Journal of Economic Entomology, 113(4), 2020, 1796–1803
doi: 10.1093/jee/toaa114
Advance Access Publication Date: 2 June 2020
Research

Electropenetrographic Comparison of Feeding Behavior of *Dichelops furcatus* (Hemiptera: Heteroptera: Pentatomidae) on Soybean and Spring Cereals

Tiago Lucini[1,3] and Antônio R. Panizzi[2]

[1]Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, PR 82590-300, Brazil, [2]Laboratório de Entomologia, Embrapa Trigo, Passo Fundo, RS 99050-970, Brazil, and [3]Corresponding author, e-mail: tiago_lucini@hotmail.com

Subject Editor: Charles Burks

Received 29 January 2020; Editorial decision 5 May 2020

Abstract

We used electropenetrography to quantify and compare counts and durations of selected waveforms, produced by adult females of the stink bug *Dichelops furcatus* (F.). Insects fed on immature soybean pods and immature seed heads of four spring cereals: wheat, black oat, barley, and rye. On all foods, bugs spent over 60% of their plant access time in non-probing activities. This total waveform duration was significantly longer on barley and rye compared to those on soybean and oat; wheat was intermediate. Considering only probing activities, bugs spent longer durations (ca. 2×), on soybean and oat compared to barley, rye, and wheat plants. Bugs produced significantly more pathway events on soybean and rye than on wheat and barley; with a significantly shorter duration per event on rye. The counts and durations of xylem ingestion did not differ among foods. Cell rupturing activities on seeds were longer on soybean (ca. 23%) and oat (ca. 21%), than on barley and rye (ca. 6%). The durations of ingestion events on seeds were significantly shorter on soybean (over 3×) compared to those on barley and wheat; oat and rye were intermediate. However, the ingestion duration per insect did not show significant difference among foods. Results demonstrated that *D. furcatus* spent more time overall in probing activities on soybean and oat; whereas, rye and barley presented the worst feeding behavior. This study provides important background information for further quantitative studies of stink bugs on different plants, such as development of resistant host plants.

Key words: crop plant, feeding behavior, electrical penetration graph, electropenetrography

Phytophagous stink bugs (Pentatomidae) are known to be key pests on several cultivated plants (Panizzi et al. 2000). They feed on different parts of their host plants, although they prefer reproductive structures, such as fruits and seeds, for nymph development and adult reproduction (Slansky and Panizzi 1987, Panizzi et al. 2000, Olson et al. 2011).

The so-called (in Brazil) green-belly stink bug, *Dichelops furcatus* (F.) is a Neotropical pentatomid primarily found in the southern region of Brazil (Pereira et al. 2010, Chiaradia et al. 2011), Argentina, and Uruguay (Grazia et al. 2015). It is polyphagous, reported on 27 different plant species from 11 botanical families, including cultivated and non-cultivated plants (Smaniotto and Panizzi 2015). Cultivated plants include soybean, *Glycine max* (L.) Merrill (Fabaceae), where the bug has been known as a secondary pest for a long time (Panizzi et al. 1977), as well as, common oat, *Avena sativa* L., and wheat, *Triticum aestivum* L. (Poaceae) (Chocorosqui and Panizzi 2004, Pereira et al. 2010, Panizzi et al. 2018). In the last 10+ years, *D. furcatus* has increased in abundance in southern Brazil and it has been reported feeding on seedlings and seed heads of different spring cereal plants (Pereira et al. 2010, Panizzi et al. 2018). This increase in the population is attributed to the extensive adoption of no-tillage cultivation systems over the years.

Feeding activities of stink bugs cause damage on vegetative and on reproductive structures of their host plants. Those damages result from the mechanical action of stylets and chemical action induced by injection of digestive enzymes within plant tissue (Hori 2000). During the feeding behavior of *D. furcatus* on soybean plants, the most severe damage is reported to occur at the reproductive stage (pod-filling) (Panizzi et al. 1977). On wheat plants, it occurs during vegetative (seedlings) and reproductive stages (booting and milk-grain) (Panizzi et al. 2016).

Improved pest management methods such as host plant resistance or transgenic resistance require better knowledge of feeding behavior of heteropterans. The most rigorous technique available to study feeding is electropenetrography (EPG) (Tjallingii 1978, Backus and Bennett 2009). In this system, the sucking insect is made part

of a simple electrical circuit together with its host plant. The circuit is closed when the insect inserts its piercing-sucking mouthparts (stylets) into the electrified plant tissue. The device graphs the stylet activities, such as ingestion, as waveforms; by measuring each waveform, one may quantify the different stylet activities (Walker 2000). This technique has been recently used to record the feeding behavior of selected pest-stink bugs species, including *D. furcatus* (Lucini and Panizzi 2018a and references therein).

The EPG might be used to make quantitative comparison of stylet behaviors on different plants to screen for possible food sources that could serve to sustain populations of a pest (e.g., Sandanayaka and Backus 2008). Therefore, in this study, we used an AC-DC EPG monitor to quantify and compare counts and durations of selected waveforms produced by adult females of the stink bug *D. furcatus* feeding on immature soybean pods (R5 stage – pod-filling) as well as immature seed heads (R11.1 stage – milk-grain) of four spring cereals: wheat, black oat, barley, and rye. Those foods were chosen based on the variable performance of *D. furcatus* nymphs and adults feeding on them (Panizzi et al. 2018).

Materials and Methods

Stink Bug Colony and Plants

Adults of *D. furcatus* were obtained from wheat plants and from crop residues at the Embrapa Trigo Research Center, Passo Fundo, RS, Brazil (28°15′ S, 52°24′ W); they were taken to the Laboratory of Entomology to establish a colony. Adults were placed inside rearing cages (25 × 20 × 20 cm) lined with filter paper, and cotton balls were added as oviposition substrate. Fresh green bean pods, *Phaseolus vulgaris* L., mature soybean seeds, and raw shelled peanuts, *Arachis hypogaea* L, were provided as food. Twice per week, food was replaced, eggs collected and placed inside plastic boxes (11 × 11 × 3.5 cm) lined with filter paper with food, as above. Nymphs were reared to adulthood to be used in the bioassays. Rearing cages/boxes were kept in a walk-in chamber at 25 ± 1°C, 65 ± 10% RH and photoperiod of L14:D10 h.

Five different crop plants were used to assess the feeding behavior of *D. furcatus*: soybean (main host) and four spring cereals: wheat, black oat, *Avena strigosa* L., barley, *Hordeum vulgare* L., and rye, *Secale cereale* L. Seeds of those plants were planted biweekly in plastic pots (2 L) and maintained in a greenhouse. Soybean plants were placed in the sunnier compartment of the greenhouse, while the cereal plants were kept under a shaded area. The following cultivars were used: soybean cv. BRS 5601 RR; wheat cv. BRS Reponte; black oat cv. BRS Neblina; barley cv. BRS Quaranta; and rye cv. BRS Serrano. For the EPG studies, potted plants were used at the reproductive stages (soybean at R5; pod-filling [Fehr et al. 1971] and spring cereals at R11.1; milk-grain [Large 1954]).

EPG Data Acquisition

A four-channel AC-DC monitor (similar to Backus and Bennett 2009; EPG Technologies, Inc., Gainesville, FL) was used to record the feeding behavior of *D. furcatus*. Adult females 15- to 25-d-old were separated from laboratory colony and placed in a plastic box (11 × 11 × 3.5 cm) without food (starved) for 5 h (4 h before wiring plus 1 h after wiring), without acclimation period on the food sources tested. The bugs were wired following the methodology described by Lucini and Panizzi (2016). On this methodology, the cuticle of the pronotum of bugs is previously sanded to improve the wire attachment. After that, the bug is wired with the insect electrode (a piece of thin gold wire; 3 cm long, 0.1 mm in diameter [Sigma-Aldrich, Saint Louis, MO] glued to a copper electrode (3 cm long) that had previously been soldered to a brass nail). To attach the gold wire in the bug pronotum, a drop of conductive silver glue (a mixture (1:1:1 wt/vol/vol) of silver flakes [Sigma-Aldrich], white glue [Elmer's Glue-All, Westerville, OH], and water) was deposited in the sanded area and the tip of the gold wire was sunk into the glue.

The experiment was conducted in a complete randomized block design. For that, two AC-DC EPG monitors, each one containing four channels (eight channels in total), were used. Wired insects and the five host plants were randomly assigned to one of the EPG channels (we used five channels per day). The recordings were carried applying an input impedance level of 10^7 Ohms in all four channels, and 50 mV alternating current (AC) was supplied via plant electrode. This setting has previously been found to be optimal to record the waveforms produced by *D. furcatus* (Lucini and Panizzi 2017).

After wiring, each bug was individually connected to the EPG probe (head stage amplifier) and placed on the reproductive structure of the plant tested (pod or seed head). To close the electrical circuit, another electrode (plant electrode) was inserted in the moistened soil of the potted-plant. EPG amplifiers, insects, and plants were kept inside a Faraday cage to protect the system from external electrical noise. Inside the Faraday cage, the soybean plants were kept with stems, containing pods, vertically positioned; whereas for spring cereals, the stem, containing a single seed head, was laid down along a glass microscope slide (7.6 × 2.6 cm) and held in place using strips of Parafilm (Pechiney Plastic Packaging, Menasha, WI). The glass slide was held horizontally by an alligator clip connected to a 'helping hand' holder (LojaLab, Piracicaba, São Paulo, Brazil).

The changes in electrical origins of the waveforms (resistance – R, and electromotive force – emf; more details in Backus et al. 2019), during stylet activities in the plant were acquired, rectified, and digitized at a sample rate of 100 Hz per channel (per insect) using a WinDaq DI-710 analog-to-digital board (DATAQ Instruments, Akron, OH) and recorded by a HP Pentium notebook with WinDaq Lite software (also from DATAQ). Each bug was continuously recorded during 15 h under laboratory conditions (25 ± 1°C) and continuous light. In total, 15 replications per plant were successfully recorded and analyzed.

Waveform Descriptions

A waveform library produced by stylet activities of *D. furcatus* have been previously described on wheat plants for both vegetative (seedlings) and reproductive structures (milk-grain stage) (Lucini and Panizzi 2017). Although, *D. furcatus* feeding behavior was not recorded on soybean and on the other three spring cereals tested (oat, barley, and rye), we observed that the stereotypical patterns of the waveforms recorded on those foods were similar to the ones recorded on wheat plants.

The waveform library generated for *D. furcatus* on stem (seedlings) and on seed head of wheat plants is composed by nine EPG waveform types and subtypes. The waveforms were grouped as non-probing (Z and Np) and probing waveforms (Df1a, Df1b, Df2, Df3a, Df3b, Df4a, and Df4b) (Lucini and Panizzi 2017). Brief descriptions of the waveforms recorded on seed heads of wheat, at 10^7 Ohms, and their biological meanings are summarized in Table 1 (except waveform Df3 which was recorded only on stems of wheat seedlings). We set up the EPG so that bugs could not reach stems, since reproductive structures (seed heads) are the preferred feeding site.

Table 1. Waveforms recorded during the feeding behavior of *Dichelops furcatus* on seed heads of wheat plants, and their biological meanings

Phase	Family	Type/subtype	Biological meanings
Non-probing	–	Z	Standing still on the plant surface
		Np	Walking on the plant surface
Pathway	P	Df1a	Stylet penetration and salivary sheath secretion
		Df1b	Bug encountering a rigid cell layer requiring stylet protraction and retraction
		Df1w[a]	Stylet withdrawal from plant tissue
Ingestion	I	Df2	Xylem sap ingestion
Salivation	I	Df4a	Stylet laceration, and enzymatic maceration of seed endosperm
Ingestion	I	Df4b	Ingestion of macerated seed endosperm

Source: adapted from Lucini and Panizzi (2017).
[a]Waveform not separated in Lucini and Panizzi (2017).

Statistical Analysis

For analysis, we combined the non-probing waveforms (Z and Np) to only one type, named 'Np', and the probing waveforms Df1a and Df1b, named 'Df1'. In addition, we designated another waveform (named Df1w) which was not separated in Lucini and Panizzi (2017); Df1w represents stylet withdrawal from plant tissue. Therefore, we calculated EPG variables for the follow waveforms: Np, Df1, Df1w, Df2, Df4a, and Df4b.

To measure the counts and durations of each waveform described above, the WinDaq Waveform Browser software was used to obtain the notepad files. Hereafter, the term 'probe' was used to define the period that includes all activities performed by the bug from stylet insertion in the plant tissue until their withdrawal. The term 'event' was used to define a continuous and uninterrupted occurrence of one waveform type/subtype within a probe.

Eight nonsequential EPG variables were calculated for each waveform type/subtype, as follows: 1) total probing duration (TPD; cohort-level), 2) total waveform duration (TWD; cohort-level), 3) (mean) number of probes per insect (NPI; insect level), 4) (mean) probing duration per insect (PDI; insect level), 5) (mean) waveform duration per insect (WDI; insect level), 6) (mean) probing duration per probe per insect (PDPI; probe level), 7) (mean) number of waveform events per insect (NWEI; event level), and 8) (mean) waveform duration per event per insect (WDEI; event level) (according to Backus et al. 2007). Practically speaking, the term 'probing duration' means the general duration of time, regardless of behaviors performed, whereas 'waveform duration' applies to only one waveform type/subtype (see Backus 2000).

Data were analyzed for significant differences using the Backus 2.0 program for Statistical Analysis Software (SAS, Cary, NC; complete program is downloadable from the EPG Workshop website http://www.crec.ifas.ufl.edu/extension/epg/epg_workshop. shtml). The data were subjected to mixed model analysis of variance (ANOVA) using restricted maximum likelihood estimation (REML-ANOVA; PROC GLIMMIX) (SAS 2009) to verify whether the counts and durations of each waveform type/subtype differed significantly among the five food sources tested. Before ANOVA, count and duration data were square root (x) and log (x) transformed, respectively, to reduce heterogeneity of data. Means were separated using the least significant difference (LSD) (SAS 2009) test and considered significant at $\alpha = 0.05$.

Results

Dichelops furcatus adults spent a larger percentage of their 15-h plant access time performing probing activities (sum of the durations of all probing waveforms: Df1, Df1w, Df2, Df4a, and Df4b) when confined on soybean and oat than on barley and rye plants; TPD on wheat was intermediate (Fig. 1; TPD percentages). Bugs spent almost one third (on oat) or over that (on soybean) of their time with stylets inserted in the plant tissue, i.e., probing. In contrast, on barley and rye, bugs spent far more than two thirds (>80%) of their access time resting or walking on the plant surface (waveform Np), i.e., with the stylets not inserted in the plant (Fig. 1).

Comparisons for each waveform type showed large differences between the food sources for the TWD (represented by the pie slices) of non-probing waveform (Np) and cell laceration and enzymatic maceration of seed endosperm (waveform Df4a); in the remaining waveform types, slight differences were noted (Fig. 1; TWD). On all food sources, bugs spent over 60% of their time in non-probing activities, on soybean and oat with the lowest values (63 and 69%, respectively), whereas on barley and rye, the highest (84%) (Fig. 1). The total duration per cohort of cell rupturing activities on seeds was high on soybean (ca. 23%) and oat (ca. 21%), more than three times higher compared to barley and rye (ca. 6%), and double compared to wheat (ca. 12%) (Fig. 1).

The average NPI, i.e., the average number of times that bugs inserted stylets in the plant tissue, was significantly higher on soybean and rye compared to barley and wheat; NPI on oat was intermediate ($F = 3.29$; df = 4, 70; $P = 0.0156$) (Fig. 2A). However, the PDPI, i.e., the average duration of each probe performed by each bug, was significantly shorter on rye compared to the other food sources ($F = 4.32$; df = 4, 70; $P = 0.0035$), and numerically longer on wheat and oat (Fig. 2B). The PDI during the entire recording period was ca. 2× longer on soybean and oat compared to rye, barley, and wheat plants ($F = 5{,}43$; df = 4, 70; $P = 0.0007$) (Fig. 2C).

The average overall duration of each EPG waveform performed per insect during the entire recording period (WDI) showed significant differences among host plants for non-probing (Np), as well as for probing activities of pathway (Df1 and Df1w) and cell rupturing (Df4a). Non-probing waveform lasted significantly longer per insect on barley and rye than on soybean and oat; wheat was intermediate ($F = 4.20$; df = 4, 70; $P = 0.0042$) (Table 2).

For probing waveforms, in general, bugs spent significantly more time in pathway activities (Df1 and Df1w) on soybean compared to the remaining food sources (Df1: $F = 5.10$; df = 4, 70; $P = 0.0012$; Df1w: $F = 2.81$; df = 4, 70; $P = 0.0319$). Numerically, on barley, bugs spent the shortest time in pathway activities (Table 2). For cell rupturing activities (Df4a), bugs spent ca. 3× longer duration on soybean and oat plants, compared to barley and rye ($F = 4.53$; df = 4, 58; $P = 0.0030$); wheat presented an intermediate value (Table 2). Waveforms associated with xylem sap ingestion (Df2) and ingestion

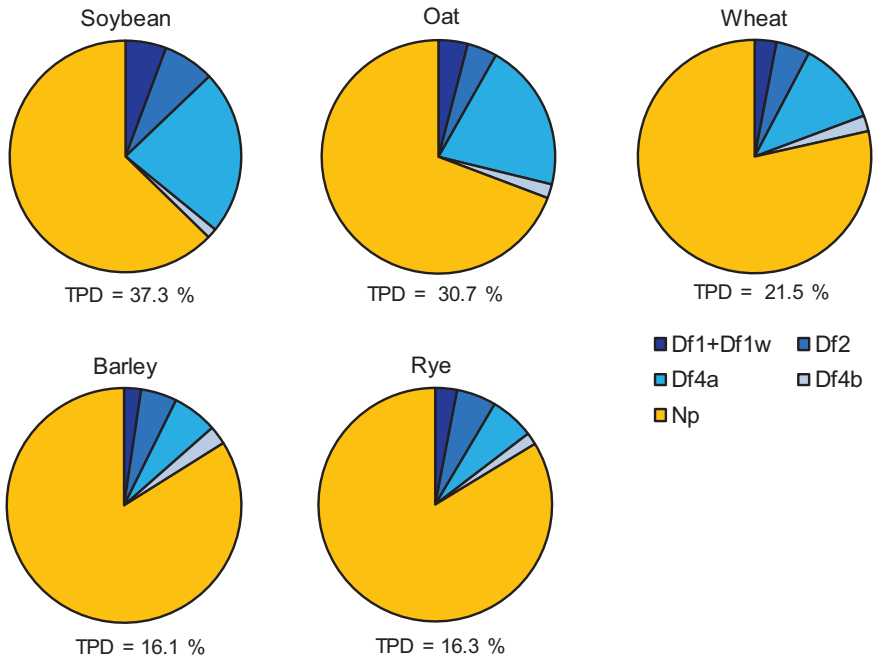

Fig. 1. Total probing duration (TPD; numerically %) and total waveform duration (TWD; pie slices) and total waveform duration (TWD-pie slices) recorded during the feeding behavior of *Dichelops furcatus* on immature soybean pod and immature seed heads of oat, barley, wheat, and rye. Df1 = combined pathway waveforms (Df1a, Df1b) = stylet penetration into the plant tissue; Df1w = stylet withdrawal from tissue; Df2 = xylem sap ingestion; Df4a = cell laceration, enzymatic maceration of seed endosperm; Df4b = ingestion of macerated seed endosperm; Np = combined non-probing waveforms (Np, Z) = insects resting/walking on plant. Color figure in online version and black and white in print version.

of macerated seed endosperm (Df4b) were performed for similar lengths of time on the five plant species tested (Table 2).

Distinct differences among the crop plants tested were observed when we examined the NWEI, and the WDEI, i.e., the mean number and duration of each individual waveform type/subtype performed during the entire recording period (Tables 3 and 4, respectively). Bugs performed a higher number of non-probing events on rye and on soybean than on wheat and barley ($F = 3.16$; df = 4, 70; $P = 0.0190$) (Table 3); however, the average duration was significantly shorter on soybean compared with wheat and barley ($F = 3.85$; df = 4, 70; $P = 0.0069$) (Table 4).

In the probing waveforms, bugs produced significantly more events of the Df1waveform per insect on soybean and rye compared to wheat and barley ($F = 3.03$; df = 4, 70; $P = 0.0230$) (Table 3); the duration of those events were the shortest on rye ($F = 3.90$; df = 4, 70; $P = 0.0064$) (Table 4). The average number of Df1w events performed was higher on soybean than on the other foods ($F = 6.01$; df = 4, 70; $P = 0.0003$) (Table 3), but their duration did not differ among the crop plants (Table 4).

The counts and durations of xylem sap ingestion events (Df2) did not differ among the crop plants (Tables 3 and 4, respectively). On seeds, the only difference observed were for the event durations for ingestion of macerated endosperm (Df4b), which were significantly longer (over 4×) on barley and wheat compared to soybean; the durations of Df4b on oat and rye were intermediate ($F = 8.27$; df = 4, 56; $P < 0.0001$) (Table 4). The average counts and durations of cell rupturing events (Df4a) did not show significant differences, although the counts and durations were numerically higher on soybean and oat, respectively (Tables 3 and 4, respectively).

In summary, lacerate/macerate activities (Df4a) of *D. furcatus* on seeds of soybean and oat showed the overall (i.e., WDI) highest durations, caused by a combination of large numbers of events (i.e., NWEI) of long duration (i.e., WDEI). On seeds of barley and rye, the bugs showed significantly lowest feeding overall duration (WDI for Df4a), caused by a combination of low numbers of events (NWEI) of short duration (WDEI). In contrast, ingestion activity on seeds (Df4b) on all crop plants did not show significant difference for overall duration (WDI). Despite, soybean presented the shortest event duration (WDEI) among crops, the overall duration was compensated by the large NWEI.

Discussion

This study is the first to use the new AC-DC EPG monitor, for large insects like stink bugs, for a quantitative experiment statistically comparing feeding on different host plants. The EPG recordings of *D. furcatus* adults on immature pods of soybean and immature seed heads of four spring cereals showed that waveforms were nearly identical in appearance and biological meanings to the waveforms previously recorded on seed heads of wheat plants. In addition, bugs used the same feeding strategies on all five crops tested, i.e., salivary sheath strategy to ingest water from xylem vessels, and cell rupture strategy to feed in the seeds, employing the lacerate and macerate tactics to break reserve cells for later ingestion (Lucini and Panizzi 2017).

On all five plants tested, bugs spent most of their access time in non-probing activities (walking and/or standing still); they spent less time non-probing on soybean and oat compared to the remaining foods. The long duration of non-probing activities is consistent with previous studies with quantitative EPG measurements in other pentatomid species (Lucini et al. 2016, Lucini and Panizzi 2018b). The number of probes was significantly higher on soybean and

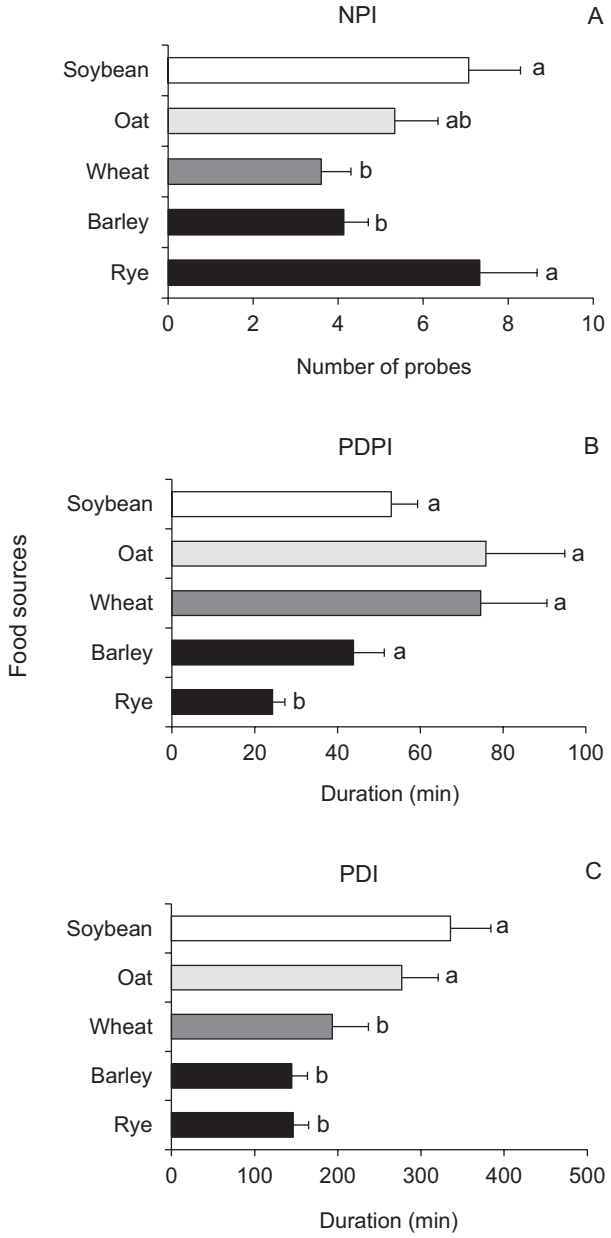

Fig. 2. Means (±SE) of the number of probes per insect (NPI) (A), probing duration per probe per insect (PDPI; min) (B), and probing duration per insect (PDI; min) (C) of *Dichelops furcatus* feeding on immature soybean pod and immature seed heads of oat, barley, wheat, and rye. Means in the bars followed by the same letter, at each EPG variable, are not significantly different at $\alpha = 0.05$ (LSD means test).

rye. On soybean, the majority of the stylet insertion attempts (over 70%) were complete, i.e., bugs inserted their stylets and reached the feeding site (xylem and/or seed endosperm). In contrast, on rye, almost half of attempts (~ 49%) were unsuccessful, i.e., bugs withdrew their stylets quickly after initial penetration, indicating difficulty in finding a proper feeding site or to accept the food.

Bugs spent significantly more time in pathway activities (penetration of the stylets in the plant tissue to reach the feeding site) on soybean, oat, and wheat. This was probably caused by the presence of physical barriers making it difficult and causing delay. Previous studies with soybean showed a rigid cell layer (sclerenchyma cells) in the pod wall (observed in histological sections), which bugs need to overcome to reach the seed endosperm (Lucini and Panizzi 2018b).

Similarly, in cereals, seeds are wrapped by rigid and individual overlapped structures, the glume (outer layer), and the lemma (layer below the glume that surrounds the seed) (Li et al. 2010). Lucini and Panizzi (2017) observed that *D. furcatus* adults forced their stylets down onto immature seed heads of wheat before reaching the seed endosperm, indicating the presence of a physical barrier. However, in general, insects quickly probed on barley and rye. Perhaps these structures are not too rigid as observed on wheat and on oat, or sometimes the seeds may be not completely wrapped by those structures, and areas of the seed remain exposed and easily reached by bugs.

On all crop plants tested, none of the nine EPG variables evaluated were significantly different for xylem sap ingestion (Df2). In general, on all foods, the bugs showed a similar NWEI with similar event duration (WDEI) during the 15-h access time. This result suggest that bugs do not use this site to obtain nutrients, but to obtain water either to maintain body hydration, as reported for aphids, psyllids, and stink bugs (e.g., Spiller et al. 1990, Bonani et al. 2010, Rivera and Mitchell 2020), or/and for nutrient balance purposes, after feeding in the highly nutrient-concentrated seed endosperm (Lucini et al. 2016). Primarily, it is thought that seed-feeders obtain water on vegetative structures of their host plants (Saxena 1963); however, some species of seed-feeders, such as, *Piezodorus guildinii* (Westwood), *D. furcatus*, *Euschistus heros* (F.), and *Nezara viridula* (L.) might reach and ingest water from xylem vessels from reproductive structures (see Lucini and Panizzi 2018a, and discussion therein).

On soybean and on oat, *D. furcatus* adults spent over 23% of their access time feeding on seeds, followed by wheat with ca. 14%, and the lowest values on barley and rye (< 9%). Considering each seed activity separately (i.e., lacerate/macerate behavior—wave Df4a, and ingestion—wave Df4b), we observed that on soybean and oat, the main time (>90%) was spent to 'prepare' the food via lacerate and macerate activities and less than 8% was used to ingest food. In contrast, on barley, rye, and wheat, the ingestion period was longer, over 16%, reaching 29% on barley. However, Panizzi et al. (2018) observed that *D. furcatus* adults feeding on the same cultivars as tested in this study, performed much better on soybean, where nymphs developed faster and females had significantly greater fecundity and tended to attain higher body weight gain. In general, oat was the better-quality spring cereal for *D. furcatus*, followed by wheat, rye, and barley, in this order.

Those results demonstrate that soybean, and, to a lesser extent, oat, despite presenting a short duration of ingestion on seeds, allow bugs to develop and reproduce much better compared with barley and rye, even though bugs performed longer ingestion duration on the latter hosts. These might be caused by the nutritional quality of the foods tested (selected components of seeds of the plants tested are reviewed in Table 5). Seeds of soybean contain a higher amount of nitrogen, an essential nutrient, lipids, and lower amount of carbohydrates compared to seeds of the spring cereals tested, which in turn contain a higher amount of carbohydrates, relatively low amount of nitrogen and are poor in lipids. Therefore, soybean seeds seem to present a more balanced concentration of major nutrients compared to spring cereals, which make them a more suitable food for stink bugs, as reported in several biological studies (Panizzi 2007 and references therein).

Allelochemicals present in the plants might also play an important role in the plant defense towards stink bugs and affect their

Table 2. Means (±SE) of the waveform duration per insect (WDI; min) of different EPG waveforms performed by *Dichelops furcatus* fed with selected food sources (immature soybean pod and immature seed heads of oat, barley, wheat, and rye)

Waveforms and biological meanings		Food sources[a]					*P* values
		Soybean	Oat	Wheat	Barley	Rye	
Np	Insect walking/ stand still on plant surface	564.5 ± 48.7 c	623 ± 44.0 bc	705.9 ± 44.1 ab	755.1 ± 18.7 a	752.3 ± 18.3 a	0.0042
Df1	Stylet penetration into plant tissue	47.6 ± 7.5 a	35.7 ± 5.9 ab	25.5 ± 6.9 c	20.4 ± 2.7 c	25.9 ± 4.1 bc	0.0012
Df1w	Stylet withdrawal	3.7 ± 1.6 a	0.8 ± 0.3 b	1.9 ± 0.7 ab	0.6 ± 0.1 b	1.4 ± 0.4 ab	0.0319
Df2	Xylem sap ingestion	74.5 ± 11.4 a	56.0 ± 6.6 a	48.2 ± 7.1 a	51.6 ± 9.4 a	56.8 ± 9.2 a	0.2426
Df4a	Cell rupture of seed endosperm	207.2 ± 41.1 a	198.7 ± 40.7 a	156.8 ± 39.3 ab	69.9 ± 12.6 b	68.4 ± 13.4 b	0.0030
Df4b	Ingestion of macerated seed endosperm	12.5 ± 4.4 a	18.7 ± 3.9 a	36.9 ± 9.2 a	28.8 ± 14.0 a	18.9 ± 7.2 a	0.1582

[a]Means within a row followed by the same letter are not significantly different at α = 0.05 (LSD means test).

Table 3. Means (±SE) of the number of waveform events per insect (NWEI) of different EPG waveforms performed by *Dichelops furcatus* fed with selected food sources (immature soybean pod and immature seed heads of oat, barley, wheat, and rye)

Waveforms and biological meanings		Food sources[a]					*P* values
		Soybean	Oat	Wheat	Barley	Rye	
Np	Insect walking/ stand still on plant surface	8.1 ± 1.2 a	6.3 ± 1.0 ab	4.6 ± 0.7 b	5.1 ± 0.6 b	8.3 ± 1.4 a	0.0190
Df1	Stylet penetration in the plant tissue	7.7 ± 1.3 a	5.4 ± 1.0 ab	4.1 ± 0.8 b	4.3 ± 0.6 b	7.6 ± 1.4 a	0.0230
Df1w	Stylet withdrawal	5.1 ± 0.7 a	2.5 ± 0.4 bc	2.2 ± 0.4 c	2.9 ± 0.3 bc	3.6 ± 0.5 b	0.0003
Df2	Xylem sap ingestion	1.9 ± 0.3 a	1.5 ± 0.3 a	1.7 ± 0.3 a	1.3 ± 0.2 a	1.5 ± 0.2 a	0.6187
Df4a	Cell rupture of seed endosperm	46.5 ± 10.0 a	37.5 ± 8.2 a	36.6 ± 11.7 a	23.1 ± 7.5 a	34.0 ± 11.5 a	0.3801
Df4b	Ingestion of macerated seed endosperm	42.5 ± 10.0 a	35.8 ± 8.0 a	43.5 ± 12.7 a	20.7 ± 7.4 a	30.9 ± 11.3 a	0.2284

[a]Means within a row followed by the same letter are not significantly different at α = 0.05 (LSD means test).

Table 4. Means (±SE) of the waveform duration per event per insect (WDEI; min) of different EPG waveforms performed by *Dichelops furcatus* fed with selected food sources (immature soybean pod and immature seed heads of oat, barley, wheat, and rye)

Waveforms and biological meanings		Food sources[a]					*P* values
		Soybean	Oat	Wheat	Barley	Rye	
Np	Insect walking/ stand still on plant surface	92.8 ± 13.8 b	121.3 ± 14.5 ab	218.4 ± 32.8 a	190.0 ± 31.4 a	131.2 ± 20.9 ab	0.0069
Df1	Stylet penetration into plant tissue	6.8 ± 0.7 a	7.5 ± 1.2 a	6.5 ± 0.7 a	6.4 ± 1.3 a	4.0 ± 0.5 b	0.0064
Df1w	Stylet withdrawal	0.6 ± 0.2 a	0.2 ± 0.1 a	1.0 ± 0.4 a	0.2 ± 0.0 a	0.5 ± 0.2 a	0.0828
Df2	Xylem sap ingestion	47.3 ± 7.2 a	44.3 ± 7.3 a	35.8 ± 7.9 a	47.8 ± 10.3 a	38.5 ± 4.8 a	0.5925
Df4a	Cell rupture of seed endosperm	5.0 ± 0.5 a	6.9 ± 1.7 a	5.5 ± 0.7 a	4.7 ± 0.8 a	3.6 ± 1.0 a	0.0863
Df4b	Ingestion of macerated seed endosperm	0.3 ± 0.1 c	0.6 ± 0.1 b	1.1 ± 0.4 ab	1.5 ± 0.5 a	0.6 ± 0.2 b	<0.0001

[a]Means within a row followed by the same letter are not significantly different at α = 0.05 (LSD means test).

behavior, inducing the bug abandoning the feeding attempt due to chemical cues picked up during stylet penetration. For example, some cereal plants, such as wheat and rye, produce hydroxamic acids (e.g., DIBOA and DIMBOA), which are secondary metabolites involved in the chemical defense against insects, including piercing-sucking species (Niemeyer 2009 and references therein). In contrast, oat and cultivated barley, along with soybean, do not synthesize any of these hydroxamic acids (Hanhineva et al. 2011).

This EPG study, coupled with the biological results, demonstrated that the green-belly stink bug *D. furcatus* performed much better on soybean pods (which is the most suitable food for the stink bug), followed by seed heads of oat, and, in less extend, wheat; whereas on barley and rye, the performance declined substantially. Populations of *D. furcatus* do not yet seem to have attained economically damaging levels on spring cereals in Southern Brazil. However, the EPG results demonstrate that those crop plants, during reproductive period, might serve as alternate food source. Thus, the spring cereals might sustain populations of the *D. furcatus* that will later colonize summer crops, mostly soybean; i.e., they form a kind of 'green-bridge' linking the food sources.

In summary, the results clearly show that *D. furcatus* adults on soybean pods and on oat seed heads spent more time overall in stylet probing activities with shorter events of non-feeding activities between probes. The main difference observed among crops was during the feeding behavior in seeds (preferred feeding site), where on soybean and on oat, bugs spent more time preparing the food (i.e., breaking a pocket of reserve cells via cell rupturing activities—wave Df4a); ingestion event durations, however, were significantly shorter on both foods. In contrast, bugs showed least preference on rye and barley seed heads, with longer periods of non-feeding and fewer activities in the seed endosperm. On barley, insects attempted to compensate for likely poor nutrition by performing longer duration of ingestion events.

The preliminary EPG studies conducted with stink bugs provide a solid background information for future quantitative EPG studies on these insects, which are essential, for example, to develop

Table 5. Composition of seeds of the crop plant species tested in this study

Nutrients/allelochemicals	Soybean	Oat	Wheat	Barley	Rye
Nitrogen (% dw)	37.0[a]	9.0[b]	12.0[a]	11.0[b]	9.0[b]
Lipids (% dw)	17.0[a]	6.0[b]	2.0[a]	3.0[b]	1.5[b]
Carbohydrates (% dw)	26.0[a]	63.0[b]	75.0[a]	56.0[b]	72.0[b]
Saponin	Yes[c]	Yes[d]	No[d]	No[d]	No[d]
Hydroxamic acids (DIBOA/DIMBOA)	No[e]	No[f]	Yes[f]	No[f]	Yes[f]

[a]Bewley and Black (1985).
[b]Alais and Linden (1991).
[c]Leming and Lember (2005).
[d]Du Fall and Solomon (2011).
[e]Pratt et al. (1995).
[f]Hanhineva et al. (2011).

resistant host plant. In this context, analysis of stylet behavior may be used to estimate tolerance of genotypes via developing a Stylet Penetration Index (SPI), which aid breeders to identify resistant accessions to breeding programs, as well as developed by Serrano et al. (2000) for a leafhopper species.

Acknowledgments

We thank Elaine A. Backus and Paula Levin Mitchell for critical reading of the manuscript and improving it. This study was partially supported by a Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant 302293/2017-5 to A.R.P., and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by a postdoctorate scholarship to T.L. (process number 40001016005P5). We also thank the Embrapa Trigo for support. The research activity has been registered at the Sisgen databank under number AA93702, and at the Publication Committee of Embrapa Trigo under number 21205.000168/2020-41.

References Cited

Alais, C., and G. Linden. 1991. Food biochemistry. Ellis Horwood Ltd., New York.

Backus, E. A. 2000. Our own Jabberwocky: clarifying the terminology of piercing-sucking behaviors of homopterans, pp. 1–13. *In* G. P. Walker and E. A. Backus (eds.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.

Backus, E. A., and W. H. Bennett. 2009. The AC-DC correlation monitor: new EPG design with flexible input resistors to detect both R and emf components for any piercing-sucking hemipteran. J. Insect Physiol. 55: 869–884.

Backus, E. A., A. R. Cline, M. R. Ellerseick, and M. S. Serrano. 2007. Behaviour: *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. Ann. Entomol. Soc. Am. 100: 296–310.

Backus, E. A., F. A. Cervantes, R. N. C. Guedes, A. Y. Li, and A. C. Wayadande. 2019. AC–DC electropenetrography for in-depth studies of feeding and oviposition behaviors. Ann. Entomol. Soc. Am. 112: 236–248.

Bewley, J. D., and M. Black. 1985. Seeds: germination, structure, and composition, pp. 1–27. *In* J. D. Bewley and M. Black (eds.), Seeds: physiology of development and germination. Plenum Press, New York.

Bonani, J. P., A. Fereres, E. Garzo, M. P. Miranda, B. Appezzato-da-Gloria, and J. R. S. Lopes. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. Entomol. Exp. Appl. 134: 35–49.

Chiaradia, L. A., A. Rebonatto, M. A. Smaniotto, M. R. F. Davila, and C. N. Nesi. 2011. Arthropods associated with soybean crop. Rev. Ciênc. Agrovet. 10: 29–36.

Chocorosqui, V. R., and A. R. Panizzi. 2004. Impact of cultivation systems on *Dichelops melacanthus* (Dallas) (Heteroptera: Pentatomidae) populations and damage and its chemical control on wheat. Neotrop. Entomol. 33: 487–492.

Du Fall, L. A., and P. S. Solomon. 2011. Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen interactions. Metabolites 1: 64–78.

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11: 929–930.

Grazia, J., A. R. Panizzi, C. Greve, C. F. Schwertner, L. A. Campos, T. de A. Garbelotto, and J. A. M. Fernandes. 2015. Stink bugs (Pentatomidae), pp. 681–756. *In* A. R. Panizzi and J. Grazia (eds.), True bugs (Heteroptera) of the Neotropics. Springer, Dordrecht, The Netherlands.

Hanhineva, K., I. Rogachev, A. M. Aura, A. Aharoni, K. Poutanen, and H. Mykkänen. 2011. Qualitative characterization of benzoxazinoid derivatives in whole grain rye and wheat by LC-MS metabolite profiling. J. Agric. Food Chem. 59: 921–927.

Hori, K. 2000. Possible causes of disease symptoms resulting from the feeding of phytophagus Heteroptera, pp. 11–36. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of economic importance. CRC Press, Boca Raton, FL.

Large, E. C. 1954. Growth stages in cereals. Illustration of the Feekes scale. Plant Path. 3: 128–129.

Leming, R., and A. Lember. 2005. Chemical composition of expeller-extracted and cold-pressed rapeseed cake. Agraarteadus 16: 103–109.

Li, X. F., D. Bin, and W. Hong-Gang. 2010. Awn anatomy of common wheat (*Triticum aestivum* L.) and its relatives. Caryologia 63: 391–397.

Lucini, T., and A. R. Panizzi. 2016. Waveform characterization of the soybean stem feeder *Edessa meditabunda* (F.) (Hemiptera: Heteroptera: Pentatomidae): overcoming the challenge of wiring pentatomids for EPG. Entomol. Exp. Appl. 158: 118–132.

Lucini, T., and A. R. Panizzi. 2017. Probing behavior of *Dichelops furcatus* (F.) (Heteroptera: Pentatomidae) on wheat plants characterized by electropenetrography (EPG) and histological studies. J. Insect Sci. 17: 1–15.

Lucini, T., and A. R. Panizzi. 2018a. Electropenetrography (EPG): a breakthrough tool unveiling stink bug (Pentatomidae) feeding on plants. Neotrop. Entomol. 47: 6–18.

Lucini, T., and A. R. Panizzi. 2018b. EPG monitoring of the Neotropical brown-stink bug, *Euschistus heros* (F.), on soybean pods: an electrical penetration graph-histology analysis. J. Insect Sci. 18; 1–14.

Lucini, T., A. R. Panizzi, and E. A. Backus. 2016. Characterization of an EPG waveform library for redbanded stink bug, *Piezodorus guildinii* (Hemiptera: Pentatomidae), on soybean plants. Ann. Entomol. Soc. Am. 109: 198–210.

Niemeyer, H. M. 2009. Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: key defense chemicals of cereals. J. Agric. Food Chem. 57: 1677–1696.

Olson, D. M., J. R. Ruberson, A. R. Zeilinger, and D. A. Andow. 2011. Colonization preference of *Euschistus servus* and *Nezara viridula* in transgenic cotton varieties, peanut, and soybean. Entomol. Exp. Appl. 139: 161–169.

Panizzi, A. R. 2007. Nutritional ecology of plant feeding arthropods and IPM, pp. 170–222. *In* M. Kogan and P. Jepson (eds.), Perspectives in ecological theory and integrated pest management. Cambridge University Press, Cambridge, United Kingdom.

Panizzi, A. R., B. S. Corrêa, D. L. Gazzoni, E. B. Oliveira, G. G. Newman, and S. G. Turnipseed. 1977. Insects of soybean in Brazil. Bol. Tec., n. 1. Embrapa Soja, Londrina, Brazil. http://www.infoteca.cnptia.embrapa.br/infoteca/ handle/doc/445546. Accessed 12 October 2019.

Panizzi, A. R., J. E. McPherson, D. G. James, M. Javahery, and R. M. McPherson. 2000. Economic importance of stink bugs (Pentatomidae), pp. 421–474. *In* C. W. Schaefer and A. R. Panizzi (eds.), Heteroptera of economic importance. CRC Press, Boca Raton, FL.

Panizzi, A. R., A. Agostinetto, T. Lucini, and P. R. V. S. Pereira. 2016. Effect of green-belly stink bug, *Dichelops furcatus* (F.) on wheat yield and development. Crop Prot. 79: 20–25.

Panizzi, A. R., T. Lucini, and T. Possebom. 2018. Development of *Dichelops furcatus* (Hemiptera: Heteroptera: Pentatomidae) reared on spring cereals versus soybean. J. Insect Sci. 18; 1–7.

Pereira, P. R. V. S., J. R. Salvadori, and D. Lau. 2010. Trigo: Manejo integrado de pragas. Embrapa Trigo, Passo Fundo, Brazil. https://www. embrapa.br/ busca-de-publicacoes/-/publicacao/887068/trigo-manejo-integrado-de-pragas. Accessed 12 October 2019.

Pratt, K., P. Kumar, and W. S. Chilton 1995. Cyclic hydroxamic acids in dicotyledonous plants. Biochem. Syst. Ecol. 23: 781–785.

Rivera, K. L., and P. L. Mitchell. 2020. Probing behavior of *Nezara viridula* first instars: EPG analysis and effect of food availability on subsequent development. J. Agric. Urban Entomol. 36: 47–63.

Sandanayaka, W. R. M., and E. A. Backus. 2008. Quantitative comparison of stylet penetration behaviors of glassy-winged sharpshooter on selected hosts. J. Econ. Entomol. 101: 1183–1197.

SAS. 2009. SAS/STATuser's guide. SAS Institute, Cary, NC.

Saxena, K. N. 1963. Mode of ingestion in a heteropterous insect *Dysdercus koenigii* (F.) (Pyrrhocoridae). J. Insect Physiol. 9: 47–71.

Serrano, M. S., E. A. Backus, and C. Cardona. 2000. Comparison of AC electronic monitoring and field data for estimating tolerance to *Empoasca kraemeri* Ross & Moore (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796–1809.

Slansky, F. Jr., and A. R. Panizzi. 1987. Nutritional ecology of seed-sucking insects, pp. 283–320. *In* F. Jr Slansky and J. G. Rodriguez (eds.), Nutritional ecology of insects, mites, spiders and related invertebrates. John Wiley & Sons, New York.

Smaniotto, L. F., and A. R. Panizzi. 2015. Interactions of selected species of stink bugs (Hemiptera: Heteroptera: Pentatomidae) from leguminous crops with plants in the neotropics. Fla. Entomol. 98: 7–17.

Spiller, N. J., L. Koenders, and W. F. Tjallingii. 1990. Xylem ingestion by aphids—a strategy for maintaining water balance. Entomol. Exp. Appl. 55: 101–104.

Tjallingii, W. F. 1978. Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 24: 721–730.

Walker, G. P. 2000. A beginner's guide to electronic monitoring of homopteran probing behavior, pp. 14–40. *In* G. P. Walker and E. A. Backus (eds.), Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.