf to have each group of leafhoppers separated by age. In this way, the females were left until they reached one week of age. Only leafhoppers aged 1 to 2 weeks were used for all bioassays.

Non-virgin males. To obtain non-virgin males, one-week-old individuals were obtained from the cages with weekly cohorts. Since the males were housed with females from the same cohort during that week, they were assumed to have mating experience. Using experienced males could increase the chances of successful courtship and/or copulation (Cook, 1994). Sexed males were grouped in rectangular plastic cages (5 cm high \times 4 cm long \times 2 cm wide) which were attached to a corn leaf to provide food. Males aged between 1 and 2 weeks were used in the bioassay.

Behavioral observations

Observations were carried out under controlled conditions of temperature ($26 \pm 2^\circ\text{C}$), relative humidity ($60 \pm 10\%$) and light intensity (150 lux). A four-armed olfactometer (similar to the one described in detail by Vet *et al.*, 1983) was used. The device comprised four equally sized compartments (arms), each with a humidified airflow. The four airflows were extracted through the central tube. In this study, only one compartment contained the scent of interest, while the remaining three carried humidified air without any scent. Each arm received a specific amount of humidified air (100 ml/min per area). For an arm to be associated with an aroma, the scent source was placed in the air stream before entering the olfactometer. A video camera (Sony®, CCD 560TVL, South Korea) was placed on top of the olfactometer to continuously record the insect's movement without disturbing it. Once the setting was ready, the insect was introduced through the central tube and observations started. If the insect did not move within five minutes, it was discarded and replaced. The observation time in each

bioassay was 15 minutes, as in previous studies with hemipterans in similar bioassays (Guédot *et al.*, 2009a), and in each replicate new insects were employed to avoid pseudoreplication. For each treatment, the following response variables were recorded: i) duration of visit (i.e., time spent by the insect in the scented arm), and ii) number of choices (i.e., number of times the insect selected the scented arm).

Experiment 1: Orientation behavior towards conspecific-related scents

In this experiment, we sought to determine if the corn leafhopper preferentially orients towards the scents coming from conspecifics, males or females, individually or together, in the absence of visual or acoustic stimuli. To determine this, the following treatments were tested: 1) same-sex conspecifics; 2) opposite-sex conspecifics (possible presence of sexual pheromones); 3) hexane extract; and 4) conspecifics of both sexes together. Since male behavior was observed, treatment 1 (hereafter referred to as "Males") consisted of 20 male leafhoppers (number used in previous behavioral studies [Todd *et al.*, 1990b]) placed in a modified 250 ml Erlenmeyer flask, which allowed humidified air to pass through, and connected to the olfactometer. Treatment 2 (hereafter referred to as "Females") consisted of 20 virgin female leafhoppers placed inside an Erlenmeyer flask in the same manner as treatment 1. Treatment 3 (hereafter referred to as "Female extract") was prepared following previous studies (Guédot *et al.*, 2009a; Xu *et al.*, 2019). To obtain the extract, 15 virgin female leafhoppers were placed inside a glass vial, to which 0.3 ml of hexane (Hycel®, ACS Grade, Mexico) were added. The leafhoppers in hexane were shaken for 5 minutes, after which the solvent was removed and placed in another glass vial to prevent further chemical extraction. Then, the 0.3 ml of extract were placed on filter paper (Double Rings, General Purpose Filter, 5.5 cm diameter), which in turn was put in a 250 ml Erlenmeyer flask for the olfactometer test. Treatment 4 (hereafter referred to as "Both sexes"), consisted of four virgin female leafhoppers and two non-virgin males alive and together in an Erlenmeyer flask. Prior to observation, the six individuals were left in the flask for a period of two hours. The latter because it has been observed that within this timeframe, corn leafhoppers carry out their courtship and copulation (Ramirez-Romero *et al.*, 2014). Subsequently, the flask was connected to the olfactometer, and observations were carried out. For each treatment, the procedure was replicated at least 14 times.

Experiment 2: Orientation behavior towards plant-related scents

The aim of this experiment was to determine whether the corn leafhopper preferentially orients towards different scents coming from its host plant, or in combination with the presence of conspecifics on the host plant, in the absence of visual or acoustic stimuli. The observations were carried out under the same controlled conditions as in the previous experiment. For each treatment, the same response variables (i.e., duration of visit and number of choices) were recorded. Plants were placed inside glass vessels modified in such a way that they allowed the passage of humidified air, in the same way as in the previous experiment. The treatments to be tested were: 1) artificially damaged plants, 2) plants with feeding leafhoppers, 3) maize plants, and 4) maize plant extract. Treatment 1 (hereafter referred to as “Artificial damage”) consisted of three plants with mechanically chopped leaves to half of their lengths. Treatment 2 (hereafter referred to as “Maize+Conspecifics”) consisted of three maize plants bearing 80 to 100 leafhoppers (i.e., high density). The insects were allowed to feed on these plants for 48 hours before observations were performed. Treatment 3 (hereafter referred to as “Maize”) consisted of three clean, intact plants. Finally, Treatment 4 (hereafter referred to as “Maize extract”), was prepared from the leaves of a corn plant which were macerated in 30 ml of hexane for 15 minutes, after which the extract was transferred to a 10 ml glass vial. From this first 10 ml of extract, 1 ml was taken and placed on a filter paper (5.5 cm diameter), which was placed inside the glass vessel, as in previous studies (Todd *et al.*, 1990b). For each treatment, the procedure was replicated at least 14 times.

Data analysis

The mean time leafhoppers spent in the scented arms (visit duration) was compared among treatments using ANOVA tests. For experiment 1, which involved conspecific-related scents, the data were log-transformed ($\log(x+1)$) prior to analysis to meet the assumption of homoscedasticity. The number of choices made by the leafhoppers for the scented arm was used to calculate the proportion of choices for the scented arm (i.e., the number of choices for the scented arm divided by the total number of choices across all arms). These choice proportions for the scented arms were compared among treatments using chi-square tests, with Bonferroni-corrected significance thresholds applied (Rice, 1989). The analyses were performed using R (Version 4.0.1, Team 2000).

Results

Experiment 1: Orientation behavior towards conspecific-related scents

Comparisons of the mean time spent by leafhoppers in the scented arm among treatments revealed significant differences ($F_{3,53}=3.766$; $P=0.016$). Male leafhoppers spent significantly more time in the arm containing the scent of both sexes than the arm with the scent of other males (Figure 1). In contrast, the time spent in arms containing scents from females and female extracts did not differ significantly from the other treatments (Figure 1). When the proportions of choices for the scented field were compared across treatments, no significant differences were observed when applying the Bonferroni-corrected alpha threshold ($P = 0.008$) (Figure 2).

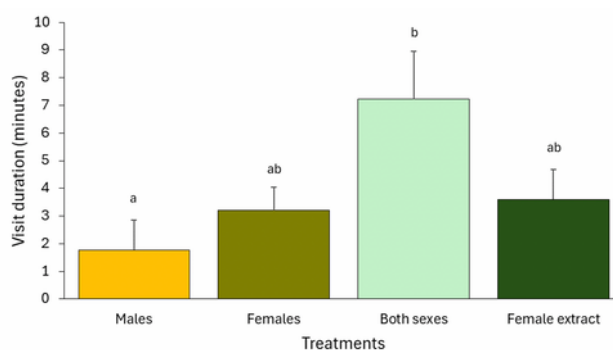


Figure 1. Mean time (\pm SE) spent by male leafhoppers in olfactometer arms characterized by different scents (treatments). Different letters indicate significant statistical differences ($p < 0.05$).

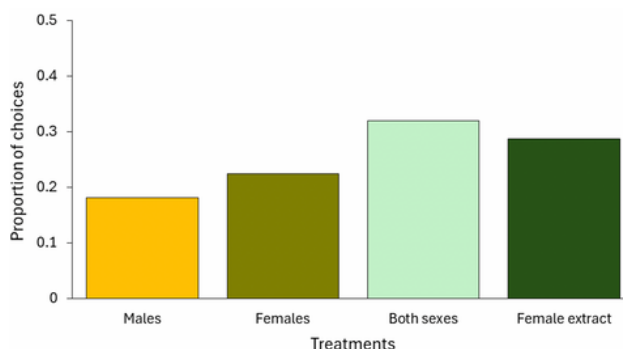


Figure 2. Proportion of choices for the scented arm (i.e., number of times the leafhopper selected the scented arm divided by the total number of choices across all arms). No significant differences were observed across treatments.

Experiment 2: Orientation behavior towards plant-related scents

When comparing the mean time that male leafhoppers spent in the scented arms across treatments, no significant differences were detected ($F_{3,53}=0.708$; $P=0.551$). The time males spent in arms with plant-related scents did not vary significantly among treatments (Figure 3). Similarly, comparisons of the choice proportions for the scented arms among treatments showed no significant differences when considering the Bonferroni-corrected significance level ($P = 0.008$).

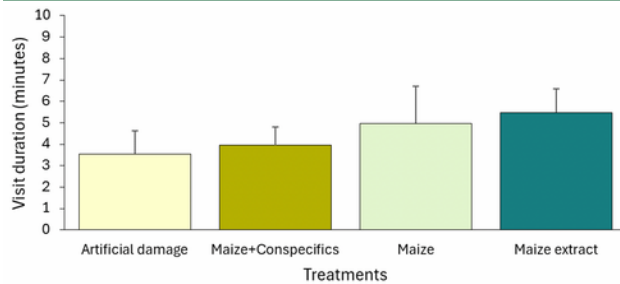


Figure 3. Mean time (\pm SE) spent by male leafhoppers in olfactometer arms characterized by different scents (treatments). No significant differences were found among treatments.

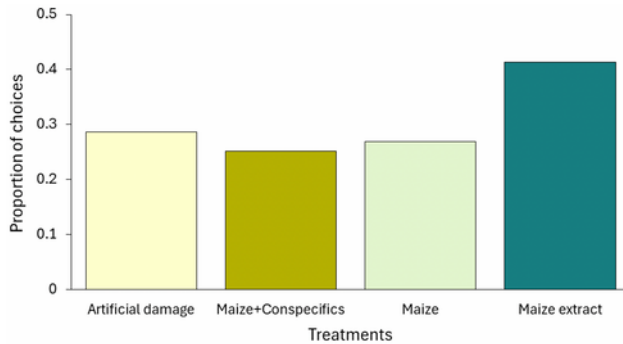


Figure 4. Proportion of choices for the scented arm (i.e., number of times the leafhopper selected the scented arm divided by the total number of choices across all arms). No significant differences were observed across treatments.

Discussion

The primary aim of this investigation was to assess the choice behavior of male *D. maidis* leafhoppers in response to various volatile stimuli, including scents from maize plants, maize plants with conspecifics, and isolated conspecifics. We found that male leafhoppers spent significantly more time in the arm with the scent of both sexes compared to the arm with scents from other males. However, the time spent in arms with female scents and extracts was not significantly different from the other treatments. We also did not find differences regarding the number of choices for the scented arm and the visit duration in response to different stimuli from conspecifics and maize plants.

Based on the findings from two separate experiments, we could infer that the choice of these leafhoppers in many cases appeared to be neutral towards the tested volatile stimuli. Our results suggest that male *D. maidis* leafhoppers do not exhibit a strong attraction to the examined scents, indicating that several volatiles alone may not play a significant role in their choice behavior. This neutral response could be attributed to various factors, including the specific blend of compounds in the tested stimuli, sex-related differences, or the possibility that male leafhoppers rely on additional sensory cues for orientation and host plant selection.

Response to scent from conspecifics

This study was based on an experimental design that focused on the orientation behavior of males. This approach was motivated by the notion that, in natural environments or courtships, males often play a crucial role in mate selection (West-Eberhard, 1984; Ramirez-Romero *et al.*, 2014). Under this scenario, we included treatments that posited the three possibilities that a male might encounter: 1) other males, 2) females, or 3) females and males together. For the first two, we used a number of individuals that has been used in previous similar experiments (Todd *et al.*, 1990b). For the third treatment we used a combination of individuals that can occur in natural conditions, as far as we have been able to observe in the field. Additionally, we used a treatment that explored the response toward female body extract, inspired by previous studies (Xu *et al.*, 2019). We found that none of these stimuli triggered a specific and consistent orientation by males, since the proportion of choices for different scented arms of the olfactometer did not differ across treatments. However, there was a tendency for males to orient more frequently toward the stimulus coming from the female and male conspecifics together, and males indeed spent more time in the arm with the scent of both sexes, compared with the males-only treatment (Figure 1). It is possible that the presence of both sexes stimulates the presence of pheromones and that, by increasing the number of partners present, the concentration of aromatic stimuli increases and produces a stronger response (Hansson, 1999). Further investigation is required to explore this possibility by isolating the volatile compounds produced during this species' courtship phase and comparing them with those captured in the absence of courtship. Such analysis could provide valuable insights into the presence of a potential pheromone in this species.

The minimal orientation behavior towards conspecific scents by males observed in the present study contrasts with that of other experiments, where body extracts with non-polar solvents (e.g., pentane, hexane) elicited a positive response towards these scents in hemipterans of the families Triozidae and Psyllidae (Guédot *et al.*, 2009b; 2010). However, to our knowledge, the present study is a pioneer in the analysis of the behavioral response of males to conspecific scents in the family Cicadellidae. Our results might be due to that: 1) the emission source does not produce any long-range volatile compound (e.g., lipids or cuticular hydrocarbon compounds that act as sexual recognition signals [Guédot *et al.*, 2009b]) and therefore, the receiving individuals do not have appropriate structures to interpret such stimuli; or 2) aromatic stimuli alone fail to produce a positive reaction and therefore act concomitantly with other communication mechanisms such as sounds or courtship calls (Heady *et al.*, 1986).

Few studies have been published on pheromones in hemipterans of the Suborder Auchenorrhyncha. For instance, the existence of alarm pheromones has been observed in certain Membracidae species (Nault *et al.*, 1974), and the presence of aggregation pheromones has been documented in the rice spittlebug *Callitettix versicolor* (Hemiptera: Cercopidae) (Chen and Liang, 2015). In males of the Family Cercopidae, some anatomical structures have been proposed as pheromone production centers, but this activity has not been confirmed (Liang, 2004). Moreover, in a Y-tube olfactometer assay, males of *Philaenus spumarius* (Hemiptera: Aphrophoridae) were attracted to the scents of virgin females, whereas females did not respond to male scents (Sevarika *et al.*, 2022).

Low molecular weight pheromones allow a rapid diffusion of the signal, with a rapid decline in concentration, whereas high molecular weight volatile compounds act at a shorter distance, at high concentrations in the area near the emitter, forming a scent layer on the substrate, which in a few cases exceeds a few millimeters (Wyatt, 2003). Chemically, most insect pheromones comprise simple or complex volatile molecules of low molecular weight. Those of long range, or low molecular weight, act over limited time at specific times (Hermann, 2010). According to our results and observations made during the courtship of *D. maidis* (Ramirez-Romero *et al.*, 2014), it is possible that females do not produce long range sex pheromones, as in some groups of Orthoptera where acoustic signals play a more important role (Otte, 1977). But rather, that this species produces volatiles that act at a short distance. It is important to note that, within the olfactometer, the scents travel approximately half a meter before reaching the area where insect observations are conducted. Alternatively, it is possible that they produce low molecular weight volatiles, but with a short release time (Wyatt, 2003), so that for example, the chemical signals emitted by the female are not sexually specific by themselves, but are so within the context of courtship, as occurs in some cockroach species (Schal *et al.*, 1984); that is, olfactory cues alone may not be sufficient to elicit a positive response in the orientation behavior towards conspecifics of *D. maidis*. Therefore, capturing and analyzing the volatile compounds potentially produced during the courtship phase at varying distances from the insect pairs could provide additional insights into the nature of these scents.

In the case of cycadellids, acoustic signals appear to be a determining factor in mate choice and copulation (Heady *et al.*, 1986; Nihardiyati and Bailey, 2005). Given that visual and acoustic stimuli were eliminated in the experiments conducted here, male leafhoppers might had

needed this synergy of signals to show a significant response to the scents of conspecifics. Detailed observations on the anatomy or electroantennographic response in *D. maidis*, as well as analysis of the volatiles possibly emitted during these phases, are required to determine the full significance of scents in intraspecific communication in the corn leafhopper.

Response to plant scents

As for the attraction to volatiles from conspecifics, the attraction to a plant depends on several factors of both the plant and the phytophagous species (Meiners, 2015; Davidson-Lowe and Ali, 2021; Zhou and Jander, 2022). Different intrinsic characteristics of the host plants, such as color, scent, flavor, or the size and shape of their leaves, influence the preference and choice of insects (Finch and Collier, 2000; Zhang *et al.*, 2022; Czarnobai De Jorge *et al.*, 2023). Although it has been previously suggested that Auchenorrhyncha do not predominantly rely on volatile signals for communication, some studies have presented evidence that challenges this paradigm (Mazzoni *et al.*, 2009; Coll-Aráoz *et al.*, 2019; Hill *et al.*, 2023).

Coll Aráoz *et al.* (2019) evaluated olfactory responses of *D. maidis* and *Peregrinus maidis* in dual-choice tests (with no air flow) to three maize germplasms: a temperate hybrid, a tropical hybrid, and a landrace. The responses of the insects to Volatile Organic Compounds (VOC) were measured in the absence of visual cues. The temperate hybrid released significantly more VOCs than the other two germplasms, with linalool being the dominant compound. *Dalbulus maidis* preferred the odors emitted by the temperate hybrid, while *P. maidis* preferred the odors from the tropical hybrid and the landrace. Addition of linalool to the tropical hybrid's VOCs attracted *D. maidis*, suggesting that this monoterpene could be part of a blend of attractants for this species, independent of visual stimuli. It has also been noted that preferences for certain host plants can be influenced by sex-specific learning (Stockton *et al.*, 2016). *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), a phloem-feeding citrus pest, can associatively learn cues in both olfactory and visual contexts: males and females displayed differing discriminatory abilities, with males exhibiting recognition of a compound stimulus alone and females capable of learning individual components. The response to olfactory cues was affected by bacterial infection of plants, which changes the type of volatiles released (Stockton *et al.*, 2016).

Considering all the above, it could be argued that volatile signals independently could play a role during the localization of these plants in monophagous insect specialists, such as the maize leafhopper (Nault, 1998; Coll Aráoz *et al.*, 2019). However, the results of this study do

not show that maize volatiles elicit a positive choice response from male *D. maidis* toward the scents, since individuals did not exhibit a preference for the extract made from maize leaves or any of the other treatments. Although it is important to highlight that our olfactometer setup included a flow of humidified air, in contrast to the study by Coll Aráoz *et al.* (2019), which employed an olfactometer without airflow. This difference suggests that this species may be more responsive to static, short-distance scents than scents traveling over a distance (as discussed above for conspecific scents). Future studies should investigate the nature of these plant-related scents to better understand their potential use as attractants. Indeed, a recent study by Hill *et al.* (2023) demonstrated that female *D. maidis* produce significantly higher amounts of volatile compounds in maize than males, suggesting that the types of volatiles generated differ between the sexes.

The neutral orientation behavior of males towards olfactory stimuli from maize may be because the aromatic stimulus alone does not cause a positive reaction and therefore could act synergistically with other stimuli, such as leaf color, similar to what has been observed in other cicadellids (Rodríguez-Saona *et al.*, 2012; Bian *et al.*, 2020). Todd *et al.* (1990b) documented the synergy between green (or white) light and a corn leaf extract by analyzing the number of contacts *D. maidis* made with the light source, suggesting an attraction to short-distance volatile stimuli. Our results showed a neutral attraction to the volatiles of the proposed treatments; however, it must be considered that the four-way olfactometer is used to detect and measure the attraction response of insects to medium and long-distance olfactory cues (Vet *et al.*, 1983). Perhaps attraction of volatiles over medium and long distances is possible if there is a visual stimulus in conjunction, but this was not determined in our bioassays.

Intact host plants elicit a positive orientation response in *Cicadulina storeyi* leafhopper, while volatiles emitted by maize infested with their congeners repel it (Oluwafemi *et al.*, 2011), contrary to what was observed in this study, as both treatments show no significant differences from each other, and even *D. maidis* responded randomly to the stimulus of the intact plant. Different maize varieties produce volatiles associated with herbivory damage in different amounts and composition, which may or may not attract some insects (Fritzsche Hoballah *et al.*, 2002). Additionally, certain herbivores, such as the fall armyworm *Spodoptera frugiperda*, prefer the scents emitted by undamaged maize plants over those released by maize plants that have sustained herbivory damage (Signoretti *et al.*, 2012). Thus, phenology and the type of damage sustained by the plant could be of importance when *D. maidis* are oriented in a certain direction. However, further observations are necessary to investigate these factors and validate these possibilities thoroughly.

The reported olfactory preferences and their influence on the orientation behavior of male *D. maidis* appear to be primarily associated with locating conspecifics, as indicated by our findings, where responses were only elicited by the presence of conspecific pairs rather than maize plant volatiles. This finding suggests that the process of host plant identification in male *D. maidis* may require additional sensory cues, such as tactile stimuli or acoustic signals, to facilitate the localization of their host plants effectively (Sivinski and Calkins, 1986; Renou and Anton, 2020). To fully understand the specific role of olfaction in male *D. maidis*, further studies are needed to investigate how these volatiles, in combination with other sensory cues, contribute to their attraction and orientation responses. Alternatively, the response to plant-related scents may be influenced by sex. In fact, Coll Aráoz *et al.* (2019) demonstrated that females of this species exhibited a clear attraction to these scents. Therefore, in addition to exploring the nature of these scents (whether they are short- or long-range), it would be worthwhile to investigate whether the observed scent response in this species is inherently sex-dependent (Hill *et al.*, 2023). Given that females are responsible for oviposition and thus for locating suitable host plants, it is plausible that they may exhibit a more robust response to plant-related scents compared to males (Meiners *et al.*, 2005). The use of electroantennographic tools could confirm the importance of volatile stimuli in the orientation of this cicadellid species.

Conclusions

The results of this study show that olfactory signals alone play a minor role in the orientation of male *D. maidis* to conspecifics, as well as to aromatic stimuli from the host plant-conspecific complex. Future studies are needed to determine whether the minimal attraction observed in males is attributable to the nature of the scents (whether they are long- or short-range) or if the underlying reasons are linked to sex-specific factors.

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