EPG monitoring of the probing behaviour of the common brown leafhopper *Orosius orientalis* on artificial diet and selected host plants

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Key words: electrophysiology, feeding, TbYDV, Hemiptera, Cicadellidae, diet, tobacco, bean, insect, vector, phloem

Abstract

The common brown leafhopper *Orosius orientalis* (Hemiptera: Cicadellidae) is a polyphagous vector of a range of economically important pathogens, including phytoplasmas and viruses, which infect a diverse range of crops. Studies on the plant penetration behaviour by *O. orientalis* were conducted using the electrical penetration graph (EPG) technique to assist in the characterisation of pathogen acquisition and transmission. EPG waveforms representing different probing activities were acquired by EPG from adult *O. orientalis* probing *in planta*, using two host plant species, tobacco *Nicotiana tabacum* and bean *Phaseolus vulgaris*, and *in vitro* using a simple sucrose-based artificial diet. Five waveforms (O1-O5) were evident when *O. orientalis* fed on *P. vulgaris*, whereas only four waveforms

(O1-O4) and three waveforms (O1-O3) were observed when the leafhopper fed on *N. tabacum* and on the artificial diet, respectively. Both the mean duration of each waveform and waveform type differed markedly depending on the food substrate. Waveform O4 was not observed on the artificial diet and occurred relatively rarely on tobacco plants when compared with bean plants. Waveform O5 was only observed with leafhoppers probing on beans. The attributes of the waveforms and comparative analyses with previously published Hemipteran data are presented and discussed but further characterisation studies will be needed to confirm our suggestions.

Introduction

Many sap-sucking Hemiptera constitute economically important pests as a direct result of their probing damage to crop plants and/or their ability to act as efficient vectors of plant pathogens. Despite their significance, there are a lack of effective control measures for many Hemiptera and/or the pathogens they vector. In many cases, this is due to a lack of knowledge of insect probing behaviour and the mechanisms of pathogen acquisition and transmission. The development of both DC and AC Electrical Penetration Graph (EPG) systems (McLean and Kinsey 1964; Tjallingii 1978; 1985a; 1988) has provided a means of analysing the intra- and intercellular plant penetration (probing) processes of many sap-sucking insects. EPG monitoring provides real time information on probing by insects in the form of waveforms, which can be correlated to specific activities and stylet tip locations. This method has been widely used on aphids for which there are a number of well characterised EPG waveforms which represent such correlation (Tjallingii 1978; 1988; Tjallingii and Hogen Esch 1993). This approach has been used to study host-plant interactions, pathogen transmission and acquisition, insecticide mode of action, host-plant resistance and insect-induced host-plant resistance (Prado and Tjallingii 1994a; Harrewijn and Kayser 1997; Liu et al. 2005; Prado and Tjallingii 2007; van Helden and Tjallingii 2000). Although mainly used with aphids, the DC EPG technique has also been adopted for other sap-sucking insect groups including planthoppers (Khan and Saxena 1988; Kimmins 1989; Powell and Gatehouse 1996; Seo et al. 2009), whiteflies (Walker and Janssen 2000), phylloxerids (Harrewijn et al. 1998; Kingston 2007),

mealybugs (Catayud et al. 2001) and thrips (Joost and Riley 2005; Kindt et al. 2006). DC EPG studies on leafhoppers have been relatively limited (Kimmins and Bosque-Perez 1996; Lett et al. 2001; Miranda et al. 2009; Stafford and Walker 2009) and some authors have used the AC EPG system with different looking waveforms (Backus et al. 2005).

The common brown leafhopper *Orosius orientalis* (Hemiptera: Cicadellidae) is a highly polyphagous, sap-sucking insect able to feed on over 60 plant species (Trębicki et al. 2010a). Apart from probing on many economically important crops in agricultural areas where it is in high abundance in Australia (Trębicki et al. 2010a; Trębicki et al. 2010b) and worldwide, *O. orientalis* is a very effective vector of numerous plant viruses and phytoplasmas. It is considered the most important leafhopper vector in Australia where it transmits the phytoplasmas responsible for causing legume little leaf (Hutton and Grylls 1956), tomato big bud (Hill and Mandryk 1954; Osmelak 1986), lucerne witches broom (Helson 1951), potato purple top wilt (Grylls 1979; Harding and Teakle 1985) and Australian lucerne yellows diseases (Pilkington et al. 2004). In addition it transmits *Tobacco yellow dwarf virus* (TbYDV; genus *Mastrevirus*, family *Geminiviridae*) which causes summer death and yellow dwarf diseases in beans and tobacco, respectively (Ballantyne 1968). Despite the important pest status of *O. orientalis*, there is limited information available concerning its probing behaviour and the characteristics of TbYDV acquisition and transmission.

The control of *O. orientalis* currently involves the indiscriminate use of environmentally hazardous chemical insecticides. Antimetabolites may also offer a future potential alternative control strategy since these have proven effective for a range of sap-sucking insect pests including the leafhopper *Nephotettix cinciteps* (Gatehouse et al. 1992; Powell et al. 1993; Peumans and Van Damme 1995) and some exhibit antifeedant activity (Powell and Gatehouse 1996). Recently, two plant lectins were identified as potential control agents for *O. orientalis* in artificial diet studies (Trębicki et al. 2009) but the influence of these antimetabolites on the insects probing behaviour is unknown.

The main objective of this study was to utilise the DC EPG System and to characterise and interpret the waveforms produced by adult *O. orientalis*. A secondary objective was to compare the probing behaviour of adult *O. orientalis* on a simple artificial diet, a preferred host plant (*Phaseolus vulgaris* L.) and non-

preferred host plant (*Nicotiana tabacum* L.) species. Studies using the artificial diet substrate should assist in the assessment of potential antimetabolites on the insects probing behaviour, while studies of the probing behaviour of *O. orientalis* on both preferred and non-preferred host plants will improve fundamental knowledge on the epidemiology of TbYDV.

Materials and methods

Insect and plant material

All adult *Orosius orientalis* used in this study were originally obtained from stock colonies reared at Charles Darwin University, Darwin, Australia. Stock leafhopper cultures were maintained on celery (*Apium graveolens* L.) for several generations, and early instar nymphs were placed in rearing cages (Trębicki et al. 2009) until adulthood. Tobacco (*Nicotiana tabacum* L.) and beans (*Phaseolus vulgaris* L.) were maintained in an insect-free, climate-controlled environment (25 $\pm$ 3°C) and used at the 2-4 leaf stage for all plant EPG recordings.

Data collection

Probing by *O. orientalis* was monitored by the EPG system (Tjallingii 1988) using a model Giga-8 DC (direct current) amplifier (EPG-Systems, Wageningen, The Netherlands) with a 1 Giga Ω input resistance. All recordings were conducted within a Faraday cage housed within a climate-controlled laboratory room (22 $\pm$ 3 °C). EPG output was set to 100x gain, data was initially acquired at 122 Hz (later converted to 100 Hz) using a DATAQ Di700 A/D data acquisition USB device card (Dataq® instruments, Ohio, USA). Data was analysed using Probe 3.4 for Windows software (Department of Entomology, Wageningen University, The Netherlands). As *O. orientalis* were active when disturbed, each insect was immobilized prior to electrode attachment by chilling at -20°C for 90 s in a 5 ml screw top plastic tube. To ensure that the cold treatment did not affect insect probing behaviour, pilot experiments were conducted at different temperatures and durations. No negative effects were observed (data not presented). Immobilized insects were then transferred onto a vacuum device (van Helden and Tjallingii 2000) platform for tethering. Insects were tethered to the

electrode using a small droplet of water-based silver glue (EPG-Systems, Wageningen, The Netherlands) placed on the pronotum using a fine entomological pin. After 20 s, a second droplet of silver glue was added and a gold wire (12.5 µm diameter, 3 cm length) was placed in the glue and allowed to dry. The gold wire was attached by silver glue to a 0.2mm diameter copper wire attached to a brass pin that was inserted into the input connector of the first-stage amplifier. Each wired leafhopper was left tethered for 1 h and then placed in the centre of the feeding substrate (either bean, tobacco or an artificial diet).

Depending on insect availability, 20-22 samples per treatment were evaluated and each EPG wired insect was recorded for 8h. All recordings were conducted using the same setting, with plant voltage adjusted for each channel ensuring the first insect probe was always positive with maximum amplitude of around +4 V. For each recording, the quality of silver glue connection between leafhopper and insect electrode was tested by using a calibration pulse after the first probe was initiated and a good contact was determined by an output signal in the form of a square pulse. The electrical origin for most of the waveforms was determined by changing the plant voltage above, below and on the 0 V output level. In addition, the electrical origin of each waveform was also investigated by using a dual EPG system that recorded two types of EPG simultaneously, the normal or full EPG (formerly called DC system) containing components of resistance (R) and electromotive force (emf) origin and the R-PG, which contains R components only (formerly called the AC system) (Tjallingii 2000; Tjallingii et al. 2010). This dual mode EPG amplifier (EPG systems, Wageningen, The Netherlands) allowed direct comparison at the same time point the two EPG's with different electrical origin backgrounds.

Artificial diet and diet chamber

An artificial diet comprising a 5% (w/v) sucrose solution was used to study the probing behaviour of *O. orientalis.* Sucrose was dissolved in sterile ultra-pure water using gentle heat (25°C) on a magnetic stirrer hotplate, and the pH adjusted to 6.5 with 1M KOH. After filtration through a 0.2 µm Millipore disposable filter, diet solutions were dispensed into 250 ml plastic containers as stock solutions and further dispensed into 50 ml tubes as working solutions and stored at -20°C prior to use. An artificial diet feeding platform was constructed from a small plastic

Petri dish (1 cm x 3.5 cm) with an access hole in the side to allow contact with the EPG diet electrode (Figure 1). The Petri dish was filled to capacity with the sucrose diet and a single layer of Parafilm M™ was stretched over the chamber carefully to prevent the occurrence of air bubbles. A single adult *O. orientalis* was attached to the EPG electrode as previously described and placed on the top of the Parafilm M™ layer in the centre of the feeding platform. *O. orientalis* stylet movement inside and outside of the feeding platform was observed using a light microscope.

<Insert Figure 1>

Data analysis

Analyses were done on each individual insect EPG recording as well as on combined recordings for each treatment. Online resources to calculate EPG parameters were used (Giordanengo 2009) as well as summary statistics using Genstat software (10th Edn© 2007, Lawes Agricultural Trust, Rothampsted, UK). Student *t*-test was performed on certain parameters to determine statistical difference.

Results

Waveform description

Replicated eight hour EPG recordings from *Orosius orientalis* on a preferred, a non-preferred host plant (bean and tobacco, respectively) and on a simple artificial diet allowed us to distinguish five distinct waveforms, designated O1-O5 (Figure 2) in accordance to their order of general appearance. In addition, a non-probing phase (np) was observed together with potential drops (Opd) (Figure 3, 4A). EPG waveforms were distinguished based on voltage level, frequency, amplitude and shape (Table 1).

<Insert Figure 2>

<Insert Figure 3>

<Insert Figure 4>

<Insert Table 1>

EPG waveforms on bean, a preferred host plant

After an initial non-probing phase (np), stylet penetration always commenced with waveform O1 (Figure 2). With a positive plant voltage adjustment, this waveform was characterised by sharp positive peaks, very often accompanied by a relatively large number of positive voltage spikes with a gradual decline in voltage level. Amplitude, mean voltage level and shape was highly variable over the experimental period but the waveform remained positive (above 0 V) for its entire duration (Table 1).

Waveform O2 (Figure 2) always occurred after O1 but less frequently than O1 (Figure 5, 6). The waveform remained at the same (generally positive) voltage level (Table 1) with relative amplitude ranging from 20-80% and was characterised by a very regular periodicity with smooth peaks and sharp downward spikes. The rather high amplitude at the beginning of the waveform remained constant for durations of up to 30 minutes, but for longer time periods the amplitude gradually decreased.

<Insert Figure 5>

<Insert Figure 6>

Waveform O3 (Figure 2) usually followed waveform O2. The mean voltage level of this waveform was similar to O2 (Table 1). This waveform was characterised by rapid, sharp peaks at irregular intervals, during which a smoothly fluctuating signal was shown with the same amplitude but significantly slower. After the peak, the waveform gradually decreased and then increased again. Waveform O3 occurred only occasionally and less frequently than waveform O2.

Waveform O4 (Figure 2) appeared not earlier than one hour (Figure 5) and always occurred after O1 or O2 (Figure 4). It was absent on diet and in most recordings from tobacco (Figure 6). On beans it occurred less frequently than other waveforms, with the exception of waveform O5), but was sustained mostly longer than 10 minutes and sometimes exceeded one hour (Figure 5). The mean duration was highly affected by very short periods of O4 in early probes (Figure 7). The O4 waveform always started with a rapid negative voltage shift (from

positive in the proceeding O1 or O2 to a negative voltage) and remaining negative in general with the exception of a small number of recordings in which it had a tendency to move gradually towards 0 V without ever exceeding it (Figure 4A). The waveform is relatively complex, characterised by a more or less steady voltage level with a constant high frequency component of around 14 Hz on which superimposed regular 2-3 time repeated upward peaks followed by one downward trough (reverse 'saw tooth' shape) at low frequency (repetition rate) of ≤1 Hz (Figure 4B, Table 1).

<Insert Figure 7>

Waveform O5 (Figure 2) was the least frequent of all waveforms (Figure 6) and was mainly preceded by waveform O4. In a few recordings, this was interrupted by short periods (less than 3 min) of waveform O2. The voltage level of waveform O5 was always low, similar to O4, and relatively constant. It was characterised by small relative amplitude of around 5% (peak-peak/O1 max.) and a 2 Hz frequency. This waveform showed two signal components, a small amplitude high frequency baseline with sharp negative peaks that were mostly regular and larger but varied in amplitude (Figure 2, Table 1).

When comparing all waveforms, O1 was not only the most frequently observed waveform but also had the shortest mean duration of all waveforms. In contrast, waveforms O4 and O5 had the longest mean duration but were only observed infrequently; waveform O5, for example, was only recorded on average once per insect/plant combination (Figure 6, 7).

EPG waveforms on tobacco, a non-preferred host plant

With *O. orientalis* probing on tobacco plants, only four distinctive probing activities were identified, represented by the previously described waveforms O1-O4, as well as potential drops and non-probing (np) phases. Excluding the np phases, the duration of waveform O3 was the longest followed by waveforms O2 and O1 (Figure 7). Waveform O1 occurred most frequently (an average 25 times per recording), while O4 was the both the shortest (mean duration of 13 s) and

least frequently (mean number of probes = 0.3) observed waveform (Figure 6, 7). Waveform O5 was not observed in any EPG recording on tobacco.

EPG waveforms on an artificial diet

On artificial diet periods of non-probing (np) and only three of the distinguished waveforms (O1, O2 and O3) were identified in all recordings. Potential drops were absent. Of all the waveforms observed, O2 occurred for the longest period with a mean duration of 2517 seconds followed by waveforms O3 and O1, respectively (Figure 7). During waveform O1, a clear watery secretion was observed on the outer surface of the Parafilm membrane covering the diet soon after contact with the insect's labium (data not presented).

Comparison of probing *behaviour on an artificial diet and host plants*

EPG recordings always commenced with non-probing (np) but there were quite marked differences in waveform numbers and durations of np periods between bean, tobacco and the artificial diet. The mean number of np periods was three fold lower on the artificial diet than on bean and tobacco plant (Figure 6). In contrast, the mean duration of np on the artificial diet was markedly longer, almost double that recorded on both tobacco and beans (Figure 7). The shortest mean duration of np was recorded on the preferred host bean.

The duration of waveforms O1-O3 periods, particularly O2 and O3, was relatively longer with insects probing on the sucrose diet compared to both host plants (Figure 7). In terms of numbers, periods of waveform O1 occurred less frequently on the artificial diet than on tobacco and bean (Figure 6). On bean, this waveform was the most frequent (Figure 6) and had the shortest mean time till first recorded whilst the mean duration was also shortest in comparison to both diet and tobacco. On beans O1 was recorded twice as often compared with the artificial diet and the total time spent as O1 was almost double when probing on plants (bean and tobacco) compared to diet (Figures 6 & 7).

More waveform O2 periods were recorded (up to threefold) on the artificial diet than on either bean or tobacco (Figure 6). In addition, the mean period of O2 lasted almost five times longer on diet than on beans and tobacco plants (Figure 7). Additionally, *O. orientalis* took longer to produce this waveform on tobacco than on artificial diet and bean plants (Figure 5).

The duration of waveform O3 periods was over two and three times longer on the artificial diet than on tobacco and bean, respectively (Figure 7). The mean number of O3 events on the artificial diet was more than double that on tobacco and beans (Figure 6), although the time to reach this waveform was longer on the artificial diet (Figure 5).

Waveform O4 was never observed on diet and was only recorded on beans and tobacco. On tobacco, the mean number observed and duration was very low with the duration significantly different ($P<0.005$) to bean (0.3 times and 13 s, respectively). *O. orientalis* took less time to reach this waveform on beans than on tobacco (Figure 5).

Waveform O5 was only recorded from *O. orientalis* probing on beans. On average, at least one O5 event was recorded per bean EPG recording. Despite a relatively low number of O5 events on bean, the mean duration was relatively long compared to other waveforms (Figures 6 & 7). This waveform usually occurred relatively late in the probing process (Figure 5).

Discussion

This is the first published EPG study describing probing behaviour of *Orosius orientalis.* Five distinctive waveforms, O1-O5, along with potential drops and non-probing phases, were observed depending on which substrate the insects were recorded. The characteristics of the five waveforms showed some similarities to those observed for other leafhoppers, planthoppers and aphids (Tjallingii 1978; Kimmins and Bosque-Perez 1996; Lett et al. 2001; Stafford and Walker, 2009).

Due to a large variation in waveform characteristics between leafhopper species, there is no universally accepted nomenclature for leafhopper EPG waveforms although within aphids the variation seems smaller, thus allowing a standard labelling system to be used (Tjallingii 1978). EPG waveforms for the leafhopper *Cicadulina storey,* have been named L1-L5 (Kimmins and Bosque-Perez, 1996) while those from *C. mbila* are designated as 1-5 (Lett et al. 2001). For the leafhopper *Circulifer tenellus* a more complicated waveform labelling has been described including a pathway phase (waveforms A, B1, B2 and C), a non-phloem ingestion phase (waveform G) and a phloem ingestion phase (waveforms

D1, D2, D3 and D4) (Stafford and Walker 2009). In our study, we tentatively propose labels O1-O5, based on the first letter of the genus name (*Orosius*) with a subsequent number representing the sequence in time more or less and representing distinct differences with respect to probing activity and possible stylet tip position on the basis of our results as will be discussed in the following.

Waveform O1 always occurred after a non-probing phase and was recorded from insects probing on all three substrates. Similar waveforms have been observed with aphids (Tjallingii 1978) and other leafhopper species (Kimmins and Bosque-Perez 1996; Lett et al. 2001; Stafford and Walker 2009) and are reported to represent the pathway phase. During this phase, the insect produces gelling saliva that creates the salivary sheath lubricating the stylets while advancing inside the tissues. Interestingly, *O. orientalis*-derived secretions were clearly observed on the diet side of the Parafilm membrane, covering the artificial diet during waveform O1, soon after labial contact with the membrane. This supports the hypothesis that waveform O1 corresponds to the pathway phase. On both plant species, but not on the artificial diet, only waveform O1 was frequently interrupted by potential drops. Potential drops are known to occur in aphid EPGs, as a result of intracellular punctures during pathway phase before stylets reach the phloem (Tjallingii 1985b; Tjallingii and Gabryś 1999) and, as such, would explain their absence from the artificial diet recordings. The membrane potential in all living plant cells, including phloem cells, is positive outside and negative inside. A stylet tip puncture of the membrane is similar to a microelectrode puncture and the rapid voltage changes in the EPG is recorded (Tjallingii 1985b). Potential drops in aphid EPGs are characterised by a drop of around −100 mV on stylet insertion and a steep rise to the extracellular potential during stylet withdrawal from the cell. The potential drops in all *O. orientalis* recordings on bean and tobacco had a similar leading edge as aphid pd waveforms but, in contrast this edge was followed by a slow and gradual rise in voltage onto the extracellular level. A possible explanation is that damage to the cell membrane by the relatively large stylet of *O. orientalis* is much greater than with aphids and this may cause a membrane leakage and a successive gradual collapse of the membrane potential.

Waveform O2 was observed in all artificial diet and plant EPG recordings. It was very similar to waveform G observed in aphids (Tjallingii 1988; Prado and Tjallingii 1994a) and whiteflies (Lei et al. 1999), waveform N5 reported in the

rice brown planthopper, *Nilavarvata lugens* (Seo et al. 2009) and waveforms 1, G and Xc from the leafhoppers *C. mbila*, *C. tenellus* and *Bucephalogonia xanthophis*, respectively (Lett et al. 2001; Miranda et al. 2009; Stafford and Walker 2009). In all cases, these waveforms have been correlated with active feeding from xylem and/or the mesophyll. The presence of waveform O2 from *O. orientalis* probing on the artificial diet, which has a negative hydrostatic pressure, as well as from both plants, indicates that *O. orientalis* may be actively ingesting fluid and that this pattern may be associated with the rhythmic activity of cibarial muscle when ingesting fluid, in the form of either diet or sap. The fact that no changes in voltage level were recorded, which typically indicates the stylet puncturing a living cell, suggests that this waveform is most likely to be a xylem-related activity *in planta*. This waveform was also the most prevalent form recorded from leafhoppers probing on the artificial diet. Presumably, the sucrose in the diet influences this waveform production as it is a strong sucking stimulant for most sap-sucking insects including other planthoppers such as *Nilaparvata lugens* (Sogawa 1982).

Waveform O3 was recorded from leafhoppers probing on all three substrates but was most commonly seen using on artificial diet recordings. A similar waveform has been reported for other leafhoppers and planthoppers, including *C. mbila* (Lett et al. 2001), but both the probing activity and stylet tip location associated with the waveform remains unknown.

Waveform O4 was only recorded from leafhoppers probing on plants, although the occurrence and duration of this waveform on tobacco was relatively low (Figure 4 & 5). This waveform was always initiated with a drop in voltage, of similar magnitude to a potential drop and remained low (often 0 V in recordings) for its entire duration. Similar waveforms have been recorded for the leafhoppers, *C. mbila* and *C. storeyi* (Kimmins and Bosque-Perez 1996; Lett et al. 2001), with waveform L2 of *C. storeyi* associated with the transmission of the phloem-restricted geminivirus, *Maize streak virus*. As such, this waveform may be associated with the salivary pump action of injecting saliva-containing virus particles into phloem cells (Kimmins and Bosque-Perez 1996). Another waveform similar to O4 is waveform E1 reported in aphids, which has been correlated with watery phloem salivation and associated with *Barley yellow dwarf virus* transmission (Prado and Tjallingii 1994a). If this waveform reflects phloem

salivation, triggered by the high hydrostatic pressure in the phloem sieve elements, the absence of waveform O4 in the artificial diet might provide circumstantial evidence for phloem salivation. Although the probing behaviour of *O. orientalis* and many other leafhopper species is poorly understood it is possible that many leafhopper species need to react to a number of phloem-related plant defenses to enable phloem sap ingestion. In particular, coagulating plant proteins play a role in these wound responses. Watery salivation into the phloem sieve elements prior to sap uptake by aphids seems necessary to suppress the wound responses and cascade reactions caused by cell disruption (Tjallingii 2006; Will et al. 2007; Will et al. 2008).

Waveform O5 was only present in recordings from *O. orientalis* probing the preferred host plant, bean, and although it was relatively less common than other waveforms it was relatively long in duration. This waveform occurred after O4 and showed a similar low voltage level. Waveform O5 resembles phloem ingestion waveforms reported for *C. mbila* and the E2 phloem waveform observed with aphids (Lett et al. 2001). Many phloem feeders, especially aphids, take advantage of the high hydrostatic pressure in the phloem to passively ingest the sap (Prado and Tjallingii 1994b; Lett et al. 2001). To achieve this, the large cybarial muscles that are used during active feeding (i.e. during xylem phase) are substituted by a precybarial valve that, by opening and closing might regulate the amount of phloem entering the food canal (McLean and Kinsey 1984). The fact that the O5 waveform was not recorded on the artificial diet is presumably due to the lack of sufficient hydrostatic pressure, thus forcing the insect to ingest actively.

Orosius orientalis is a successful vector of many viruses and phytoplasmas including the phloem-restricted *Tobacco yellow dwarf virus*. Although *O. orientalis* can acquire the virus from bean and other host plants, it cannot acquire the virus from TbYDV-infected tobacco plants (Helson 1950). The presence of waveform O4 and absence of waveform O5 in leafhoppers probing on tobacco provides further circumstantial evidence that O5 represents phloem ingestion.

The recent development of an artificial diet for *O. orientalis* (Trębicki et al. 2009) will enable further studies to be undertaken under negative and positive hydrostatic pressure to further elucidate the mechanism of action of antimetabolites. Additional research also needs to be conducted to further

characterise the waveforms and correlate them to specific probing activities. Such studies would require a histological approach for the plant and insect combined with laser stylectomy and time lapse videophotography of the insect. An understanding of the physiological meaning of the waveforms will be critical to understanding the probing behaviour and the mechanisms of pathogen transmission/acquisition of this very important vector.

Acknowledgements

This research was funded by Horticulture Australia Limited and the Tobacco Research and Development Corporation with in-kind support from the Department of Primary Industries (DPI), Victoria, Australia. The assistance of Dr Kim Andrews (DPI Rutherglen), Gary Baxter (DPI Ovens) and Lucy Tran-Nguyen (Charles Darwin University, Australia) is gratefully acknowledged.

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Tables and Figures

Table 1 Major characteristics of the waveforms recorded using a DC EPG system for adult *Orosius orientalis* probing on *Phaseolus vulgaris* (waveforms O1-O5), *Nicotiana tabacum* (O1-O4) or sucrose-based artificial diet (O1-O3).

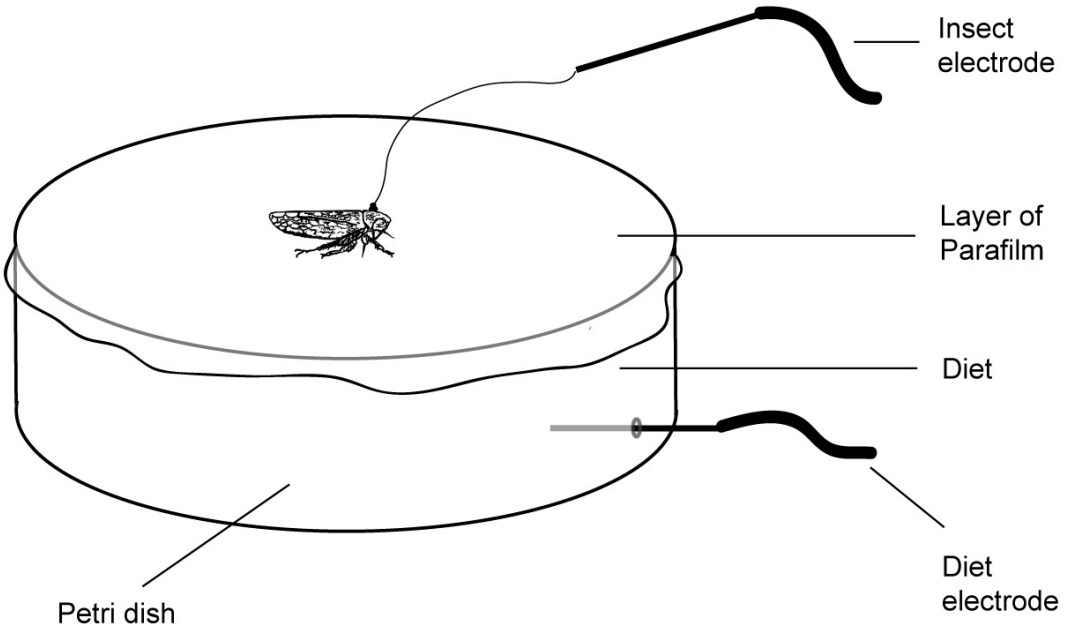

Figure 1 Chamber to study the probing behaviour of *Orosius orientalis* on an artificial diet. One electrode was connected to the leafhopper while the other was placed in the diet through a side-opening, sealed post electrode insertion to prevent diet leakage.

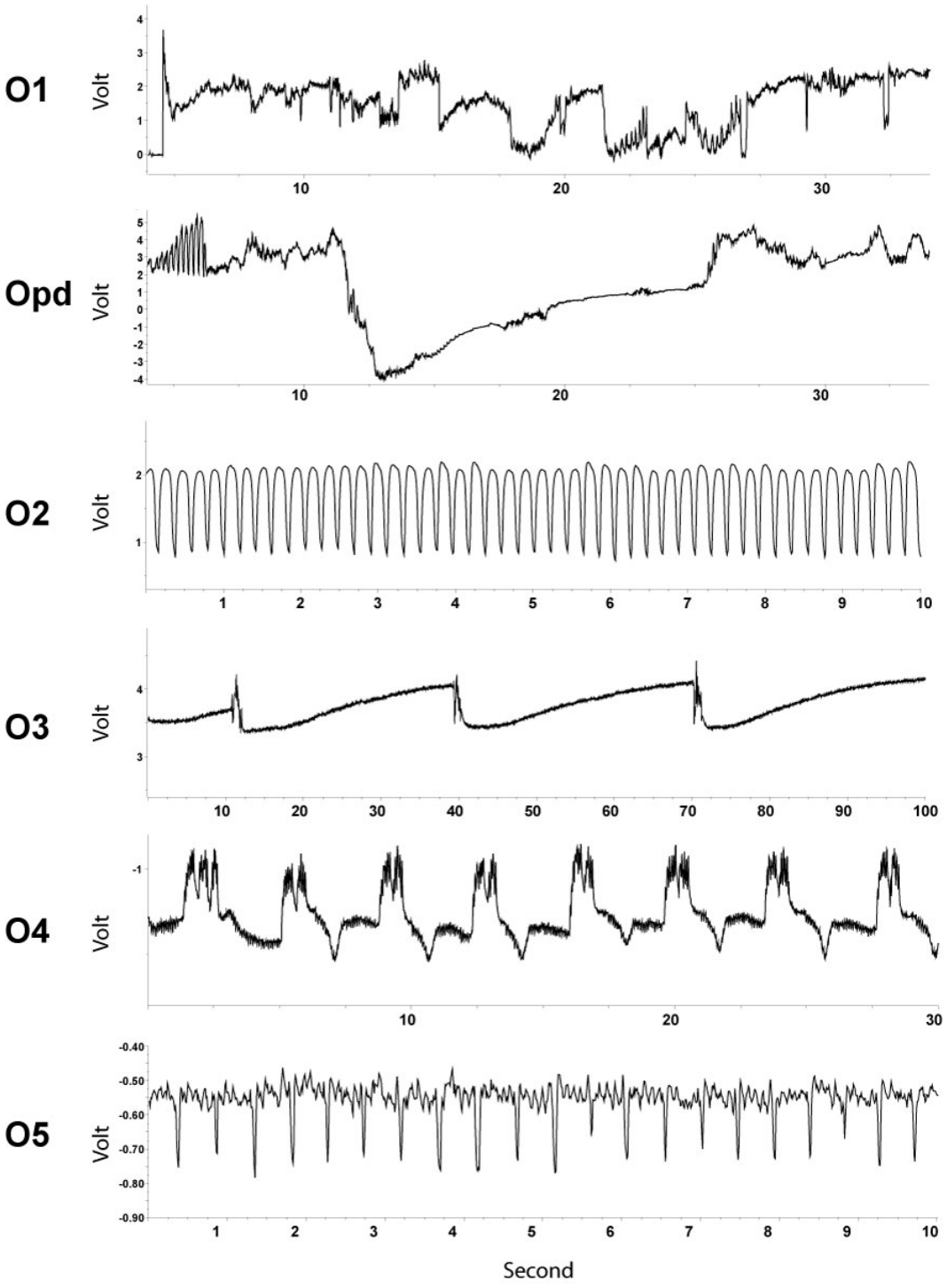

Figure 2 Visual representation of the distinctive electrical penetration graph waveforms produced by adult *Orosius orientali*s probing on bean (O1-O5), tobacco (O1-O4) and an artificial diet (O1-O3); Opd = potential drop.

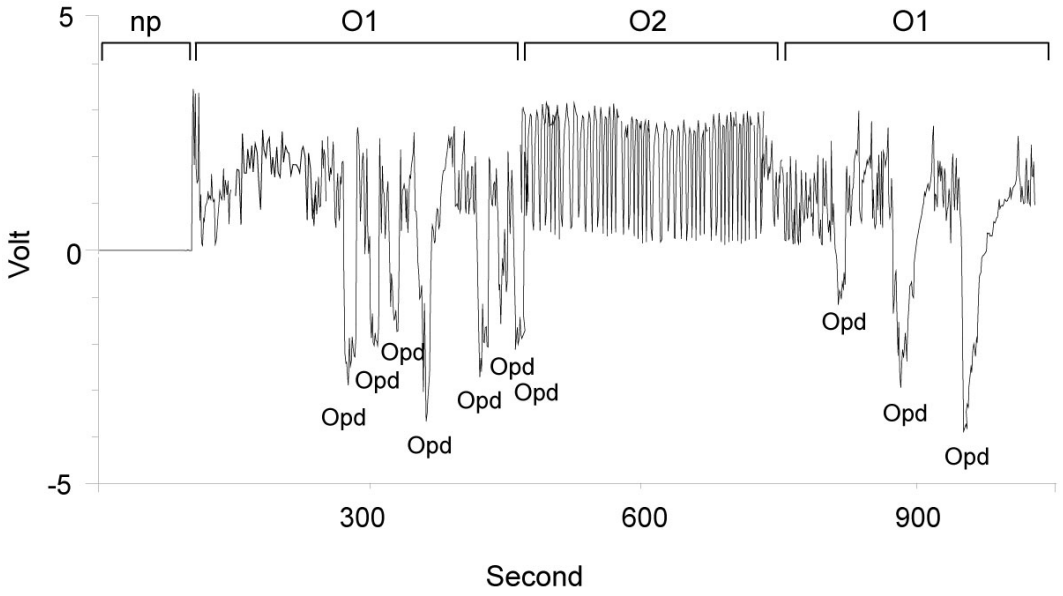

Figure 3 Visual representation of non-probing and waveforms O1 and O2 and potential drops (Opd).

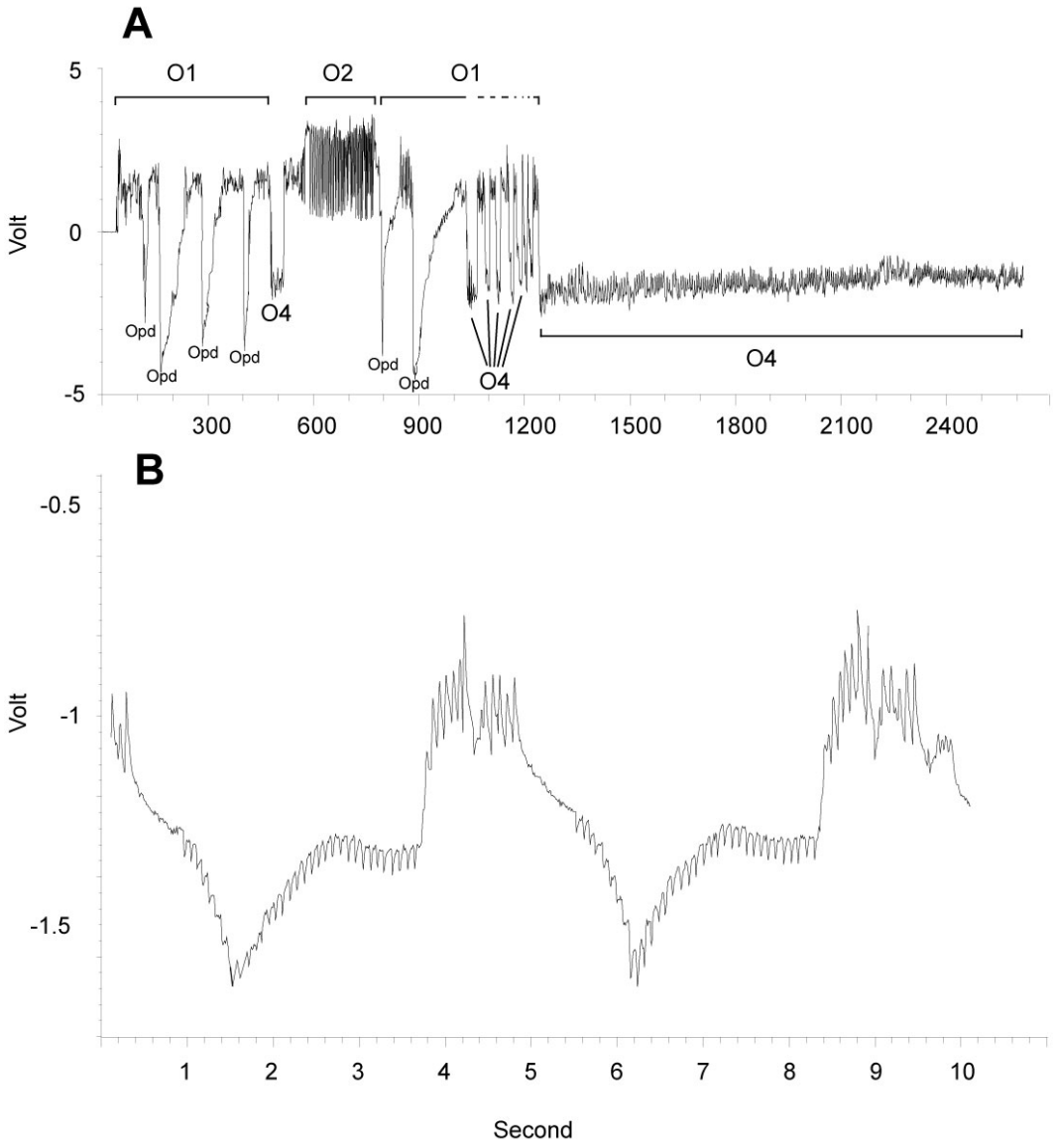

Figure 4 **(**A) transition from waveform O1 to O2 and from O1 to O4, showing the negative voltage shift and O1 interrupted by Opd and short O4 prior to long (>10mm) O4 period (B) detailed representation of complete O4 waveform.

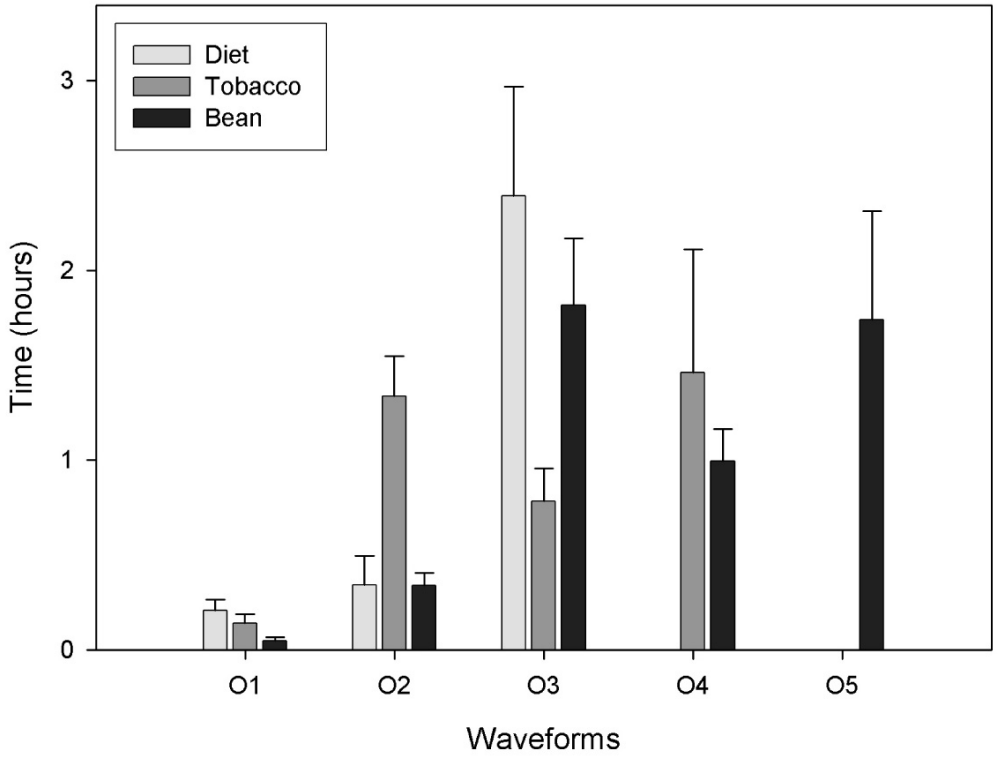

Figure 5 Mean times until waveforms O1-O5 appeared when placed on an artificial diet, tobacco plants and bean plants.

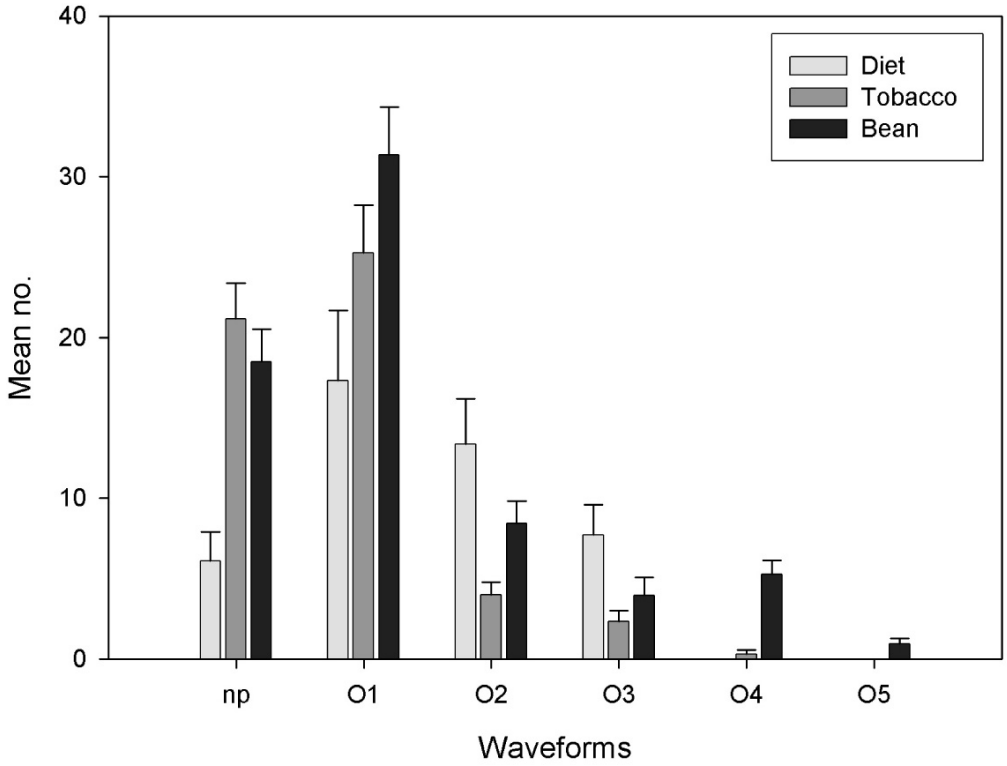

Figure 6 Mean number of non-probing (np) and waveform O1-O5 periods occurring on an artificial diet, tobacco plants and bean plants.

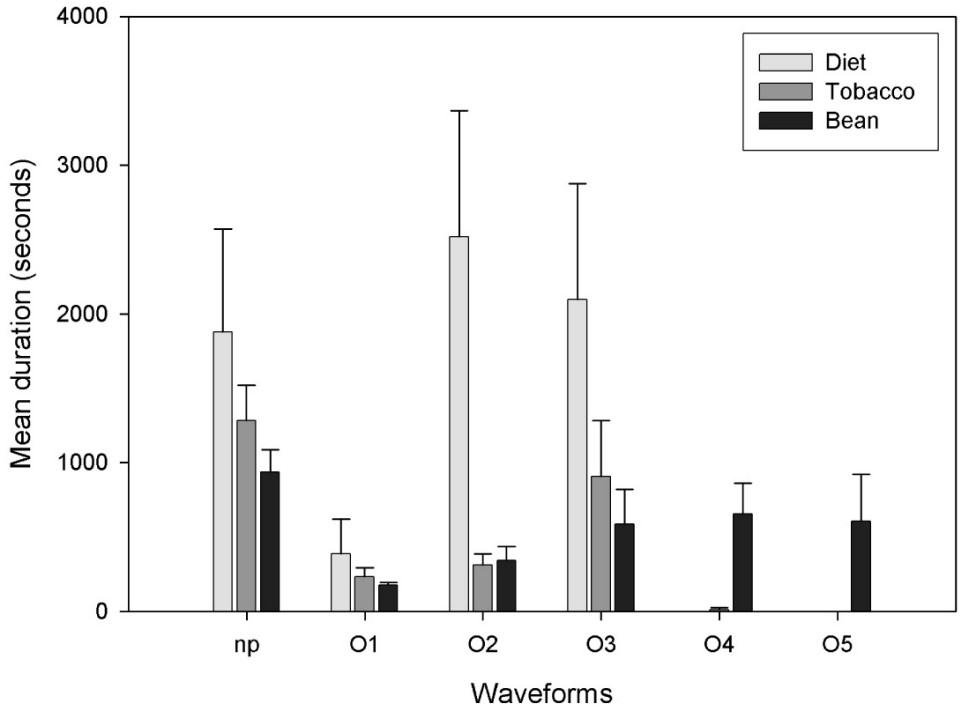

Figure 7 Mean duration of waveforms (O1-O5) and non-probing (np) phases occurring on an artificial diet, tobacco plants and bean plants.

Table 1 Major characteristics of the waveforms recorded using a DC EPG system for adult *Orosius orientalis* probing on *Phaseolus vulgaris* (waveforms O1-O5), *Nicotiana tabacum* (O1-O4) or sucrose-based artificial diet (O1-O3)

	Waveform characteristics				Proposed correlation[c]	
EPG waveform	Relative amplitude (%)	Repetition rate (Hz)	Voltage level[a]	Electrical origin[b]	Plant tissue	Remarks
O1	100	Variable	E	R	Epidermis, mesophyll, all tissues	Cuticle penetration, sheath salivation and other pathway activities
Opd	-	na	I	emf	All living plant cells	Cell puncture by stylets, possibly with salivation and ingestion
O2	35	4-5	E	R/emf	Xylem and/or mesophyll	Active feeding probably from xylem or mesophyll
O3 LF**	50	0.02-0.05	E	R	Undetermined	Unknown
O4 LF HF	5 1	0.6-1 14	I I	Emf R	Sieve elements	Unknown – possibly watery salivation
O5 LF HF	5 1	2 5-8	I I	Unknown Unknown	Sieve elements	Unknown - possibly phloem ingestion

Opd = potential drop, na = not applicable' LF low-frequency, HF high-frequency component
[a] E = extracellular (positive), I = intracellular (negative)
[b] R = resistance, emf = electromotive force * based on comparison with published studies on other Hemipteran insects.