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Penetration and feeding damage produced by *Delphacodes kuscheli* on maize and barley leaves (Hemiptera, Fulgoromorpha, Delphacidae)

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Abstract – *Delphacodes kuscheli* Fennah is at present the only species demonstrated as vector of the virus that produces «Mal de Río Cuarto». This is a maize viral disease, nowadays seriously affecting this crop in Argentina. Stylet penetration and feeding damage on *Zea mays* L. and *Hordeum vulgare* L. leaves revealed that *D. kuscheli* is a typical "salivary-sheath-feeder", with salivary flange and sheath formation. The lipoproteinaceous nature of the sheath material was determined using fluorochromes and specific dyes. Stylets penetrate epidermal cells through the cuticle and produce cellular disruptions and morphological alterations in penetrated and non-penetrated adjacent cells. The pathway of stylets through mesophyll towards phloem is both intracellular and intercellular. The present study evaluates the various effects on foliar tissues of maize and barley, especially on chloroplasts and vacuoles.

Résumé – Altérations provoquées par la pénétration et la prise de nourriture de *Delphacodes kuscheli* (Hemiptera, Fulgoromorpha, Delphacidae) sur feuilles de maïs et d'orge. – *Delphacodes kuscheli* Fennah est actuellement le seul vecteur reconnu du 'Mal de Río Cuarto', une maladie virale du maïs qui sévit sérieusement en Argentine. L'étude de la pénétration des stylets et des altérations induites sur *Zea mays* L. et *Hordeum vulgare* L. montre que *D. kuscheli* produit une gaine salivaire typique. La nature lipoprotéique de la gaine est révélée par flurochromes et colorations spécifiques. Les stylets, en traversant la cuticule, pénètrent à l'intérieur des cellules épidermiques et produisent des altérations cellulaires et des modifications morphologiques s'étendant aux cellules adjacentes. Dans le parenchyme chlorophyllien, la trajectoire des stylets vers le phloème est intra- et intercellulaire. Les conséquences induites sur les tissus foliaires des deux plantes, en particulier sur les chloroplastes et les vacuoles, sont également analysées.

The Hemiptera Fulgoromorpha, commonly known as "planthoppers", represent a large group of insects, which are economically important as agricultural pests. Within, the Delphacidae occupy the third place in the world in order of importance of vectors, i.e. insects capable of transmitting viruses and other pathogens (Nault & Ammar 1989).

The delphacid *Delphacodes kuscheli* Fennah is at present the only species in Argentina with demonstrated vector capability of the disease known as "Mal de Río Cuarto del maíz" (Remes Lenicov *et al.* 1985). This is the most important viral disease presently affecting this crop. The agent is a fijivirus called Maize Río Cuarto Virus (MRCV) which causes important losses on maize crop and also affects other cultivated Poaceae (Remes Lenicov *et al.* 1999). *D. kuscheli* has more than 12 known alternate host plants which belong to the family Poaceae (Ornaghi *et al.* 1993; Remes Lenicov *et al.* 1999).

Backus (1988) reported that hemipterans specialize on a certain range of host plants, on certain parts of the host plant, and also on a preferred feeding tissue within the plant. Miles (1968, 1972) identified two behavioural strategies employed by phytophagous hemipterans to achieve this specialization. They are: 1) lacerate-and-flush feeding: the insects in this group produce copious amounts of watery saliva containing digestive enzymes. They usually insert stylets into the plant, lacerating mesophyll and parenchyma cells. These insects are primarily mesophyll-feeders, and do not produce a salivary sheath. This behaviour is typified by most of the phytophagus Heteroptera, notably cimicomorphs (e.g. tingids and phytophagous mirids) and some of the pentatomorphs (e.g. lygaeids and

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pyrrhocorids). Some homopterans also feed in this way, eg. leafhoppers in the subfamily Typhlocybinae. 2) stylet-sheath feeding: insects in this group produce at least two types of salivary secretions. One of them is the watery saliva, a vehicle primarily for digestive enzymes secreted during ingestion, accomplishing the digestion of sap and liquifying plant cellular contents and cell walls. The other type of saliva is lipoproteinaceous and is secreted while the stylets are penetrating plant tissues and solidifies rapidly on ejection. These insects generally ingest from vascular tissue, specializing on either xylem or phloem as their primary food sources. The sheath-feeding behaviour is typified by almost all homopterans but a few heteropteran taxa have also adopted this system (e.g. pentatomids). Backus (1985, 1988) discusses each strategy in detail. *D. kuscheli* was identified, preliminarily, as a phloem-feeder using the location of the sheath to determine the tissues from which the insect was ingesting (Brentassi *et al.* 1998). That identification was made on studies on Auchenorrhyncha feeding made by Pollard (1968) for Cicadomorpha Cicadellidae and by Sogawa (1982) and Spiller (1990) for Fulgoromorpha Delphacidae.

According to Virla & Remes Lenicov (1991) and Costamagna (1998) maize as compared to barley is not a prefered host plant for oviposition and feeding on the basis of low density, and absence of immature and brachypterous forms.

In this paper, microscopical examination of salivary sheaths provides evidence of the mechanism of penetration and feeding damage of *D. kuscheli* on maize and barley leaves.

Materials and methods

Insects and plants rearing

D. kuscheli were reared in growth chambers under controlled conditions of light, temperature and humidity (L 16: D 8 ; 24.9 ± 3.6 ° C; 60-70 % HR). *Z. mays* L. and H. *vulgare* L. plants were grown from seeds in a greenhouse. Plants with two leaves were used for every assay.

Vector exposure on host plants – Ten specimens of 1-2 day old, adult female planthoppers were exposed individually for 48 h on the apical zone of the second leaves on each of two host plants. For this, planthoppers were put in glass tubes 2.5 cm long by 0.5 cm diameter. The insects were starved for 4 h before they were placed on leaves. Normal leaves were used as control.

Light Microscopy (LM)

Paraplast sections – Small blocks of leaves were fixed overnight at room temperature in formaldehyde-acetic acid-alcohol (FAA) solution, dehydrated in standard ethanol series and embedded in Paraplast, then serial sectioned, at 18 µm. Sections were stained with 0.5 % aqueous safranin red and 0.1 % ethanolic fast-green and coverslipped with Eukkit.

Resin sections – Small blocks of leaves (3-4 mm thick) were fixed at 4°C for 2h in 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.5) and postfixed for 4h in 1% osmium tetroxide. Blocks were dehydrated in a graded ethanol-propylene oxide series and embedded in Spurr's resin. Sections (1µm thick) were stained with toluidine blue (Roland & Vian 1991).

Histochemical studies – In order to detect the composition of salivary secretions, staining procedures used included, Sudan black B for lipids (Bronner 1975), calcofluor

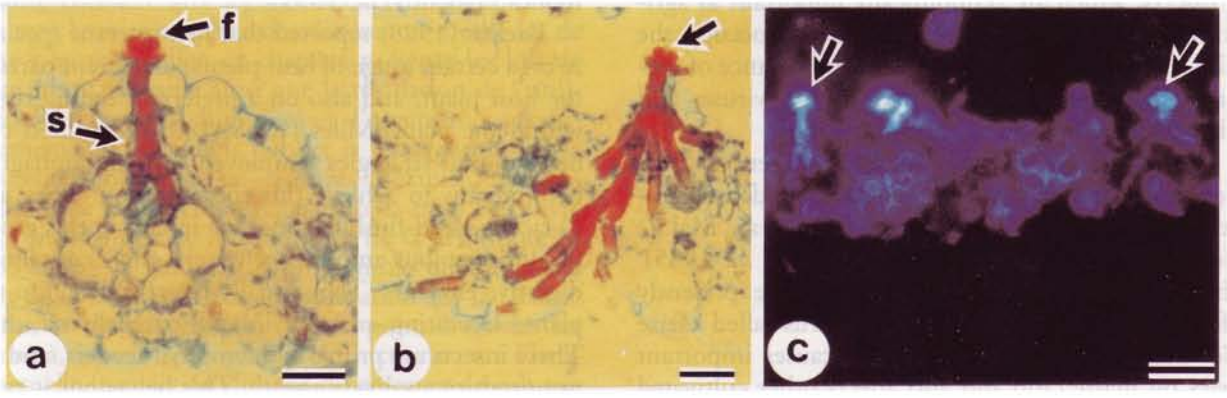

Figure1
Light (a, b) and epifluorescent (c) micrographs from Paraplast sections. – a) Flange (f) and salivary sheath (s) deposited by *D. kuscheli* on maize leaf. The salivary sheath terminates in the phloem; bar = 20 µm. – b) Branched pattern on barley leave. The salivary sheath (arrow) reaches the opposite epidermis; bar = 20 µm. – c) Fluorescence microscopy of the salivary deposit (arrow) using ANS fluorochrome on maize leaf; bar = 50 µm.

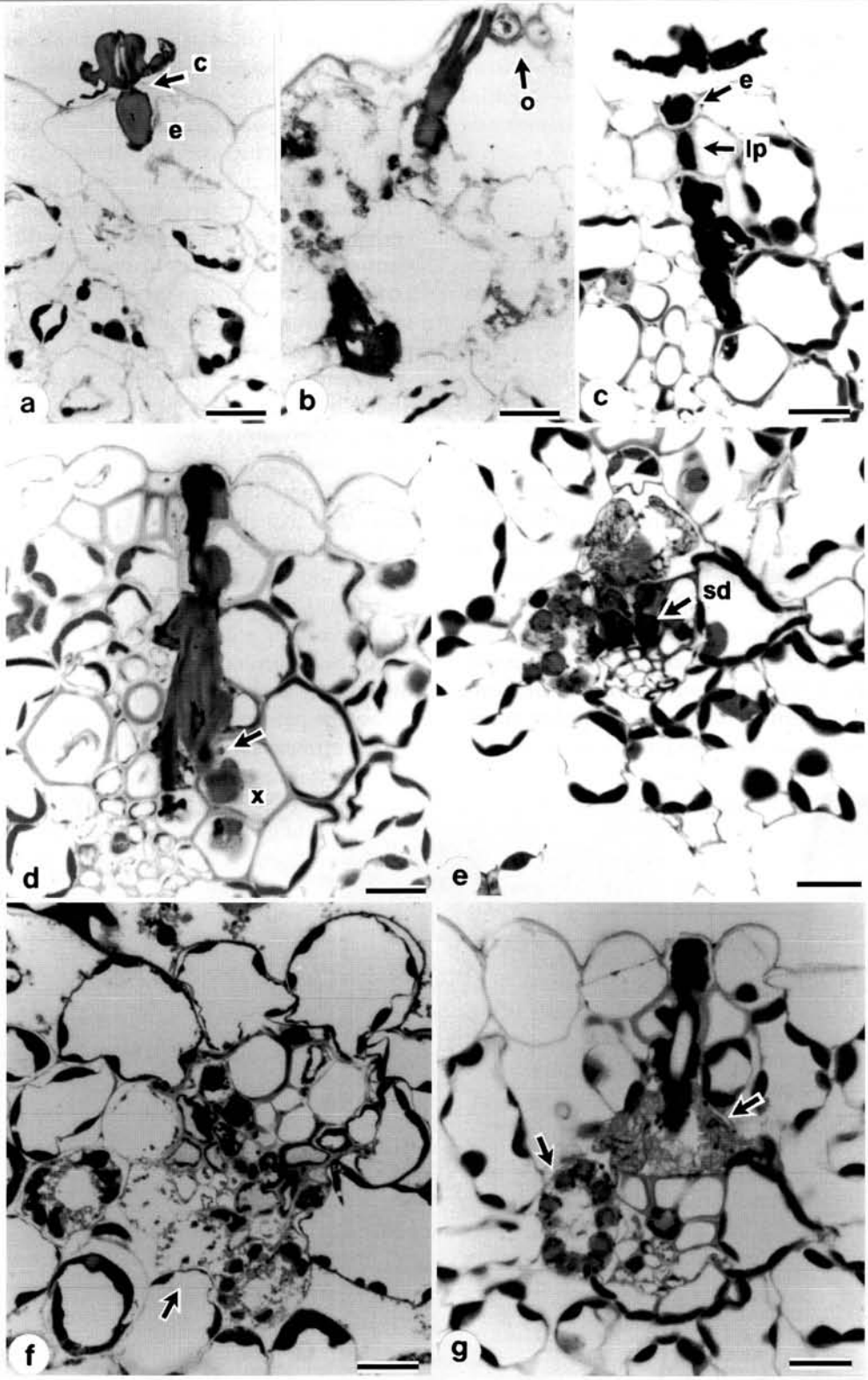

Figure 2
Light micrographs from Spurrs resin sections. Barley (a, b, f) and maize leaves (c, d, e, g); bars= 10 µm (a-g). – a) Epidermal cell (e) partly occluded with sheath material; cuticle (c). – b) Penetration through epidermal cell adjacent to a stomata (o). – c) Epidermal cell (e) totally occluded. Intercellular path of secretion (ip) in the mesophyll. – d) Degradation of cell wall (arrow) of a xylematic vessel (x). – e) Vascular tissue occluded with salivary deposit (sd). – f) Abnormal vascular bundle appearance (arrow). – g) Alteration and collapse of penetrated cell and reaction of adjacents cells (arrows).

white for polysaccharides (O'Brien & McCully 1981) and toluidine blue and fast green for proteins (Feder & O'Brien 1968; Fulcher *et al.* 1972). Proteins were also identified under fluorescence microscopy using ANS fluorochrome (Fulcher 1982).

Transmission Electron Microscopy (TEM)

Tissues embedded for semithin sections were also used for ultrastructural studies. Ultrathin sections were cut, then mounted on grids, stained with uranyl acetate followed by lead citrate (Roland & Vian 1991) and examined with a Turbo Zeiss EM 109.

RESULTS

Macroscopic observations of the general condition of healthy and injured leaves made just before they were excised, revealed that the area exposed to the vector appeared normal in both host plants. No difference in the mechanism of penetration was found between maize and barley leaves.

The feeding mark or salivary flange produced by *D. kuscheli* was observed on the epidermis of both species. These salivary deposits were spherical in form, approximately 30 µm in diameter. Deposits continued into plant tissues with the salivary stylet-sheath, a tubular structure that represents the pathways of stylets. Salivary deposits remained within the plant tissue even after withdrawal of the stylets; they stained deeply with safranin (fig. 1a). The salivary sheaths were single or branched, straight or curved and in general terminated in the phloem (fig. 1a). However, they sometimes did not reach the phloem, but instead ended in the mesophyll, or even near the opposite epidermis (fig. 1b). The sheath was most often branched and curved suggesting that stylet insertion was repeatedly intended in different directions, from the initial point of entry.

A positive reaction of the sheath material with Sudan Black and ANS fluorochrome (fig. 1c) indicated that the salivary sheath was mainly made of lipoproteic material.

The study of the salivary sheath in semithin sections (fig. 2 a-g) determined that the stylets always penetrated through the cuticle (fig. 2a). Even though penetration could occur next to the stomata, the entrance through the ostiole was never observed (fig. 2b). According to the type of cell (typical epidermal cell or bulliform cell), the sheath material either partly or totally occluded the lumen, respectively (fig. 2 a, c). Stylets pathway through mesophyll was predominantly intracellular, however intercellular penetration

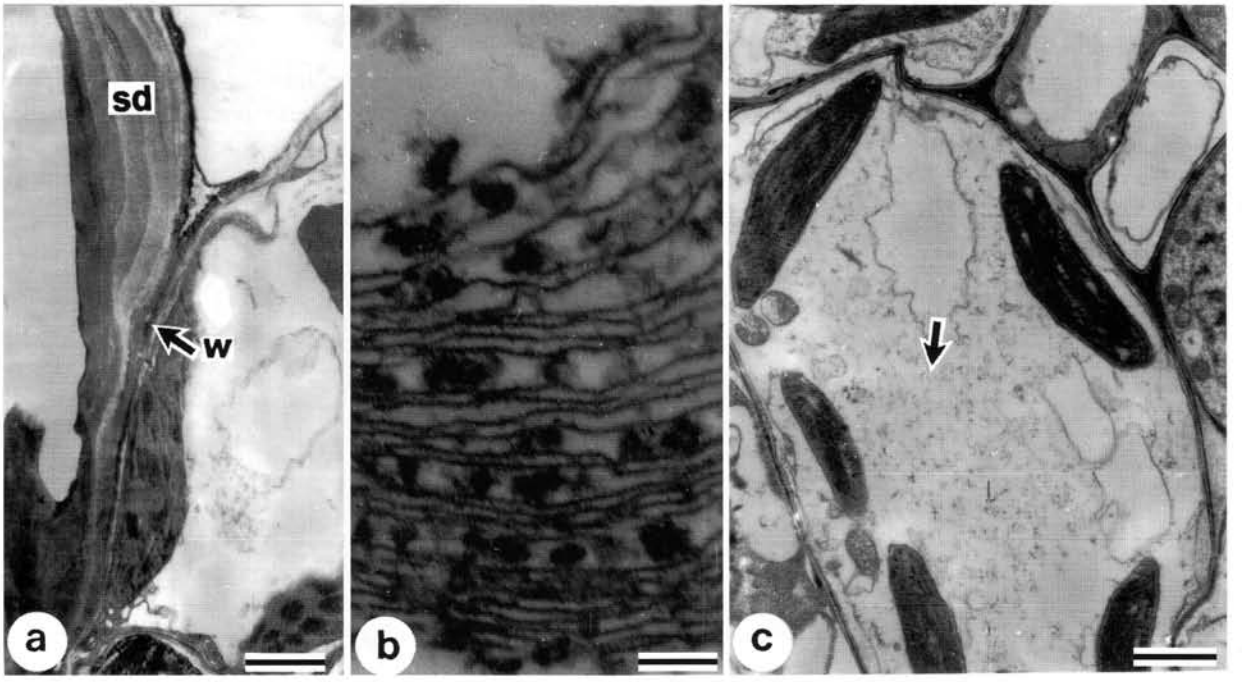

Figure 3

Transmission electron micrographs. – a) Intercellular penetration in a mesophyll cell. Salivary deposit (sd) and cell wall (w) (maize leave); bar = 0.6 µm. – b) Distorted chloroplast with degenerated membranes (maize leave); bar = 0.2 µm. – c) Vacuole of a mesophyll cell with dark staining material (arrow) (barley leave); bar = 1.21 µm.

has been also observed (fig. 2c). Once the stylets reached phloem cells, they did not advance further. If penetration occurred through the adaxial epidermis, the xylem vessels were crossed (fig. 2d). Sheath material often occluded wholly the lumen of cells of mesophyll, xylem and phloem (fig. 2e). Penetration was followed by disorganisation of vascular bundles. Non-penetrated cells next to the salivary sheath reacted by altering their normal morphology, especially causing abnormal appearance and aggregation of chloroplasts (fig. 2 f, g).

Ultrastructural studies confirmed the intercellular penetration occurring in some mesophyll cells (fig. 3a). Affected cells neighbouring the stylet pathway showed spherical chloroplasts, different from normal with ellipsoidal shape. Thylakoid membranes often appeared disorganized (fig. 3b). Vacuoles stained densely (fig. 3c), and contained coagulated lipids and proteins originating from the denatured cytoplasm.

DISCUSSION

According to Miles (1968, 1972), the salivary sheath is moulded by the action of the stylets to form a tubular lining to the path taken by them in plant tissues and the salivary flange is the external deposit on epidermal tissue. This study shows that *D. kuscheli*, as other Auchenorrhynchous vectors of plant pathogens, is a typical salivary-sheath-feeder. The salivary flange and the salivary sheath of *D. kuscheli* remaining after stylet withdrawal are well-formed and resemble those of other delphacids as *Nilaparvata lugens* (Sogawa 1982) and *Peregrinus maidis* (Backus 1985). The lipoproteinaceous nature of the sheath of *D. kuscheli*, detected by histochemical tests, agrees with the findings of Sogawa (1982) for *N. lugens*.

The large number of branches of the sheath starting from a single entrance point indicates the existence of repeated advances and partial withdrawals of stylets in order to search the host plant, as well as extending the affected area to a large number of cells. In the vascular tissues, phloem and xylem are often penetrated by repeatedly branched tracks. In this respect, Brentassi *et al.* (1998) reported that the majority of salivary sheaths produced by *D. kuscheli* terminate in the phloematic tissue.

Stylet insertion into leaves tissues is predominantly intracellular, in epidermal cells, mesophyll cells and vascular tissues. This is in agreement with studies for *N. lugens* (Sogawa1982; Spiller1990). However, on a few occasions, penetration was intercellular through mesophyll tissues.

The direct feeding damage caused by Auchenorryncha is commonly referred to as hopperburn, a noninfectious disease of many crop plants which causes yellowing of host plant. Sogawa (1982) and Ecale & Backus (1995a, b) described the causes and symptoms of hopperburn produced by *N. lugens* and *Empoasca fabae* (Cicadomorpha Cicadellidae) on rice and alfalfa plants, respectively. The present study shows the absence of macroscopic symptoms of damage on barley and maize leaves during the time of feeding of the vector in this assay. However, cellular alterations were observed under LM and TEM. The damage inflicted by *D. kuscheli* on maize and barley leaves consists essentially of occlusion of penetrated cells and disruption of non-penetrated cells next to the salivary sheath. Reactions occurring in non-penetrated adjacent cells suggest that *D. kuscheli* saliva contains enzymes that are capable of interfering with cellular metabolism. Structural symptoms include morphological changes of chloroplasts followed by thylakoid disorganization and changes in the vacuolar contents by deposition of material originating from disrupted cytoplasms. These alterations are similar to the ones produced by other phloem feeders, such as the salivary-sheath feeder *N. lugens* (Spiller 1990) and the lacerate-and-flush feeder *E. fabae* (Kabric & Backus 1990).

According to Backus (1985), the studied species of Homoptera secrete digestive enzymes that help in the feeding process by liquefying cellular contents and cell walls, i.e. carbohydrases, pectinases, hemicellulases and cellulases. Collmer & Keen (1986) suggest that a convincing role in pathogenesis has been established for enzymes that attack the pectin fraction of plant cells walls because some fragments released from the cell walls by pectic enzymes can elicit plant defence reactions. The intracellular and intercelullar pathways of the stylets of *D. kuscheli* in the tissues of host leaves indicate the presence not only of enzymes that attack the pectin fraction of the cell wall but also enzymes of the fibrillate fraction, i.e. cellulase and/or hemicellulases. We propose to investigate these topics further.

Salivary deposits of *E. fabae* on alfalfa stems occur too infrequently to mechanically block vascular tissue; feeding damage is therefore caused by a substance in the saliva of the insect that induces plant cells to enlarge and block the phloem (Kabric & Backus 1990). In *D. kuscheli,* the occlusion of the cellular

lumen with sheath material could produce the direct blockage of vascular tissues.

Miles (1972) reported that salivary sheath feeding is not accompanied by immediate damage. Deposits of solidifying components of the saliva of many species which persist in the food plants modify the long term effects of feeding by the insects. Kabric & Backus (1990) demonstrated that the time of exposure of *E. fabae* to plants could influence the degree of damage. In our study, though macroscopic symptoms of damage have been not observed after vector feeding, chlorotic spots on barley leaves used for insect maintenance in the laboratory were observed. This suggests that larger durations of vector exposition on hosts as well as sheath material persistence in plant tissues could influence the degree of damage produced.

The present study shows that both stylet penetration pattern of the vector and alterations produced consequently, are similar on maize and barley. In the field, the different behaviour of the vector on these host plants could be related to differences in their internal structure or physiology. Cook & Denno (1994) reported that the failure of a planthopper species to feed on a certain host plant species most likely results from the presence of feeding inhibitors or toxins and/or the absence of feeding stimulants. The identification of any substance that works as feeding inhibitor or toxin would allow finding the causes of non-preference for maize.

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