Feeding behavior of the vector *Delphacodes kuscheli* (Hemiptera: Fulgoromorpha: Delphacidae) on maize and oat

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Abstract. The planthopper *Delphacodes kuscheli* Fennah 1955 is an important pest affecting maize in Argentina. It transmits, in a persistent way, *Mal de Río Cuarto virus* (MRCV) (Reoviridae, *Fijivirus*) which has been reported to cause a severe loss in maize crops (*Zea mays* L.). The present study reports on the feeding behavior of the vector (adults and immature instars) on oat (preferential host) and maize (non-preferred host). Feeding sites and salivary sheaths were detected through histological sections of leaves. The location of salivary sheath terminations and the honeydew excretion analysis revealed that *D. kuscheli* feeds on phloem sap in both hosts. On maize, the high number of sheaths ending in the mesophyll and their particular disposition (parallel to the leaf surface and across the vascular bundles) strongly suggest the non-preference of the vector to feed on this species. On both hosts, immature instars showed similar feeding behavior to adults indicating the capacity to transmit the virus along the life period.

Résumé. Comportement alimentaire du vecteur *Delphacodes kuscheli* (Hemiptera : Fulguromorpha : Delphacidae) sur le maïs et l'avoine. La cicadelle *Delphacodes kuscheli* Fennah 1955 est un important ravageur du maïs en Argentine. Elle transmet de manière persistante le *Mal de Rio Cuarto virus* (MRCV) (Reoviridae, Fijivirus) qui est connu pour provoquer des pertes sévères dans les cultures de maïs (*Zea mays* L.) La présente étude traite du comportement alimentaire de ce vecteur (adultes et stades immatures) sur l'avoine (hôte préfèré) et sur le maïs (hôte non préfèré). Les sites d'alimentation et les gaines salivaires on été détectées grâce à des coupes histologiques dans les feuilles. La localisation des orifices de gaines salivaires et l'analyse des miellats exsudés ont montré que *D. kuscheli* se nourrit de sêve du phloëme chez les deux espèces-hôtes. Sur le maïs, le grand nombre de gaînes salivaires qui aboutissent au mésophylle et le disposition particulière (parallèles à la surface des feuilles et perpendiculaire aux nervures) suggère fortement la non-préférence du vecteur pour celle espèce. Chez les deux espèces hôtes, les stades immatures ont montré un comportement alimentaire identique jusqu'au stade adulte, ce qui indique leur capacité de transmettre le virus tout au long de leur vie.

Keywords: MRCV vector, *Delphacodes kuscheli*, feeding behavior, maize, oat.

The planthopper *Delphacodes kuscheli* Fennah 1955 (Hemiptera: Delphacidae) is the most important well known vector for *Mal de Río Cuarto virus* (MRCV) in Argentina (Remes Lenicov *et al*. 1985), disease that causes a severe loss in maize crops (*Zea mays* L.) (Lenardón *et al.* 1998). The MRCV belongs to serogroup II of the genus *Fijivirus*, family Reoviridae, and is persistently transmitted (Arneodo *et al.* 2002a). This virus was initially assumed as a geographically distant strain of Maize rough dwarf virus (MRDV, *Fijivirus*) which occurs in Europe and the Eastern Mediterranean region (Nome *et al.* 1981; Milne *et al.* 1983). However, molecular probes indicate that MRCV is a different entity (Marzachi *et al.* 1995; Conci & Guzmán *in lit.*). Research on transmission biology carried out with adults and nymphs of *D. kuscheli* also revealed differences in minimum acquisition access period (AAP) and minimum inoculation access period (IAP) which were lower than those registered for MRDV (Arneodo 2002a).

D. kuscheli is a native species widely distributed in Argentina (latitude 32° to 35° S) (Remes Lenicov *et al.* 1999); field studies have shown that it is multivoltine, with four generations from spring up to early autumn; it shows density dependent wing dimorphism with macropterous and brachypterous forms (Remes Lenicov *et al.* 1991). It develops outbreak populations in oat (*Avena sativa* L.), and also breeds on wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and several

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wild grasses (Remes Lenicov & Virla 1999), most of these hosts have been demonstrated as reservoirs of MRCV (Truol *et al.* 2001; Laguna *et al.* 2002). *D. kuscheli* does not breed successfully on maize, so the virus transmission occurs when the adults macropterous migrates to feed on juvenile plants due to the senescence or harvest of oat, which is its most important winter host (Tesón *et al.* 1986; Remes Lenicov *et al.* 1991; Virla & Remes Lenicov 1991; Ornaghi *et al.* 1993 a,b; Ornaghi *et al.*1999). Diverse aspects of *D. kuscheli* biology observed in laboratory (host-plants range, life tables, survivorship and life expectancy, longevity and oviposition behavior) reveal that *Zea mays* L is not a preferred host while *Avena sativa* L., *Sorghum halepense* (L.) Pers., *Bromus unioloides* L., *Cynodon dactylon* (L.) Pers., *Triticum aestivum* L., *Hordeum vulgare* L. and *Secale cereale* L. are the most suitable (Virla & Remes Lenicov 1991; Maragliano & Virla 1992; Costamagna *et al.* 1998; Brentassi & Remes Lenicov 1999). Concerning to the direct feeding damage of *D. kuscheli*, the cytological alterations caused by the stylet penetration and salivary deposits on maize and barley leaves are known (Brentassi & Maldonado 2002).

Among Hemiptera, Miles (1972) discussed the ways of feeding in relation with the salivary function and Backus (1985) described the typical behavior sequence involved in auchenorrhynchan feeding. Studies about feeding sites by means of salivary sheaths termination within plant tissues were recorded on important pests such as *Saccharosydne saccharivora* (Westwood) (Metcalfe 1969), *Nilaparvata lugens* Stål (Sogawa 1973, 1982; Kimms 1989), *Laodelphax striatellus* Fallen (Sonku & Sakurai 1973) and *Peregrinus maidis* (Ashmead) (Fisk *et al.* 1981). In addition the analysis of honeydew excretion was used as a complementary study of the feeding activity of planthoppers (Paguia *et al.* 1980; Heinrichs *et al.* 1985; Padgam & Woodhead 1988; Karim & Saxena 1991; Kumar *et al.* 2001). Among other discussed issues, Mattson (1980) studied the effect of plant physiology on the insect feeding process and Hattori (2001) recorded the effect of non-host antifeedant on the *N. lugens* behavior.

The knowledge of the feeding behavior of the vector *D. kuscheli* is an important step to a further understanding of the virus-vector-plant relationship. This paper reports on the feeding behavior of adults and immature instars of *D. kuscheli* on maize and oat leaves.

For practical purposes, in this contribution we use the specific name of this vector species as it was originally proposed by Fennah. However, its taxonomic position it is still under revision.

Materials and methods

Plants

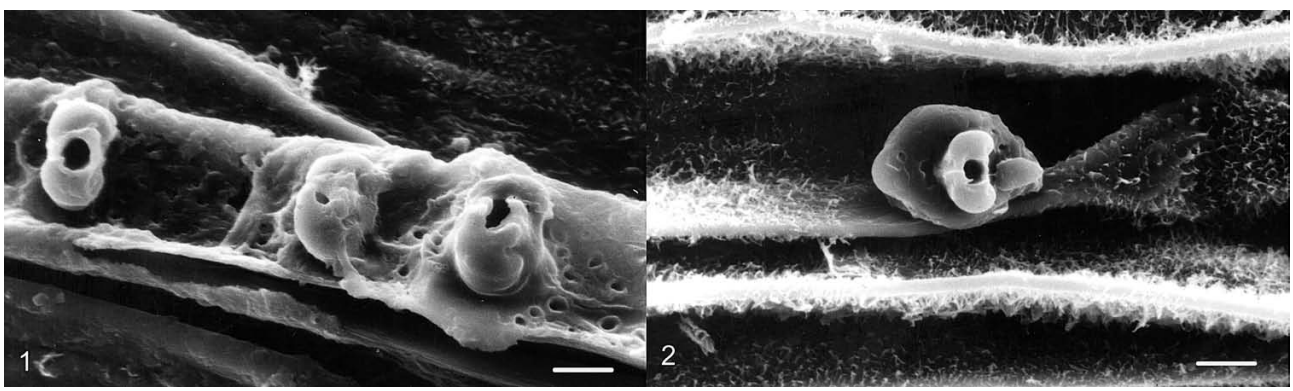
Zea mays and *Avena sativa* (avena cv Tambera), were grown in a greenhouse under natural light. All plants were used at the third leaf stage.

Insects

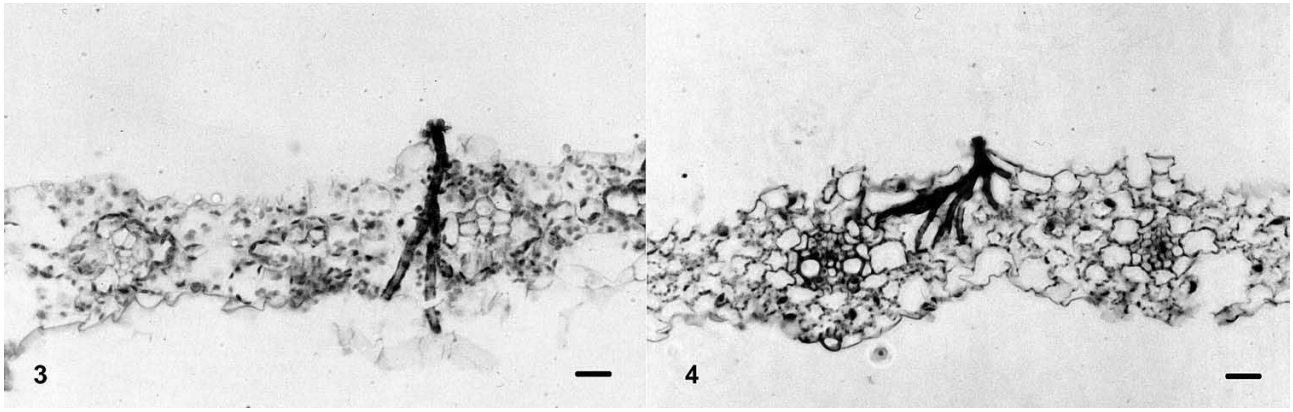
D. kuscheli was taken from stock colonies reared successively on oat seedlings at 24 ± 2 °C, 70–80% r.h. and L16:D8 photoperiod at the laboratory of the Departamento Científico de Entomología, Facultad de Ciencias Naturales y Museo, Universidad de La Plata. The vector was collected in oat fields in Río Cuarto, (Córdoba province, Argentina). In all experiments, 0-1 d old adult macropterous females and immature instars were used.

Feeding behavior

One hundred and twenty adults and 20 nymphs of each instar were caged singly for 24 h on the apical zone of the second leaves of each one of the host plants. For this purpose, planthoppers were put in glass tubes (2.5 cm long and 0.5 cm diameter). The



Figures 1–2
Salivary flanges on plant surface produced by, **1**, adults and, **2**, nymphs of *Delphacodes kuscheli*. Scale bar = 5 μm.



Figures 3–4
3, Salivary sheath produced by an adult of *D. kuscheli* traversing completely a vascular bundle of maize. **4**, Sheath of a second nymphal instar reaching the vascular tissue of oat. Scale bar = 25 µm.

insects were starved for 4 h before they were placed on leaves. Others leaves were used as control.

The feeding sites of adults and immature instars were observed on 40 leaves of each host. They were sectioned in small blocks and the Backus *et al*. (1988) staining technique was used. The sections were examined using light microscopy. The measures were carried out on 20 flanges chosen at random. In order to register the feeding sites with scanning electron microscopy, 20 leaves of each host were sectioned, dehydrated in an ethanol series, dried in air, mounted on stubs and coated with gold according to Swearingen *et al*. (1997).

In order to describe the salivary sheaths produced during feeding, 60 leaves of each host were cut in small blocks, fixed in formaldehyde-acetic acid-alcohol (FAA) solution during 12 hours at room temperature, dehydrated in standard ethanol series, embedded in Paraplast and serial sectioned at 10 µm. Sections were stained with 0.5 % aqueous red safranin and 0.1% ethanolic fast-green and coverslipped with Eukkit. The feeding tissue was detected by the location of salivary sheaths termination.

Data was analysed by using contingency tables, assuming a uniform random distribution of the three considered strata (mesophyll, phloem and xylem), and the test for the significance of difference between two proportions was run using normal distribution. The Chi Square test (X^2) was applied for the first case. The level of significance used was 0.05 % (Sokal & Rohlf 1981; Spiegel 1991).

Honeydew excretion analysis

In order to complement previous studies, the honeydew excretion was analyzed following the procedure of Khan & Saxena (1984). Groups of five macropterous females and ten nymphs of each instar were placed in honeydew collection chambers. One plant was used in each chamber and the insects were allowed to feed for 24 h. Ten replicates were made for each assay.

The excretion was collected on a 9 cm diameter Whatman filter paper disk placed around the base of seedlings which were enclosed in a cylindrical PET cage (20 cm high - 9 cm in diameter). Papers were treated with 0.1 % nynhydrin in acetone and bromocresol green (Paguia *et al*. 1980; Heinrichs *et al*. 1985; Karim & Saxena 1991).

For chemical analysis, honeydew droplets from the first and second instars nymphs were collected from the plant surface with micropipettes. The weight was calculated by differential weighing of the micropipettes before and after the collection of honeydew on a 0.0001-mg sensitive weighing balance. The excretion was analyzed using a Waters HPLC gas chromatograph with a refractive index detector. A Microsorb MV column and amino and acetonitrilo-water (78:22) were used as eluyente. Four replicates were made for each host.

Ph estimation was also carried out using Whatman test paper.

Results

Feeding behavior

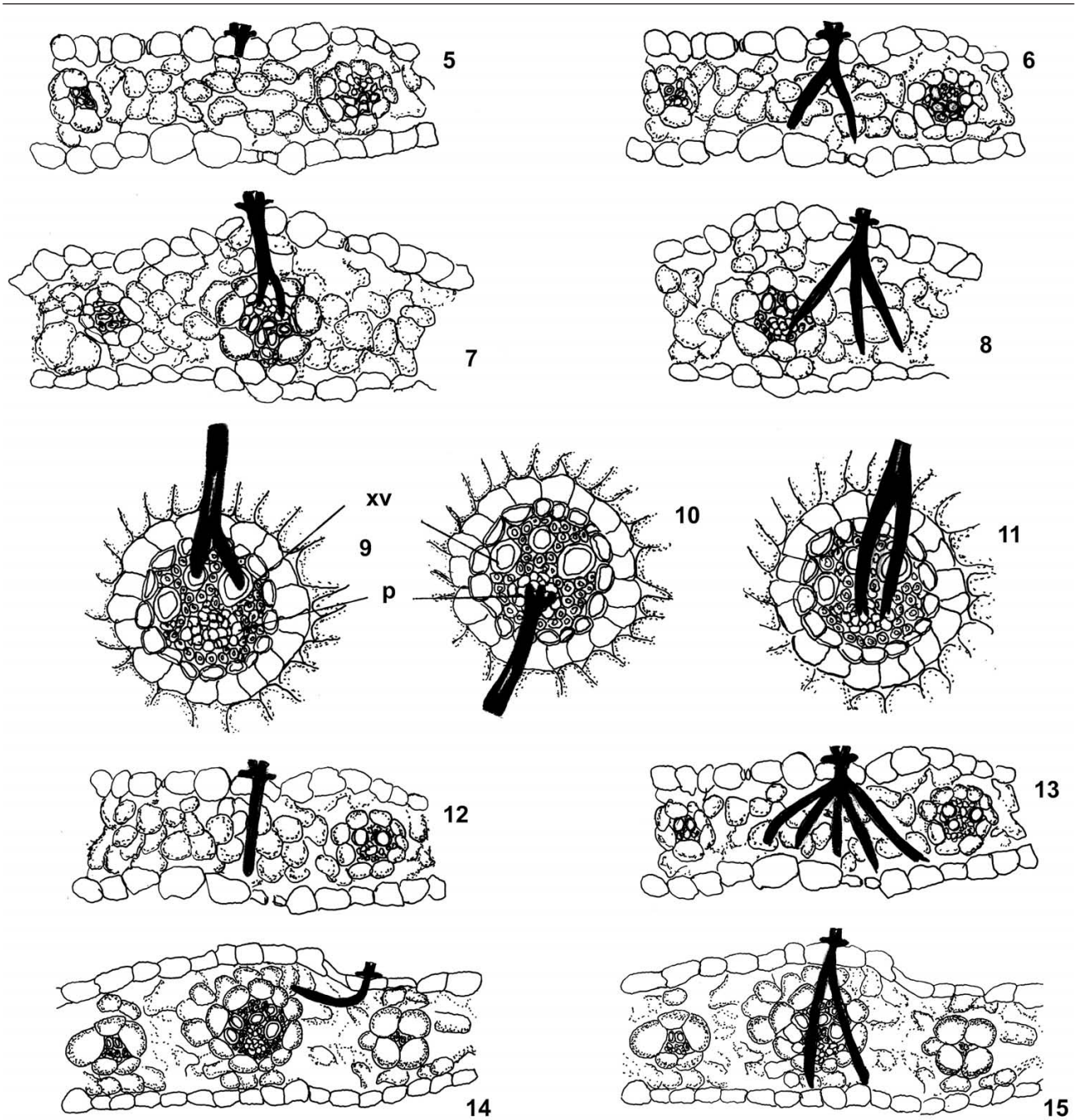
On both hosts, feeding sites were detected by the *flange-salival* formation. They have a circular shape with a variable diameter depending on the vector stage (tab. 1). Deposit appearance observed in the scanning electronic microscopy is shown in figs 1–2. The *flange-salival* is continuous with saliva ejected wherever the

Table 1. Flange-salival diameter of immature instars and adult stage of *Delphacodes kuscheli*.
Values (mean ± standard error) based on random examination of 20 feeding sites of each developmental stage.

Developmental stage	N 1	N 2	N 3	N 4	N 5	Adult
Flange salival diameter (µm)	11.87 ± 0.26	13.81 ± 0.62	17.15 ± 0.53	20.49 ± 0.71	25.74 ± 1.15	28.35 ± 1.35

stylets penetrate host tissue and form a conspicuous tubular structure, the s*tylet-sheath*. Typical salivary sheaths of adults and immature instars of *D. kuscheli* on maize and oat are shown in figs 3–4.

D. kuscheli produced shallow probes ending in the epidermal cells (fig. 5) and others which entered deeper reaching the parenchyma (fig. 6) or vascular tissues (figs 7–8). The penetration of the vascular bundle may



Figures 5–15
Diagrams showing the typical salivary sheaths produced by *D. kuscheli* on oat and maize leaves. **5**, shallow probes ending in the epidermal cells. **6**, probes entered deeper reaching the parenchyma. **7**, salivary sheaths reaching directly the vascular tissues or, **8**, indirectly with previous exploration in the parenchyma cells. **9**, track salival ending in the xylem; or, **10**, **11**, phloem (p); xylem vessels (xv) are crossing when the penetration occurs from adaxial epidermis. **12**, **13**, stylet sheaths showing a single or branched pattern, straight or curved. **14**, **15**, salivary sheaths frequently observed on maize. **14**, shallow stylet sheaths parallel to the epidermis and, **15**, sheaths crossing the vascular bundle.

occur directly (fig. 7) or indirectly if there is previous exploration in the parenchyma cells (fig. 8); when the vascular tissue is reached, the track ends in the xylem (fig. 9) or phloem (figs 10–11). If penetration occurs from leaf abaxial epidermis, the stylets do not usually penetrate beyond the phloem tissue (fig. 10). If penetration occurs from adaxial epidermis, xylem vessels are crossed (fig. 11). The stylet sheaths were single or branched, straight or curved (figs 12–13); 2 to 8 sheath branches were registered for adults and 2 to 6 for immature stages; in both cases 2 or 3 branches were frequently observed. Shallow stylet sheaths parallel to the epidermis as well as across the vascular bundles were frequently observed on maize (figs 14–15).

The analysis of salivary sheaths data produced by adults and immature instars on oat and maize leaves is given in tabs 2–4 and figs 16–17.

On both hosts, adults produced a significantly higher proportion of branched sheaths (oat: z = 11.85; maize: z = 2.06). The number of sheaths ending in the mesophyll, xylem or phloem were host specie depending (X^2 = 7.82, 2 gl, $p < 0.05$): on oat and maize, the proportion of sheaths ending in the phloem were higher than that in the xylem (z = 5.1) and (z = 3.31) respectively, while on maize, the proportion ending in the mesophyll was significantly higher than that in the phloem (z = 2.5) and higher than that in oat (z = 3.5).

On immature instars, the pattern of the salivary sheath was host species depending (X^2 = 16.17, 1gl, $p < 0.05$): on oat, the proportion of branch sheaths was higher than single (z = 7.28, $p < 0.05$) but on maize, the proportion of sheaths of both types was similar (z = 0.32; $p > 0.05$). The number of sheaths ending in the mesophyll, xylem or phloem too was host species depending (X^2 = 15.83, 2 gl, $p < 0.05$); on oat and maize, the proportion of sheaths ending in the phloem was higher than that in the xylem (z = 11.64) and (z = 5.5) respectively; on maize, the proportion ending in the mesophyll was significantly higher than that in the phloem (z = 8.6) and higher than that in oat (z = 5.42).

Honeydew excretion analysis

The honeydew droplets excreted by *D. kuscheli* are light in colour and 0.1–0.5 mm in diameter; they are usually deposited on the plant surface and become

Table 2. Adult stage of *Delphacodes kuscheli*. Percentage of single and branched salivary sheaths and feeding sites on oat and maize leaf tissues.

Host	Pattern of salivary sheath		Location of salivary sheath termination			Total number of salival tracks recorded
	Single	Branched	Mesophyll	Phloem	Xylem	
Oat	8.77	91.23	28.07	54.39	17.54	57
Maize	36.59	63.41	56.10	34.15	9.76	41

Table 3. Immature instars of *Delphacodes kuscheli*. Percentage of single and branched salivary sheaths and feeding sites on oat leaf tissues.

Nymphal instar	Pattern of salivary sheath		Location of salivary sheath termination			Total number of salival tracks recorded
	Single	Branched	Mesophyll	Phloem	Xylem	
N1	36.84	63.16	52.63	39.47	7.89	38
N2	30	70	40	60	0	20
N3	18.75	81.25	43.75	50	6.25	32
N4	25	75	57.14	42.86	0	28
N5	28.57	71.43	33.33	57.14	9.52	21

Table 4. Immature instars of *Delphacodes kuscheli*. Percentage of single and branched salivary sheaths and feeding sites on maize leaf tissues.

Nymphal instar	Pattern of salivary sheath		Location of salivary sheath termination			Total number of salival tracks recorded
	Single	Branched	Mesophyll	Phloem	Xylem	
N1	38.1	61.9	59.52	30.95	9.52	42
N2	50	50	61.54	26.92	11.54	26
N3	78.26	21.74	82.61	13.04	4.35	23
N4	50	50	62.5	29.17	8.33	23
N5	50	50	68.18	27.27	4.55	44

yellowish and dense. Honeydew spots produced during feeding showed ninhydrin and bromocresol green positivity indicating they were amino acid-rich. Also the values of pH = 8 showed a basic reaction on both hosts. Fructose was the major sugar detected in the honeydew (0.025 mg fructose/mg sample).

Discussion

Delphacodes kuscheli revealed the typical feeding behavior of the *salivary-sheath-feeders*, by the formation of the *flange salival* and the *stylet sheath*, terminology proposed by Miles (1972), Sogawa (1982) and Backus (1985). The *flange salival* of *D. kuscheli,* remained after the withdrawal of the stylets, showed a similar size and appearance reaching 11.87 µm for the first nymphal instar and 28.35 µm for the adults. Sogawa (1982) registered similar dimensions for *Nilaparvata lugens* adults.

Feeding strategies observed on oat and maize have evidenced that the penetration of stylets occurs perpendicularly to the leaf surface, however, most of them change directions producing several branch salivals through the initial point of entry. The large number of branches indicates the existence of repeated advances and partial withdrawals of stylets in order to search the feeding tissue.

The prevalence of sheaths related with the phloem in both hosts, and the analysis of the excretion (basic reaction and high concentration of sugars) strongly suggest that *D. kuscheli* sucks phloem sap as its primary food source. This is in agreement with studies for *N. lugens* (Sogawa 1982; Kimms 1989) and with the findings of Cook & Denno (1994) for other vector delphacid species as *Laodelphax striatellus, N. lugens, Peregrinus maidis* and *Saccharosydne saccharivora*. According to Mattsson (1980), the preference of insects for phloem sap is due to the fact that sap in this tissue has about 1000 more parts of sugar than xylem. Sogawa (1982) associates this fact, according to the experiments on *N. lugens*, with the presence of feeding stimulants and/or absence of feeding inhibitors in phloem sap.

On maize, the prevalence of sheaths in every developmental stages of the vector ending in the mesophyll cells (more than 55 %) and their particular disposition (parallel to the leaf surface and others that go throughout the vascular bundles) indicate that *D. kuscheli* withdraws its stylets before reaching the vascular tissues; this strongly suggests the presence of unsuccessful attempts of ingestion and repellence mechanisms during exploration and stylet probing phases. This result reveals a less effective feeding behavior on this host and is consistent with demographic studies on field and laboratory reported by Virla & Remes Lenicov (1991) which indicate the non-preference of *D. kuscheli* feeding and reproduction on maize. Likewise, these results could explain the observations of Arneodo *et al*. (2002b) who detected viroplasms and dispersed MRCV particles only inside the phloem (parenchyma cells cytoplasm, sieve-elements and companion cells), on wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*); whereas, in infected maize leaves, viral particles were found outside the phloem, in the bundle sheath cells that surround the vascular region. Furthermore, the feeding behavior of *D. kuscheli* observed on maize might explain the low transmission rate of MRCV reported on this species by Ornaghi *et. al.* (1999) and the long inoculation periods required by the vector in relation with winter cereals such as barley (Truol *et al*. 2001).

Based on the study of the stylets pathway of *D. kuscheli* on maize and barley leaves, Brentassi & Maldonado (2002) reported that the different behavior of the vector could not be related with structural

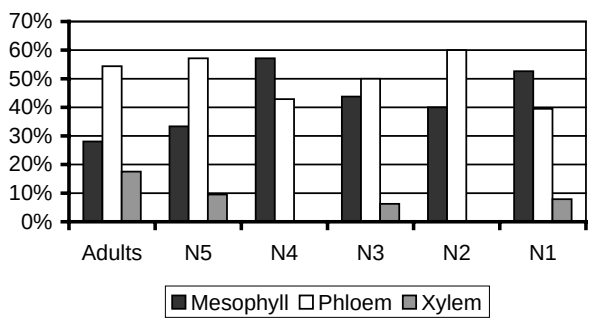


Figure 16
Oat (*Avena sativa*). Percentage of salivary sheaths, produced by adults and immature instars of *Delphacodes kuscheli*, ending in leaf tissues.

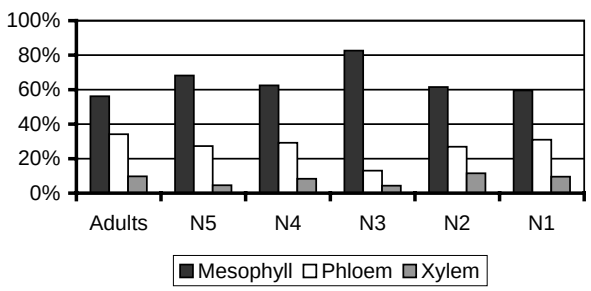


Figure 17
Maize (*Zea mays*). Percentage of salivary sheaths, produced by adults and immature instars of *Delphacodes kuscheli,* ending in leaf tissues.

differences, therefore, it should be associated with the host plant chemistry. Sogawa (1982) 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; Hattori (2001) determined that *N. lugens* feeding activity on the barnyard grass is interrupted by aconitic acid located in non-phloem tissues such as the parenchyma. More studies should be carried out to know if the non-preference of *D. kuscheli* feeding on maize is due to the inhibitors substances or toxins.

The results presented in this paper showed a similar feeding behavior along the life period of *D. kuscheli*, which would indicate -according to Nault (1994)- a higher infective capacity with severe epidemiological effects due to the persistent characteristic of the pathogen transmitted.

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