Neotropical Entomology (2025) 54:71
https://doi.org/10.1007/s13744-025-01286-2

ECOLOGY, BEHAVIOR AND BIONOMICS

Electropenetrography (EPG) Study of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) Adults Feeding on Canola Stem and Silique

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Received: 12 September 2024 / Accepted: 19 May 2025

Abstract
This research aimed to study the feeding behavior of *Nezara viridula* (L.) adults on canola stems and siliques using the electropenetrography (EPG) technique. Three distinct phases were identified: non-feeding (waveform Np), pathway (waveform Nv1—which was divided into three subtypes Nv1a, Nv1b and Nv1w), and feeding (waveforms Nv2 and Nv3), which follow previously identified waveforms for this species on soybean. The Nv3 waveform was divided into two subtypes (Nv3a and Nv3b). Biological meanings of the waveforms were proposed by correlating them with visual observations and histological studies. The waveform Np was correlated with insect resting/walking on plant surface; Nv1a with initial stylet insertion and secretion of gelling saliva to create a salivary sheath; Nv1b with deep stylet penetration plus secretion of more gelling saliva; Nv1w with stylet withdrawal, and Nv2 with xylem ingestion, as previously observed for *N. viridula* nymphs on soybean. Nv3a was correlated with laceration/maceration activities; and Nv3b with ingestion of the lacerated/macerated tissue. Regarding comparative analysis, the initial penetration and stylet withdrawal were both significantly longer on stems. Xylem ingestion did not differ significantly between substrates. Feeding events were more common on siliques, and overall duration of ingestion was greater on this substrate. Although no difference in overall duration of laceration/maceration (cell-rupture feeding strategy) was observed between stems and siliques, the average duration of each laceration/maceration event was greater on stems. Significantly more time was spent on non-feeding activities on siliques compared with stems.

Keywords Electrical penetration graph · EPG waveforms · Feeding behavior · Ingestion sites · Stink bug

Introduction

Canola (*Brassica napus* L. var. *oleifera*) is a member of the Brassicaceae (cruciferous) family and is commonly used in crop rotation systems. It is an excellent option for winter cultivation in Southern Brazil, helping to mitigate phytosanitary issues in crops such as legumes, grasses, and cereals. Beyond its agricultural benefits, canola has significant economic value due to its role in producing vegetable oils, which can be used for biodiesel production and human consumption (Tomm et al. 2009).

In Brazil, the area dedicated to canola cultivation has been increasing steadily, especially in Rio Grande do Sul. In 2023, approximately 83,000 hectares were cultivated, representing a 50% increase in sowed area compared to 2022. Production reached 103,000 tons, an increase of about 7% over the previous harvest (CONAB 2023). As the cultivated area expands, pests like stink bugs may exploit this new nutrient source during fall and winter, when their usual food crops like soybean and maize—typically grown in spring and summer—are unavailable (Panizzi 1997).

Stink bugs are considered major pests in economically important crops across all continents, particularly in tropical regions (Schaefer and Panizzi 2000). In the Neotropical

Edited by Eugenio E de Oliveira

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Published online: 31 May 2025

region, stink bugs are frequently found damaging plants from families such as Asteraceae, Brassicaceae, Fabaceae, Poaceae, and Solanaceae (Smaniotto and Panizzi 2015), making them a potential threat to canola production. The first report of damage caused by *Nezara viridula* (L.) to canola crops, impacting both yield and seed quality, was made by Dias (1992).

Other stink bug species, including *Euschistus heros* (F.) and *Piezodorus guildinii* (Westwood), have also been reported in canola fields (Tomm et al. 2009). A more recent study by Bianchi et al. (2019) identified *N. viridula* as the third most common stink bug species in southern Brazil, following *E. heros* and *Diceraeus furcatus* (F.). *Nezara viridula* is known to prefer plants from the Fabaceae and Brassicaceae families (Todd 1989). This preference can lead to increased populations in canola fields, potentially reducing grain yield, especially after silique development. However, detailed information on the feeding behavior of *N. viridula* on canola remains scarce.

Studying the feeding behavior of sucking insects is challenging due to their piercing-sucking mouthparts, which operate within plant tissues. The electropenetrography (EPG) technique is commonly used to analyze such feeding behavior, providing detailed insights into stylet activities inside plant tissue. The EPG creates a simple electrical circuit between insect and plant; then, a low electrical current flows through the circuit, and then captures and amplifies electrical signals generated by ionized fluid flow through the insect's stylets, translating these signals into waveforms that represent the insect's feeding activities (Walker 2000). Several species of stink bugs have already been studied using EPG monitoring (Panizzi et al. 2021), including early and later nymphs (first and fifth instar) of *N. viridula* feeding on vegetative and reproductive structures of soybean plants (Mitchell et al. 2018; Rivera and Mitchell 2020). But, to our knowledge, no EPG study has been conducted for *N. viridula* on canola plants.

This study applied the EPG technique to analyze the feeding behavior of *N. viridula* adults on canola stems and siliques, identifying waveform patterns, their biological meanings, and conducting quantitative analyses. These findings aim to enhance our understanding of *N. viridula*'s feeding patterns, preferred feeding sites, frequency, and duration, with the goal of developing effective pest control strategies.

Materials and Methods

Insect Colony and Plants

Adults of *N. viridula* were collected from May to June 2023 on plants of *Raphanus sativus* L. in the experimental field of Embrapa Trigo (latitude 28°15′46″S, longitude 52°24′24″W), taken to the laboratory of entomology, and placed in plastic rearing cages (20 × 20 × 25 cm) lined with filter paper. Immature pods of green beans (*Phaseolus vulgaris* L.), mature soybean seeds (*Glycine max* L.), raw shelled peanuts (*Arachis hypogaea* L.), and immature canola siliques (*B. napus*) were provided as food sources.

The food was replaced two/three times a week, as necessary, and egg masses were collected and placed in plastic cages (11 × 11 × 3.5 cm) lined with filter paper and a plastic lid (2 × 2 cm—diameter and height, respectively, containing a piece of moistened foam to maintain humidity). After hatching, the nymphs were fed with green bean pods, mature soybean seeds and raw shelled peanuts, and replaced three times/week until they reached adulthood. The rearing cages were kept in a walk-in chamber with controlled conditions (25 ± 2 °C temperature, 65 ± 2% relative humidity and 14 h photoperiod).

Canola seeds, hybrid"Hyola 575 CL", were sown every 21 days in plastic pots (2L) and kept in a greenhouse under semi-controlled conditions without application of insectides. The plants obtained were used in the EPG experiments and histological studies. The feeding activities of the stink bugs were evaluated on vegetative structures (immature stems at stage 6—flowering) and reproductive structures (immature siliques at stage 7 (pod-filling stage [over 50% of siliques have reached final size and seeds are completely developed]) (Canola Council of Canada 2024).

EPG Recordings

The feeding behavior of adult females of *N. viridula* on canola stems and siliques was recorded using two AC-DC EPG monitors, each one containing 4 channels (Backus et al. 2019) that were adjusted to apply an input impedance (Ri) of 10^7 Ohms and 50 mV alternate current (AC). Waveform rectification and gain (amplitude) were adjusted as needed. Before recording, the females (we only used females because the goal of the study was to determine the feeding behavior on different structures of canola plants, and not to compare male and female feeding) were separated from the colony and remain fasting for 18 h (enough time to stimulate the insect to feed). After that, the insects were placed on a Petri dish and immobilized with an adhesive tape for wiring protocol. For that, the electrode (a piece of gold wire; 3 cm long and 0.1 mm in diameter [Sigma-Aldrich, Saint Louis, MO, USA]) was fixed on the pronotum using a water-based silver glue (white household glue:water:silver flakes—1:1:1 [v:v:w]). The wired insect was connected to the EPG probe and placed on the plant structure evaluated (stem or immature silique). The plant electrode (10 cm long copper wire) was inserted into the soil of the potted-plant containing stems and siliques to create the electrical circuit and start the recordings.

The feeding activities of *N. viridula* were recorded continuously, using a DI-710 (Dataq Instruments, Akron, OH) with a recording software installed in a laptop, for 8 h (9am – 5 pm) under laboratory conditions with continuous light and controlled temperature of 25 ± 2 °C. In total, 20 insects were successfully recorded for each plant structure (stems and siliques). The waveforms were separated according to their appearance and comparisons with EPG studies on *N. viridula* (Mitchell et al. 2018) and other species of stink bugs (Lucini and Panizzi 2018). The feeding waveforms were named as "Nv" (abbreviation for *N. viridula*) followed by a number to designate the waveform type plus a letter, if necessary, to designate the subtype, as previously applied by Mitchell et al. (2018) studing the feeding behavior of *N. viridula* fifth instar on soybean plants. During the EPG recordings, visual observations of the insects' behavior (e.g., walking, resting, stylet movements) were made and noted; This information is essential to later determine the biological meanings of each recorded waveform.

Waveform Quantification

EPG waveforms were manually identified and measured using the WinDaq Software Waveform Browser (DATAQ Instruments, Akron, OH). Five nonsequential variables (Backus et al. 2007) were calculated for each waveform type, as follows: (1) number of waveform events per insect (NWEI); (2) waveform duration per event per insect (WDEI; mean duration of each individual event of a waveform per insect); (3) waveform duration per insect (WDI; mean of all events of a waveform per insect); (4) total waveform duration (TWD; expressed in %) and (5) total probing duration (TPD expressed in %—composed of the sum of all feeding waveforms). Descriptive statistics of EPG variables were performed using the Backus 2.0 program developed for EPG data analysis, using SAS statistical analysis software (SAS, Cary, NC).

The complete program for data analysis is available at https://www.crec.ifas.ufl.edu/extension/epg. Variables were compared using ANOVA (ANOVA; PROC GLIMMIX) and then separated by the LSD (Least Significant Difference) test using LSMEANS. Means were considered significantly different at the level of $\alpha = 0.05$.

Histological Analysis

The position of the salivary sheath/stylet tip on the canola stem and silique, and the correlation with the EPG waveforms was performed via histological studies and/or fresh cuts, according to the methodology used by Lucini and Panizzi (2016). For this, a set of *N. viridula* adults were recorded using EPG, applying an Ri of 10^7 Ohms and 50 mV of AC current. The EPG monitor was turned off when a specific waveform was observed on the computer screen; then, the stylets were carefully cut using entomological micro scissors. A piece of the stem or silique, containing the severed stylets, was detached and manually cut into thin sections using a razor-sharp blade (Wilkinson Sword, United Kingdom) under a stereomicroscope (Wild Heerbrugg, Model M5 A, Switzerland) to prepare semipermanent slides. The position of the stylet tips and/or salivary sheath was determined based on 1) Stem: one sample for waveform Nv1, seven samples for Nv2 and five for Nv3; 2) Silique: one for waveform Nv2, one for waveform Nv3 recorded in the silique wall and seven for waveform Nv3 recorded in the seed. Digital images were captured using a microscope (Digilab, Model DI-115B, Piracicaba, SP, Brazil), connected to a computer with image capture software installed.

Results

Waveform Characterization

Seven different waveform types/subtypes were recorded for *N. viridula* while feeding on stems and siliques of canola. These waveforms are summarized in Table 1 and Figs. 1 and 2. All waveforms were strongly similar between plant structures, and they were grouped into three main phases: 1) non-feeding (waveform Np), 2) pathway (waveform type Nv1),

Table 1 Summary of EPG AC-DC waveforms and their proposed biological meanings for each waveform recorded during feeding behavior of *N. viridula* adult females feeding on vegetative (stem) and reproductive (immature silique) structures of canola plants

Phase	Type or subtype	Suggested biological meaning
Non-feeding	Np	Insect standing still or walking on the plant surface
Pathway	Nv1a	Beginning of stylet penetration and secretion of gelling saliva to create a salivary sheath
	Nv1b	Deep stylet penetration and secretion of more gelling saliva to create branches of a salivary sheath
	Nv1w	Stylet withdrawal from the plant tissue
Ingestion	Nv2	Xylem sap ingestion
Salivation	Nv3a	Mechanical laceration and enzymatic maceration of the stem and endospem tissues
Ingestion	Nv3b	Short ingestion event of the lacerated/macerated stem and endosperm tissues

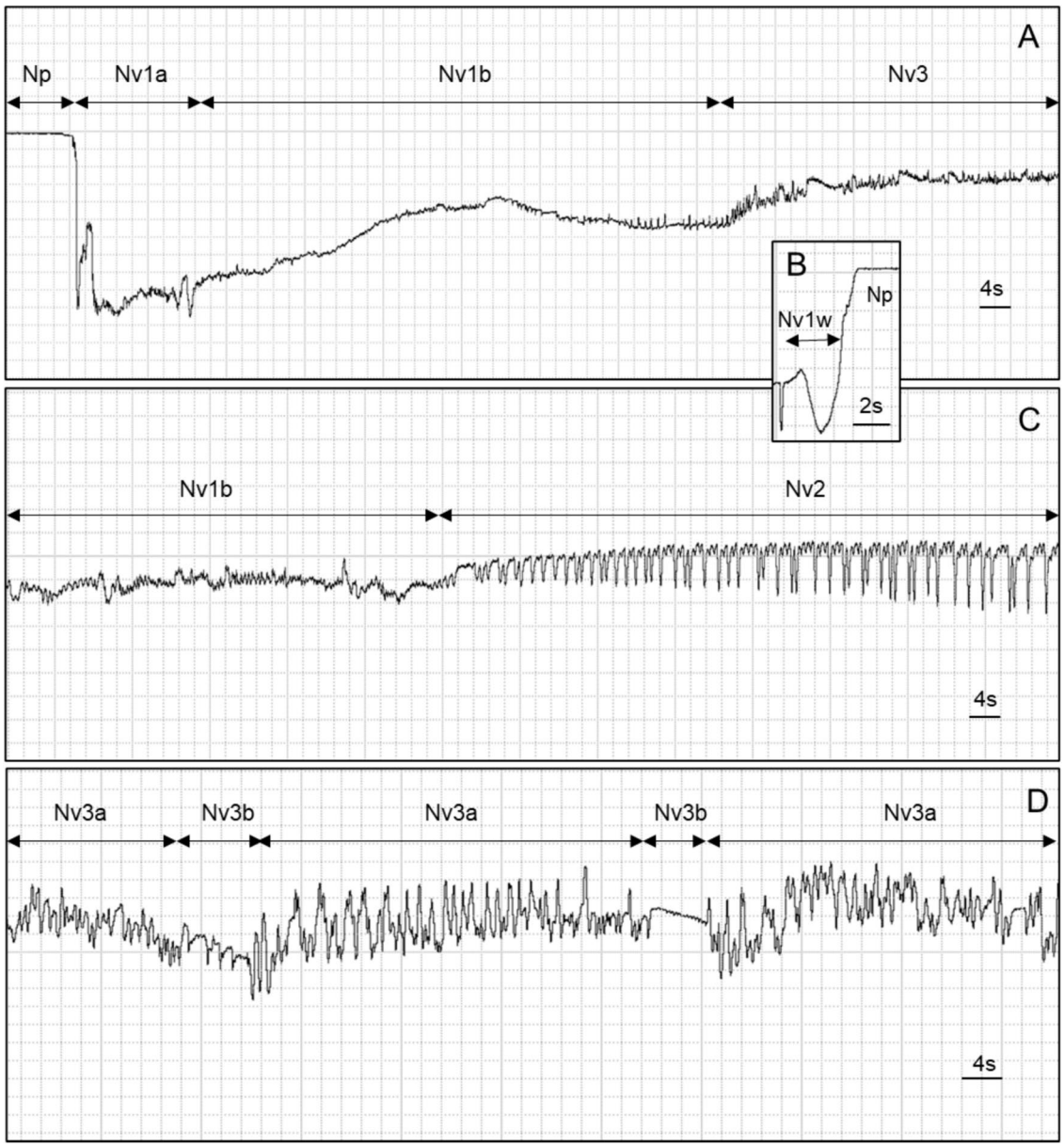

Fig. 1 Waveforms generated using AC-DC EPG recorded from *N. viridula* adult females on canola silique. Overview of the pathway waveforms Nv1a and Nv1b and beginning of the cell rupturing waveforms (Nv3) (**A**); Enlarged view of the stylet withdrawal waveform (Nv1w) (**B**); View of the transition between waveforms Nv1b and Nv2 (**C**); Detail of the waveforms Nv3a and Nv3b which occurs interspersed with each other (**D**). [Figure **A** has Windaq compression 10 (2 s vertical/division), gain 8x; **B** and **D** have compression 5 (1 s vertical/div.), gain 16x; and **C** has compression 5 (1 s vertical/div.), gain 32x]

and 3) salivation and ingestion activities (waveform types Nv2 and Nv3). The Np waveform was characterized as a flat line with none or rare alterations in the form. The Nv1 wave was divided into three subtypes (Nv1a, Nv1b and Nv1w), Nv1a was composed by irregular and high peaks randomly distributed; Nv1b was always recorded after Nv1a and it was characterized by a flatter waveform without highlighted peaks (Figs. 1A,C and 2A); Nv1w also showed an irregular appearance, with a very short duration (few seconds) and always recorded at the end of the feeding event, preceding the Np waveform (Fig. 1B).

The waveform Nv2 was always preceded by the Nv1b wave, and it presented a very regular pattern composed by downward oriented peaks sections (although in some events these peaks are upward) interspersed by a regular wave portion (Figs. 1C and 2B,C). The Nv3 wave was divided into two subtypes (Nv3a and Nv3b) that occurred interspersed with each other throughout the entire feeding activity. The Nv3a waveform was always recorded after the

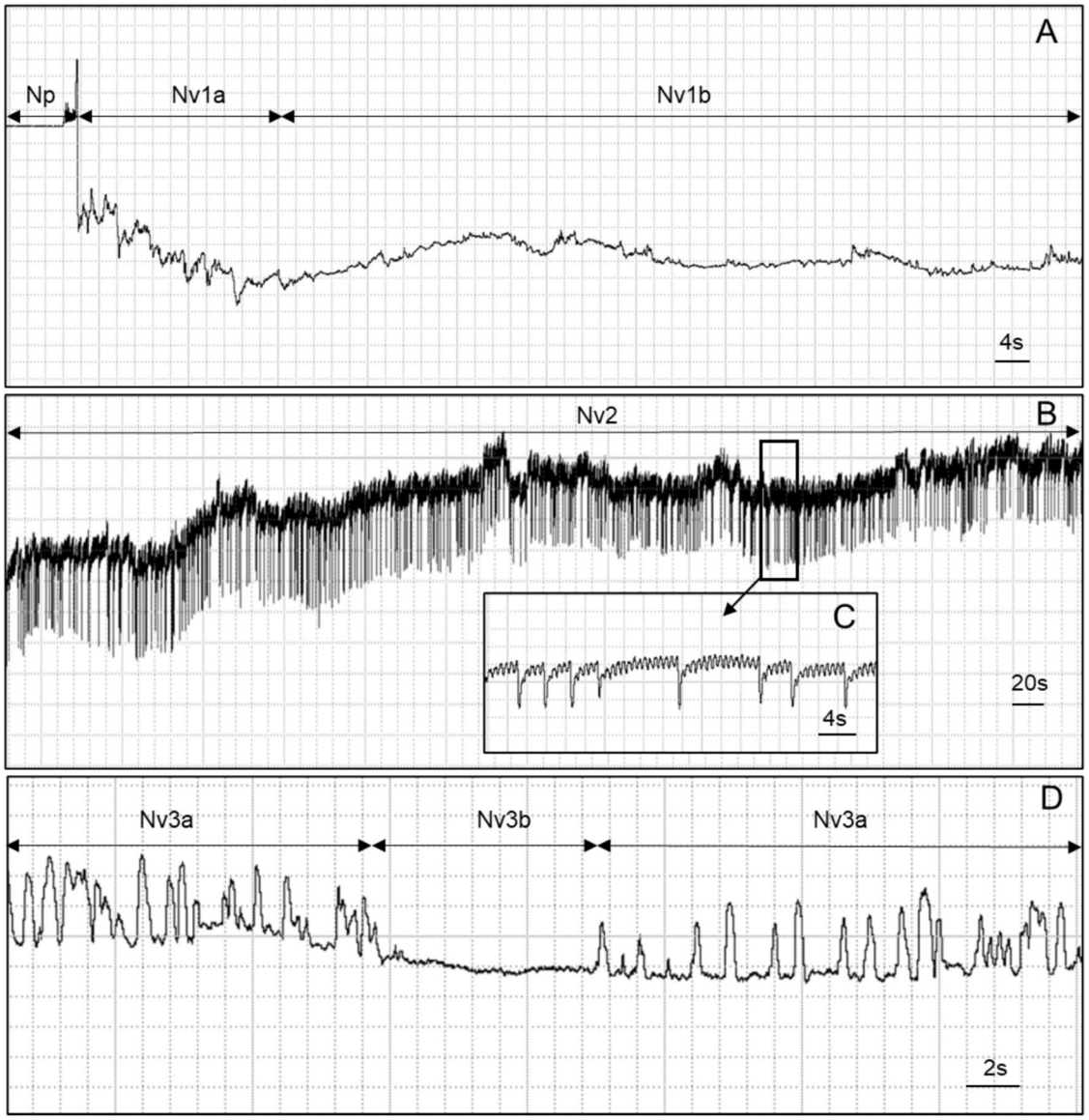

Fig. 2 Waveforms generated using AC-DC EPG recorded from *N. viridula* adult females on canola stem. Overview of the pathway waveforms Nv1a and Nv1b (**A**); Compressed view of the xylem ingestion (Nv2) (**B**); Detail of the Nv2 waveform (**C**); Enlarged view of the Nv3 waveform with subtypes Nv3a and Nv3b (**D**). [Figure **A** has Windaq compression 10 (2 s vertical/division), gain 8x; **B** has compression 50 (10 s vertical/div.), gain 8x; **C** has compression 10 (2 s vertical/div.), gain 16x; and **D** has compression 5 (1 s vertical/div.), gain 16x]

Nv1b wave; it showed a very irregular appearance with peaks randomly distributed throughout the event. In contrast, the Nv3b waveform was always recorded between Nv3a events and presented a more regular pattern and lower amplitude compared to the Nv3a (Figs. 1D and 2D).

Histological Correlation and Biological Meanings of the Waveforms

Regarding the biological meaning of the waves, the Np waveform represents the insect standing still or walking on the surface of stem and on silique of canola plants, which was visually observed. The correlation between feeding waveforms (Nv1, Nv2 and Nv3) and their respective feeding sites/meaning was determined via histological analyses and fresh sections of the stem and silique tissues.

For waveform Nv1, in the stem, we observed that the tip of the salivary sheath ended in the parenchyma cells (Fig. 3A), indicating that the waveform Nv1 represents the insertion and penetration of the stylets into the plant tissue and secretion of the salivary sheath. Based on this observation, Nv1a waveform probably represents the beginning of

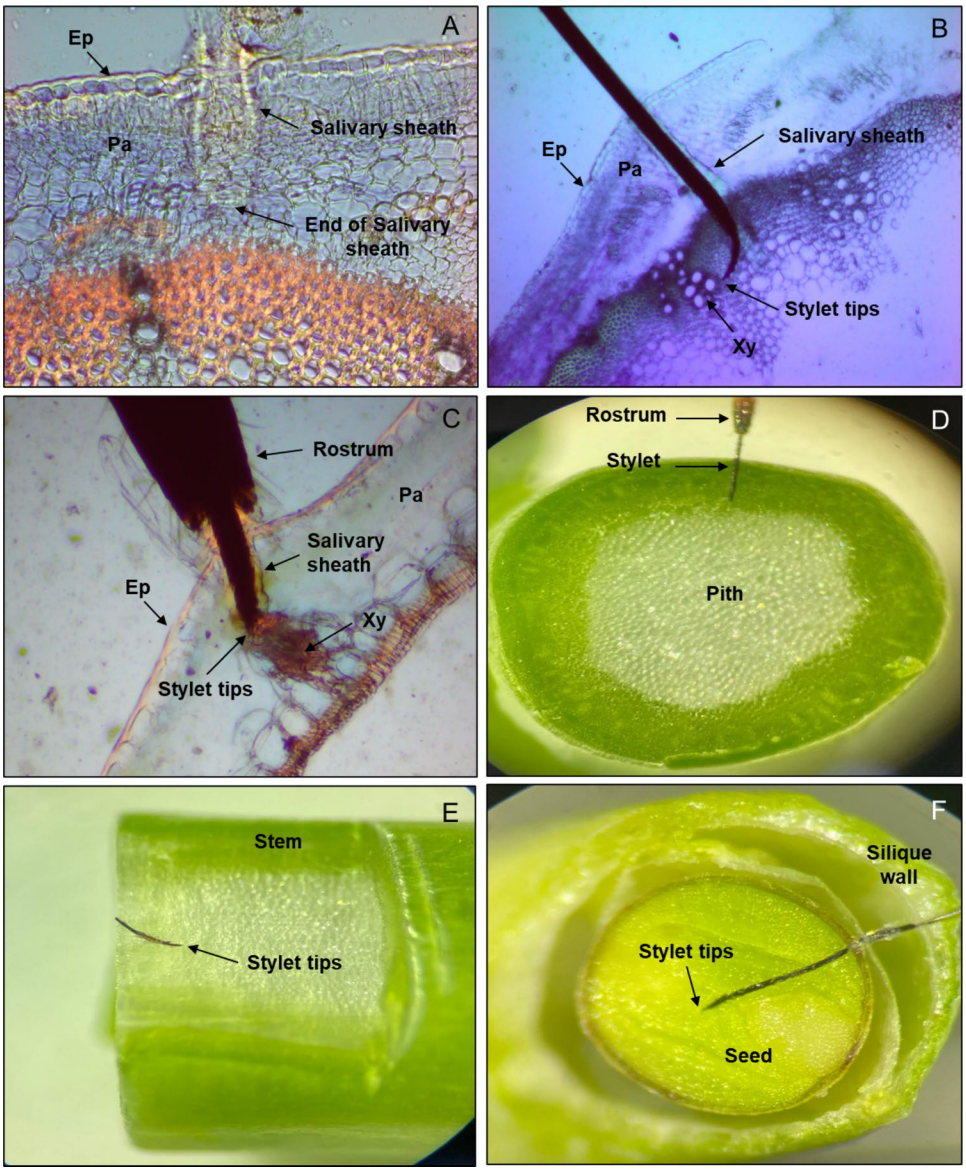

Fig. 3 Histological and fresh cross-sections of canola stem and seed showing salivary sheath and severed styles of *Nezara viridula*. Salivary sheath positioned in the parenchyma tissue of the stem during waveform Nv1b (**A**); Salivary sheath and stylet tips ending in the xylem vessels of the stem during waveform Nv2 (**B**); Stylet tips near of the xylem vessels in the silique during waveform Nv2 (**C**); Cross-section of fresh canola stem containing severed stylets positioned inside the tissue during waveform Nv3 (**D**); Stylet tip positioned longitudinally in the stem during waveform Nv3 (stylets did not reach the inner tissue – pith) (**E**); Cross-section of fresh canola seed containing severed stylets positioned in the endosperm during waveform Nv3 recorded on silique (**F**). Ep = epiderm; Xy = xylem vessels; Pa = parenchyma tissue

the stylet penetration into the plant tissue and secretion of gelling saliva to form the salivary sheath; Nv1b, represents the deep penetration of the stylets plus secretion of more gelling saliva; and Nv1w, represents the stylet withdrawal from the plant tissue at the end of a feeding event.

Histological cuts made in the stems of canola after a Nv2 event, showed the stylet tips ending very close to the xylem vessels of the plant (Fig. 3B), as well as in cuts performed in the silique wall (Fig. 3C). Therefore, we conclude that Nv2 probably represents the ingestion of sap from the vascular bundles, primarily xylem vessels Regarding waveform Nv3, fresh cuts made during this wave (histological cuts were not possible to be obtained) revealed that the stylets were positioned in the parenchyma tissue when the bugs were feeding on the stem (Fig. 3D). In this plant structure, the stylets moved more superficially in the tissue without reaching the inner part of the stem (pith) (Fig. 3E). Furthermore, Nv3 was also recorded on canola silique, with most of the cuts with stylets positioned deep into the seed (Fig. 3F). On both plant structures, a couple of insects moved their stylets longitudinally in the stem and silique wall (stylets were visually observed to move continuously and quickly during EPG recordings and noted) producing a specific kind of damage, known as "rosette" (see Discussion for more explanation).

During subtype Nv3a, we visually observed that the stylets moved in and out quickly and vigorously in the plant tissue (stem and silique); whereas, during Nv3b the stylets were motionless for a short time. Based on these observations, we proposed that Nv3a represents the laceration and maceration activites (cell-rupturing strategy), where the insect uses mechanical and chemical ways to degrade the plant tissue. In contrast, Nv3b represents the ingestion of

the previously lacerated/macerated cellular contents of stem (composed of cells from parenchyma and possibly vascular vessels) and silique (primarily seed endosperm) of canola.

Waveform Quantification

In a comparative analysis between silique and stem, the total probing duration (TPD given in %; which represents the sum of the feeding waveforms Nv1, Nv2 and Nv3) was higher when insects fed on stem (57.7%) compared to silique (43.2%). The total waveform duration (TWD; represented by the slices on the graph) showed that the insects spent most of the time in non-feeding activities (Np), with a higher value on silique. Regarding pathway and xylem ingestion, slight differences were observed between plant structures; however, the greatest discrepancy is notably observed for laceration/maceration activities (Nv3a) on stem, where the insects spent ca. 34% of the time of feeding activities on this wave, whereas, on silique they spent ca. 18% (Fig. 4).

Statistical differences were observed in the three EPG variables evaluated when comparing the plant structures (Table 2). Regarding non-feeding activities (Np), there was a significant difference in the variables WDI and WDEI. For both variables, the means were significantly higher when the bug was recorded on the silique compared to the stem. For the pathway phase, only the waveforms Nv1a and Nv1w presented statistical differences between the plant structures. For both waveforms, the insect spent more time (WDEI and WDI) when fed on stem compared to silique, although the frequency (NWEI) did not differ. Waveform Nv1b did not show statistical differences for any variable, however, the frequency and duration were numerically higher on stem than on silique (Table 2).

For xylem waveform (Nv2), there was no significant differences for the EPG variables; however, numerically, the insect ingested sap for longer time (WDI) when fed on silique (ca. 104 min) compared to stem (ca. 84 min) (Table 2). For laceration/maceration (Nv3a) and ingestion (Nv3b) waveforms, some statistical differences were observed. On silique, the stink bugs significantly performed a greater number of Nv3a and Nv3b events (NWEI; over 2X) compared to stem.

Regarding the laceration/maceration activities (Nv3a), adults of *N. viridula* spent significantly more time per event (WDEI) when fed on stem (ca. 7.8 min) than on silique (ca. 2.7 min); however, the duration per insect (WDI) did not show statistical differences, even though, the time spent on stem was numerically higher than on silique (ca. 60 min higher). In contrast, for ingestion of cell contents (Nv3b), the bug spent a significantly longer time (WDI) when fed on silique compared to stem; nevertheless, the duration of each event (WDEI) was similar between the plant structures (Table 2).

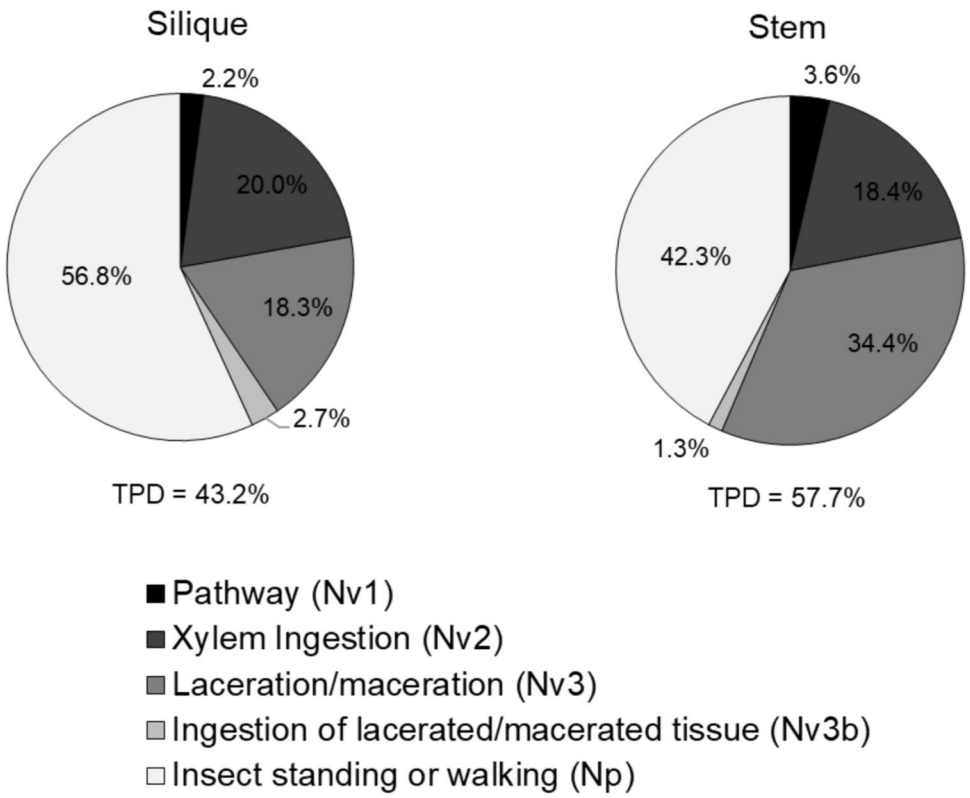

Fig. 4 Total probing duration (TPD; numerically given in %) and total waveform duration (TWD; pie slices given in %) recorded during the feeding behavior of *Nezara viridula* adult females on vegetative (stem) and reproductive (immature silique) structures of canola

Table 2 Means (± SE) of the waveform duration per insect (WDI; min), waveform duration per event per insect (WDEI; min), and number of waveform events per insect (NWEI) performed by *N. viridula* adult females feeding on vegetative (stem; n = 20) and reproductive (immature silique; n = 20) structures of canola plants

Waveform	Plant Structure	WDI (min)			WDEI (min)			NWEI		
Np	Silique		264.97 ± 19.72	a		64.25 ± 11.62	a		5.9 ± 0.8	a
	Stem		194.19 ± 24.54	b		41.0 ± 10.44	b		7.0 ± 0.8	a
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		5.24	1;38	0.0277	5.90	1;38	0.02	0.93	1;38	0.3407
Nv1a	Silique		0.8 ± 0.2	b		0.17 ± 0.2	b		4.9 ± 0.7	a
	Stem		2.4 ± 0.7	a		0.35 ± 0.07	a		6.0 ± 0.8	a
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		7.86	1;38	0.0079	15.31	1;38	0.0004	1.02	1;38	0.3199
Nv1b	Silique		9.05 ± 1.15	a		1.66 ± 0.24	a		6.3 ± 0.8	a
	Stem		13.95 ± 2.27	a		2.19 ± 0.30	a		7.2 ± 0.8	a
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		3.95	1;38	0.0542	1.65	1;38	0.2063	0.57	1;38	0.4563
Nv1w	Silique		0.20 ± 0.04	b		0.04 ± 0.01	b		4.9 ± 0.8	a
	Stem		0.37 ± 0.04	a		0.06 ± 0.01	a		6.0 ± 0.8	a
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		6.23	1;38	0.017	10.98	1;38	0.002	0.93	1;38	0.3407
Nv2	Silique		103.82 ± 14.56	a		68.56 ± 9.61	a		1.7 ± 0.2	a
	Stem		84.41 ± 11.29	a		70.48 ± 8.48	a		1.3 ± 0.2	a
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		1.11	1;36	0.2998	0.16	1;36	0.6897	1.47	1;36	0.2334
Nv3a	Silique		106.86 ± 19.24	a		2.67 ± 0.45	b		50.4 ± 9	a
	Stem		166.46 ± 25.50	a		7.76 ± 0.97	a		24.1 ± 4.1	b
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		1.65	1;33	0.2076	34.12	1;33	< 0.0001	7.42	1;33	0.0102
Nv3b	Silique		15.45 ± 5.13	a		0.32 ± 0.06	a		47.1 ± 8.7	a
	Stem		6.45 ± 1.59	b		0.32 ± 0.05	a		19.6 ± 8.9	b
		F	df	*P* value	F	df	*P* value	F	df	*P* value
		5.39	1;33	0.0265	0.01	1;33	0.9383	9.23	1;33	0.0046

Means (± SE) followed by the same letter in the column, for each waveform type/subtype on each EPG variable, are not significantly different at α = 0.05 (LSD means test)

Discussion

This study represents the first use of the EPG technique to analyze the feeding behavior of *Nezara viridula* adults on canola plants. Previous *N. viridula* investigations using EPG and histological methods focused on early and late nymphal stages (first and fifth instars) feeding on vegetative and reproductive structures of soybean plants (Mitchell et al. 2018; Rivera and Mitchell 2020). The findings reveal that *N. viridula* adults feed on vegetative and reproductive structures of canola using the same ingestion sites as fifth instars feeding on vegetative and reproductive structures of soybean plants.

Additionally, the waveforms corresponding to feeding behaviors were highly similar between nymphs and adults across both crop plants. However, a key distinction lies in the utilization of vegetative tissues by the stink bug. On canola, stems were targeted through laceration/maceration activities, while in soybean, petioles and leaves serve primarily as sources of hydration through xylem ingestion (Mitchell et al. 2018). Similar to *N. viridula*, adults of *Diceraeus furcatus* (F.) and *Diceraeus melacanthus* (Dallas) also exhibit laceration/maceration feeding behavior on wheat and maize stems, respectively (Lucini and Panizzi 2017a, b).

During pathway activity (wave Nv1), observations revealed that stink bugs spent more time on the stem than on the silique. This difference may be attributed to the rigidity of stem tissue, crucial for structural support during plant growth, particularly in the reproductive phase. Tofanica et al. (2011) noted that rapeseed (*Brassica napus*) stems share compositional similarities with hardwoods and non-woody plants, containing significant amounts of holocellulose, cellulose, and lignin. Consequently, canola stems,

enriched with these compounds, offer greater resistance to stylet penetration compared to the less rigid silique.

Feeding on siliques, specifically on seeds, adults tended numerically (not statistically different) to increase overall consumption of xylem sap compared to stems. However, xylem sap, being dilute and low in nitrogen and organic carbon, is unlikely to provide adequate nutrition (Brodbeck et al. 1993). Nevertheless, the ingestion of xylem sap by insects is an essential strategy for hydration (Spiller et al. 1990) and/or nutritional balance after ingestion of concentrated seed content (Lucini et al. 2016). Notably, all examined stink bug species utilizing EPG ingest xylem sap across various plant structures, such as leaves, stems, petioles, and pods of soybean by *N. viridula* (Mitchell et al. 2018) and *Piezodorus guildinii* (Westwood) (Lucini et al. 2016) and stems and ear heads of wheat by *Diceraeus furcatus* (F.) (Lucini and Panizzi 2017a, b). Although in our study no statistical differences were observed for xylem ingestion, Mitchell et al. (2018) found significant differences between durations of xylem ingestion on petioles and pods, ca. 2× more ingestion time on pods than on petioles.

Stink bugs are typically generalists, targeting multiple plant parts including stems, leaves, flowers, fruits, and seeds. However, they exhibit a preference for reproductive structures, particularly seeds, during immature stages (Schuh and Slater 1995; Olson et al. 2011). Seeds'endosperm provides essential nutrients like proteins, lipids, and carbohydrates, readily accessible through their stylets (Slansky and Panizzi 1987). However, during periods of reduced seed availability in the field, stink bugs adapt their feeding habits to include less nutritious food sources to maintain their development, such as the vegetative tissues of cultivated and non-cultivated plants (Panizzi and Silva 2012).

This study revealed that the most significant differences occurred during the cell rupture activity (wave Nv3), a hemipteran feeding strategy (Lucini and Panizzi 2018). Our findings highlight that *N. viridula* adults used this feeding strategy to feed on stems and siliques of canola, spending more time in laceration/maceration events on stems compared to siliques. Potentially, this could be due to the stem's lower nutritional content compared to seeds, requiring the insect to increase the degradation of cell components to fulfill its nutritional needs. This result does does not corroborate observations made on soybean vegetative structures, on wich, nymphs performed significantly less feeding activites than reproductive structures (pods); however, on soybean petioles and leaflets, *N. viridula* did not use cell rupturing to feed in the plant tissue, they only ingested from xylem vessels (Mitchell et al. 2018).

In a recent study to evaluate the biology and damage caused by adults of *N. viridula* on immature siliques of canola, Oliveira et al. (2023) reported an interesting kind of damage, probably resulting from cell rupturing activity, which was known as "rosetting". This damage is characterized by a point of insertion of the stylet into the plant tissue surrounded by whitish spots caused by multiple and rapid longitudinal movements of the stylets in the silique wall, which were visually observed during experiment; authors reported that ca. 70% of *N. viridula* adults evaluated performed this rosetting behavior on immature siliques. In our EPG study, we also observed this kind of damage while bugs were feeding on silique wall and on stems of the canola plants; However, few insects exhibited this behavior during the 9-h recording time on the EPG (one insect recorded on siliques [5%] and 5 insects on stems [25%]). This low occurence of rosetting during EPG analysis can be explained by the short period of time insects were feeding; in the Oliveira et al. (2023) study, the insects had 72 h to feed.

Another important point is that as the lower siliques of canola elongate, there is a reduction in the number of leaves, resulting in a change in the source of nutrients translocated for growth and seed filling. At this stage of plant development, the stem and siliques become the main structures responsible for photosynthesis (ca. 65%), reaching ca. 90% at ripening stage (Canola Council of Canada 2024), thus allowing canola to maintain the physiological needs of the plant and seed filling (Mogensen et al. 1997; Thomas 2003; Edwards and Hertel 2011). Consequently, rosetting activities on silique walls and on stems can diminish plant productivity by reducing photosynthetic tissue. Depending on stink bug infestation levels and resultant damage, canola yield can be significantly affected.

In conclusion, our study demonstrates that *N. viridula* adults fed on stem and silique of canola utilize xylem vessels for hydration and employ cell rupture strategies to degrade and ingest cell contents of stem, silique wall, and the seed endosperm. These findings offer insights for future research, particularly in evaluating canola cultivar/hybrid susceptibility/resistance to *N. viridula* attacks and assessing insecticide impacts on stink bug feeding behaviors, potentially informing pest management strategies.

Acknowledgements We thank anonymous reviewers for critical reading of the manuscript which improved its readability. We also thank Embrapa Trigo and IFRS (Campus Sertão) for their support.

Author Contributions ALMJ, ARP, BGL, EDB, MAS and TL contributed to the study design and wrote the paper. EDB and TL conducted the EPG tests. ALMJ, EDB, ARP and TL co-conducted insect rearing and behavior observations. All authors have read and approved the manuscript.

Funding This research work was funded in part by Embrapa Trigo, and part by Embrapa Agroenergia.

Declarations

Ethical Approval Not applicable.

Conflict of interest The authors declare no competing interests.

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