# Does the corn leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) reproduce in hosts other than maize?

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**Abstract.** The corn leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) is a specialist insect vector of the corn stunting disease complex. This leafhopper has been reported from different overwintering plants, and more information is required regarding other potential Brazilian plants that may serve as reproductive hosts for this specie. The present study aimed to evaluate possible plants for leafhopper reproduction usually found near the maize fields and the *D. maidis* reproductive differences among maize genotypes. Three trials were conducted to assess *D. maidis* survival and oviposition on different maize genotypes and other potential host plants. Survival, nymph presence, oviposition, and nymph eclosion rates were observed. No nymphs were observed in plants other than maize. The number of *D. maidis* adults was influenced by the different maize inbreds, and the mean number of laid eggs varied across the tested maize materials. Oviposition and nymph emergence was observed in pearl millet [*Pennisetum glaucum* (L.) R. Br., Poaceae] when *D. maidis* adults were offered only this plant as substrate, although significantly lower than those recorded on maize. Our results suggest that eliminating maize plants in the off-season could be used as a tool to reduce *D. maidis* reproduction, thereby minimizing the vector population size in the following season.

**Keywords:** Corn stunt; Insect vectors; Mollicutes; Oviposition; Pearl millet.

Maize production is significantly threatened in tropical and subtropical America by the specialist insect vector *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), the corn leafhopper. Damage can occur directly through feeding and oviposition in maize seedlings (Waquil 1997; Virla *et al.* 2021), or indirectly by transmitting spiroplasma mollicutes (*Spiroplasma kunkelii* Whitcomb *et al.*- Mycoplasmatales: Mycoplasmataceae) (Whitcomb *et al.* 1986), the maize bushy stunt (MBS) phytoplasma (Bedendo *et al.* 1997), and the rayado fino virus (MRFV) (Lopes & Oliveira 2004). Maize producers are concerned with the economic losses caused by *D. maidis*, which is based on the presence or absence of the vector in the fields. Corn stunting diseases often causes multiple small ears and missing kernels in plants, which is the most important limiting factor of maize production (Oliveira *et al.* 2003; Virla *et al.* 2004; Jones & Medina 2020), as yield losses in susceptible maize hybrids can be up to 90% (Sabato *et al.* 2013; Torres *et al.* 2013). Nevertheless, no control strategy targeting this phytopathogen has been established. Instead, control methods are based on attempts to suppress insect vectors (Pérez-López *et al.* 2018).

Maize is the main host of *D. maidis* (Oliveira & Lopes 2004; Oliveira & Sabato 2017). According to Scott (1981), in addition to maize and its ancestor teosinte (*Zea mays mexicana* (Schrad.) Iltis and *Zea diploperennis* H.H.Iltis Doebley & R.Guzman & Pazy B.- Poales: Poaceae) species of the genus *Tripsacum* L. (Pitre 1970) could also serve as hosts for the entire biological cycle of *D. maidis*, from immature to adult (Burckhardt *et al.* 2014). The restricted range of host plants is a major challenge for *D. maidis* survival, especially during the maize off-season (Oliveira & Lopes 2004). However, Waquil *et al.* (1999), Summers *et al.* (2004), Oliveira *et al.* (2013) and Sabato *et al.* (2018) reported that leafhoppers can use other plants close to maize crops to obtain refuge and food during the off-season; for instance, they may use shelter plants, according to the terminology of (Burckhardt *et al.* 2014). Although this varies among plant species (Ramos 2016; Sabato *et al.* 2018), the presence of these shelter plants is essential for leafhopper survival when the preferred host crop is absent. The ability of *D. maidis* to survive and oviposit on other plant species in the absence of maize plants is speculated, especially in periods greater than 3-4 months (Oliveira *et al.* 2020).

Understanding the dynamics of *D. maidis* and how it survives in the absence of the main host plant requires further study (Oliveira *et al.* 2020) and is crucial for developing effective methods for managing pest outbreaks. Hence, our study aimed to evaluate the reproductive capacity of *D. maidis* in alternative plants that are easily found near maize

fields which may be used as shelter plants, and in different maize germplasms. This study evaluated previously untested potential host plants associated with Brazilian maize fields and compared oviposition and survival between maize germplasms.

## MATERIAL AND METHODS

Three different trials were carried out in a greenhouse and laboratory to compare the potential host plants for *D. maidis* additionally to different maize germplasms, as described below.

**Comparison of the survival and reproductive capacity of *D. maidis* on different maize germplasms and other potential host species (Trial I).** Trial I was performed in Itumbiara, Goiás, Brazil (49°12'00.63" S, 18°20'05.18" W) from August to October 2016. Twelve treatments were arranged in a randomized complete block (RCB) design with eight replicates each, totaling 96 plots or cages. The tested species are listed in Table 1, and Table 2 provides details regarding the maize germplasms used in the experiments.

Greenhouse soil was prepared with conventional tillage and fertilized with 300 kg ha$^{-1}$ of 06-24-12 (NPK). The tested host plants were established using seeds and caryopses, except for sugarcane, which was planted using seedlings. Because of their slow growth compared to the other species, sugarcane and palisade grass plots were planted 15 days prior to the others, to provide similar shelter at infestation. Seedlings were enclosed in a *voile* cage (0.6 × 0.6 × 2.4 m; l × w × h) (Foresti *et al.* 2018) soon after emergence to prevent other insects from entering the plots. The cages were fixed using suspended chains attached to the top of the greenhouse. Prior to infestation, extra plants were thinned to achieve a final stand of four plants in maize plots (40 × 40 cm between rows and plants within a row); six plants in soybean, sorghum, johnson grass, and pearl millet plots (40 cm between rows and 20 cm between plants within a row); and 12 plants in palisade grass plots (40 cm between rows and 8 cm between plants within a row). Sugarcane plots had a final stand of two plants, 20 cm apart.

All experimental cages were infested on the same day, 30 days after sugarcane and palisade grass planting and 15 days after the planting of all other species. Each cage was infested with 50 healthy leafhopper adults (7-10 days old) from colonies reared in laboratory maize plants following the methodology of Oliveira *et al.* (2017). Because of the high number of insects released in each plot, the sex ratio was not verified. The insects were kept in cages until natural death (25±5 °C). The cages were kept in the plots until trial completion.

The presence/absence of nymphs in the plants (without quantification) was first verified 15 days after infestation (DAI), by visual inspection through a zippered opening in the cage. Plots in which no living nymphs were found within 2 min of assessment were considered "without nymphs". The number of adults per plot was quantified at 41 DAI. One day before counting, yellow sticky traps were installed inside the cages to facilitate adult collection. Leafhoppers not glued to the traps were manually collected.

**Alternative host plants for *D. maidis* in comparison to maize hybrids (Trial II).** Trial II was conducted in Planaltina, Distrito Federal, Brazil (47°36'35.87" S, 15°43'32.84" W) from October 2019 to January 2020. The species tested are listed in Table 3.

Sixteen treatments were arranged in an RCB design with four replicates, totaling 64 experimental plots. A similar methodology from Trial I was used, except that the number of adult leafhoppers infested per plot was increased to 150 per cage. In addition, the presence/absence of nymphs was determined at 15, 30, and 45 DAI, and the number of adults at 50 DAI.

**_Dalbulus maidis_ oviposition and nymph eclosion in selected plants (Trial III).** Trial III was conducted in Toledo, Paraná, Brazil (53°45'32.16" W, 24°40'19.74" S) from July to September 2019. Treatments were selected based on the observations from Trial I. The tested species and varieties

**Table 1.** Plant species tested in Trial I.

| Entry | Species | Variety[1] | Scientific name |
|---|---|---|---|
| 1 | Maize hybrid | 30F53 | *Zea mays* L. |
| 2 | Maize inbred | Inbred 1 | *Z. mays* L. |
| 3 | Maize inbred | Inbred 2 | *Z. mays* L. |
| 4 | Maize inbred | Inbred 3 | *Z. mays* L. |
| 5 | Maize inbred | Inbred 4 | *Z. mays* L. |
| 6 | Popcorn | - | *Z. mays* L. |
| 7 | Palisade grass | Marandu | *Urochloa brizantha* (Hochst. Ex A. Rich.) R. D. Webster |
| 8 | Pearl millet | - | *Pennisetum glaucum* (L.) R. Br. |
| 9 | Sorghum | 50A10 | *Sorghum bicolor (*L.) Moench. |
| 10 | Johnson grass | - | *S. halepense* (L.) Pers. |
| 11 | Soybean | P98Y51 | *Glycine max* (L.) Merr. |
| 12 | Sugarcane | Cana Caiana | *Saccharum officinarum* L. |

[1]Varietal names are given for test plants when known.

**Table 2.** Details of maize inbreds used in the experiments.

| Variety | Year | CRM[1] | Germplasm main origin | Corn stunt score[2] |
|---|---|---|---|---|
| Inbred 1 | 2010 | 139 | Tropical | 5 |
| Inbred 2 | 2012 | 122 | Temperate | 1 |
| Inbred 3 | 2010 | 143 | Tropical | 7 |
| Inbred 4 | 2003 | 113 | Temperate | 1 |

[1]Comparative relative maturity [2]Average score based on multiple observations during the breeding process. Corteva™ agriscience internal evaluations, using a 0-9 scale (adapted from Neves *et al.* 2021), where 0 = dead plant and 9 = no corn stunt symptoms.

**Table 3.** Plant species tested in Trial II.

| Entry | Species | Variety¹ | Scientific name |
|---|---|---|---|
| 1 | Broadleaf carpetgrass | - | *Axonopus compressus* (Sw.) P. Beauv. |
| 2 | Indian goosegrass | - | *Eleusine indica* (L.) Gaertn. |
| 3 | Soybean | P98Y51 | *Glycine max* (L.) Merr. |
| 4 | Pearl millet | Inbred 3 | *Pennisetum glaucum* (L.) R. Br. |
| 5 | Sugarcane | Cana Caiana | *Saccharum officinarum* L. |
| 6 | Sorghum | 50A10 | *Sorghum bicolor (*L.) Moench. |
| 7 | Johnson grass | - | *S. halepense* (L.) Pers. |
| 8 | Palisade grass | Marandu | *Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster |
| 9 | Palisade grass | Mg-4 | *U. brizantha* |
| 10 | Palisade grass | BRS Piatã | *U. brizantha* |
| 11 | Palisade grass | BRS Paiaguas | *U. brizantha* |
| 12 | Palisade grass | Mg-5 Xaraés | *U. brizantha* |
| 13 | Signal grass | Basilisk | *U. decumbens* (Stapf) R. D. Webster |
| 14 | Koronivia grass | Humidicola | *U. humidicola* (Rendle) Schweick |
| 15 | Congo grass | Ruziziensis | *U. ruziziensis* (R. Germ. & C.M. Evrard) Crins |
| 16 | Maize hybrid | 30F53 | *Zea mays* L. |

¹Varietal names are given for test plants when known.

were maize hybrid var. 30F53, maize inbred varieties 1-4, popcorn, and pearl millet. These seven treatments were arranged in a completely randomized design with eight replicates, totaling 56 experimental plots (4.9-L pot with one plant).

A cone-shaped *voile* cage (0.1 × 0.6 m) was used to enclose the individual plants 10 days after planting. The bottom of the cages was fixed in the pot using a PVC tube buried in the soil, and a rope was used to support the cage. Plants were artificially infested with 7-10-day-old *D. maidis* adults from the first generation of a field-collected greenhouse-reared colony. When the maize plants were in the $V_2$ growth stage (10 days after planting), each cage was infested with 10 leafhopper adults. After 72 h, both insects and cages were removed.

Five days after adult removal, the plants were cut, identified, stored in plastic bags, and taken to the laboratory (26±2 °C) for egg counting using a magnifier. As the embryos were well developed and the eyespots were evident, no staining techniques were used. Egg counting was performed on the entire plant, and an entomological needle was used to find and count the number of eggs deposited in inconspicuous places of the plant. Then, a leaf section with approximately 15 eggs was removed from each plant, stored in a square quartz petri dish with 1% agar solution, and maintained in a growth chamber (26±2 °C) until nymph eclosion. Nymphs were counted 3 and 6 days after egg counting, corresponding to 8 and 11 days after adult removal. Eggs that had not hatched by day 11 were considered unviable.

**Statistical analysis.** Nymphs were classified as present or absent. Normality and homogeneity of variance were checked using Shapiro-Wilk and Bartlett's tests. The number of adults collected, number of eggs, and percentage of nymph eclosion were subjected to Kruskal-Wallis test and comparison of means using Tukey's test (p > 0.05) using Minitab software (MINITAB 2021).

## RESULTS

**Comparison of the survival and reproductive capacity of *D. maidis* on different maize germplasms and other potential host species (Trial I).** No leafhopper nymphs were found in plots with plants other than maize (Table 4).

In contrast, all plants in plots with maize (i.e., hybrid, inbred, and popcorn) contained at least one nymph (Table 4), and the further evaluation of the number of leafhopper adults confirmed reproduction in these species (Figure 1). The grand average of adults on plants with leafhopper reproduction was 264 (CV = 21.85%; n = 48). Regarding the number of *D. maidis* adults, significant differences among the maize germplasms were found ($p < 0.0001$, H = 78.77, df = 11; Figure 1). The number of *D. maidis* adults in inbred 3 was lower than that in inbred 1 and inbred 4 (Figure 1). All insects experimentally infested on plants other than maize died between infestation and adult counting at 41 DAI.
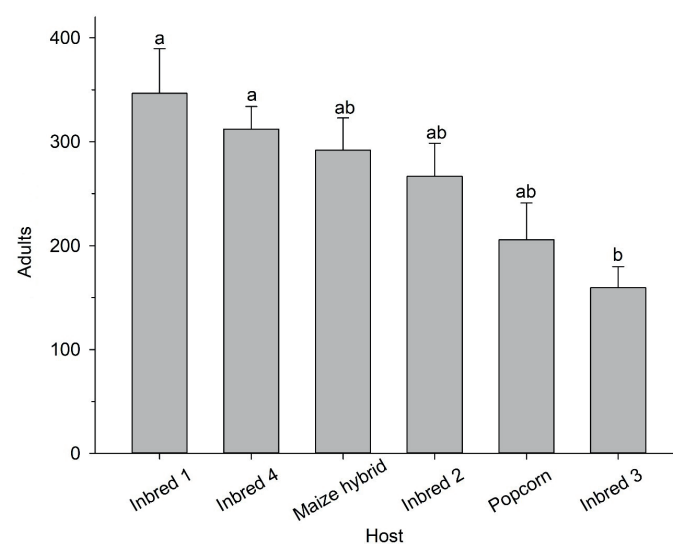


**Figure 1.** Mean number of *Dalbulus maidis* (Hemiptera: Cicadellidae) adults on different host maize (*Zea mays*) varieties at 41 days after infestation. Different letters represent statistical differences among hosts (Tukey's test; $P \leq 0.05$), Trial I.

**Alternative host plants for *D. maidis* in comparison to maize hybrid (Trial II).** As in Trial I, no *D. maidis* nymphs were found in the treatments with plants other than maize hybrid, the only maize germplasm tested in this experiment (Table 5). The number of leafhopper adults collected 50 DAI differed between the potential host plants and the maize hybrids ($p < 0.0001$, H = 45.47, df = 15; Table 5). The average number of adults on the maize hybrids was 921 (CV = 12.87%, n = 4).

**Table 4.** Observation of *Dalbulus maidis* (Hemiptera: Cicadellidae) nymphs on various plant species, Trials I and II.

| Species (trial I[1]) | Nymphs | Species (trial II[2]) | Nymphs |
|---|---|---|---|
| Maize hybrid, 30F53 | + | Broadleaf carpetgrass | - |
| Maize inbred, Inbred 1 | + | Indian goosegrass | - |
| Maize inbred, Inbred 2 | + | Soybean, P98Y51 | - |
| Maize inbred, Inbred 3 | + | Pearl millet | - |
| Maize inbred, Inbred 4 | + | Sugarcane, Cana Caiana | - |
| Popcorn | + | Sorghum, 50A10 | - |
| Palisade grass, Marandu | - | Johnson grass | - |
| Pearl millet | - | Palisade grass, Marandu | - |
| Sorghum, 50A10 | - | Palisade grass, Mg-4 | - |
| Johnson grass | - | Palisade grass, BRS Piatã | - |
| Soybean, P98Y51 | - | Palisade grass, BRS Paiaguas | - |
| Sugarcane, Cana Caiana | - | Palisade grass, Mg-5 Xaraés | - |
| | | Signal grass, Basilisk | - |
| | | Koronivia grass, Humidicola | - |
| | | Congo grass, Ruziziensis | - |
| | | Maize hybrid, 30F53 | + |

+ = present; - = absent. [1]Considering eight replicates 15 days after infestation [2]Considering four replicates at 15, 30, and 45 days after infestation.

**Table 5.** Mean number of *Dalbulus maidis* (Hemiptera: Cicadellidae) adults collected on various plant species 50 days after infestation, Trial II.

| Species | Number of adults[1] |
|---|---|
| Broadleaf carpetgrass | 0.0 b |
| Indian goosegrass | 0.5 b |
| Soybean, P98Y51 | 1.5 b |
| Pearl millet | 2.5 b |
| Sugarcane, Cana Caiana | 0.3 b |
| Sorghum, 50A10 | 0.3 b |
| Johnson grass | 3.8 b |
| Palisade grass, Marandu | 1.0 b |
| Palisade grass, Mg-4 | 1.3 b |
| Palisade grass, BRS Piatã | 0.5 b |
| Palisade grass, BRS Paiaguas | 0.0 b |
| Palisade grass, Mg-5 Xaraés | 1.8 b |
| Signal grass, Basilisk | 2.5 b |
| Koronivia grass, Humidicola | 0.3 b |
| Congo grass, Ruziziensis | 1.3 b |
| Maize hybrid, 30F53 | 921.3 a |

[1]Means. Different letters denote statistical differences among hosts (Tukey's test; $P \leq 0.05$).

***Dalbulus maidis* oviposition and nymph eclosion in selected plants (Trial III).** Leafhopper oviposition under greenhouse conditions varied among maize germplasm and pearl millet ($p < 0.0001$, H = 32.17, df = 6; Table 6), from 6 eggs/plant in pearl millet to 71 eggs/plant in inbred 2. *Dalbulus maidis* oviposition in pearl millet was lower than that in any other tested plant, and inbred 3 had fewer eggs than the other maize genotypes (Table 6). In contrast, the percentage of nymph eclosion under laboratory conditions was similar in all maize germplasms and in pearl millet ($p = 0.574$, H = 4.77, df = 6; Table 6).

## DISCUSSION

Our study showed the presence of nymphs only in maize genotypes in Trials I and II, which is consistent with the observations of Sabato *et al.* (2018), who did not find *D. maidis* nymphs on other Poaceae plants; however, in the present study, additional plant species were tested. Ramos (2016) also evaluated the survival of leafhoppers on different weeds and found no nymphs in any species. In contrast, Pitre (1967) found nymphs of *D. maidis* on various cultivated and non-cultivated

plants similar to those tested in our trials, such as sorghum, Johnson grass, pearl millet, and signal grass [*Brachiaria platyphylla* (Griseb.) Nash- Poales: Poaceae]. However, in their study, nymphs completed their development only on maize.

The reproduction rates of *D. maidis* on the maize hybrids of Trials I and II were almost identical, confirming that the test conditions were similar across experiments. The adults on non-host plants in Trial II probably remained from the initial infestation, as no nymphs were observed in any plot without maize. According to Larsen *et al.* (1992), *D. maidis* females can survive for 56 days with water only, without any plant for feeding or shelter. Pitre (1967) observed the complete development of *D. maidis* only on maize plants, but the author observed adult survival for more than 30 days on non-host plants. Sabato *et al.* (2018) also found that leafhopper adults survived for 35 days on alternative hosts. Trial II suggested that some adults can survive over 50 days on alternative plants, since only broadleaf carpetgrass and palisade grass (BRS Paiaguas) had no insects across all replications, reinforcing that alternative plants can provide shelter and food for *D. maidis*, although they are not suitable

**Table 6.** Mean number of *Dalbulus maidis* (Hemiptera: Cicadellidae) eggs per plant and percentage of eclosion in different hosts, Trial III.

| Species | Number of eggs[1] | Percent of nymph eclosion |
|---|---|---|
| Maize hybrid, 30F53 | 69.87 a | 67.59 a |
| Maize inbred, Inbred 1 | 70.12 a | 71.36 a |
| Maize inbred, Inbred 2 | 71.00 a | 74.36 a |
| Maize inbred, Inbred 3 | 41.87 b | 72.04 a |
| Maize inbred, Inbred 4 | 66.87 a | 70.33 a |
| Popcorn | 48.62 ab | 74.54 a |
| Pearl millet | 6.00 c | 67.59 a |

[1]Means. Different letters denote statistical differences among hosts (Tukey's test; $P \leq 0.05$).

for reproduction (Larsen *et al.* 1992; Summers *et al.* 2004; Oliveira *et al.* 2020). *Dalbulus maidis* only reproduced in maize plants, confirming its dependence on this primary host; therefore, it is essential to develop management practices that reduce vector populations across crop seasons.

Sabato *et al.* (2018) found *D. maidis* eggs on pearl millet plants; however, no nymph eclosion was observed. Although we found eggs and nymph eclosion on pearl millet plants under laboratory conditions, the number of eggs was significantly lower on pearl millet compared to that on maize. Our laboratory results suggested that pearl millet is suitable for *D. maidis* oviposition, and no nymphs were found in our greenhouse trials. These results may have resulted from the low number of eggs or viability of nymphs when restricted to feeding only on pearl millet plants. More information is needed regarding *D. maidis* nymph development in pearl millet plants compared to maize to understand the effect of this specie on the leafhopper population dynamics. No difference was found in the percentage of nymph eclosion among the plant species, including maize. Apparently, there is no effect from physical leaf structure on *D. maidis* nymph eclosion, which may also be related to good adult nutrition before infestation.

The difference in the number of eggs laid and adults in maize inbred varieties may be related to the volatile organic compounds released by plants, which are involved in host recognition. Coll Aráoz *et al.* (2019) found that *D. maidis* adults preferred odors emitted from temperate hybrids over tropical ones. Inbred 3 is the most tropical variety tested, showing characteristics such as late cycle and leaf disease resistance. In addition, it has the highest resistance to corn stunt among the tested genotypes (Corteva Agriscience internal research data). In addition to chemical compounds, physical leaf defenses and tolerance to direct vector damage can also influence *D. maidis* reproduction in different maize lines (Waquil 1997; Bellota *et al.* 2013; Virla *et al.* 2021). Waquil (1998) evaluated 42 hybrids and found significant differences in the number of adults and eggs laid per plant. Recently, Faria *et al.* (2021) found variable levels of resistance in maize genotypes (different genotypes used for our study), and the reduced oviposition rate was associated with antixenosis related to leaf hardness.

Our results indicate that *D. maidis* reproduction depends on maize plants in the field; the elimination of volunteer maize plants should be used as a tool to reduce *D. maidis* reproduction, leading to a reduced vector population size in the following season. Further studies are necessary to verify the survival capacity of corn leafhoppers under the availability of pearl millet plants only. Furthermore, other plants should be tested to identify those that may act as hosts for this important vector and determine the role of these plants in the survival strategy of *D. maidis*.

In summary, the number of *D. maidis* eggs and adults varied among the maize varieties under greenhouse conditions. The

oviposition of *D. maidis* was lower in pearl millet plants than in maize plants; however, nymph eclosion was observed in this species under laboratory conditions. *Dalbulus maidis* only reproduced in maize plants, and no nymphs were observed in plants other than the maize varieties in the greenhouse trials.

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