Characterising the Root-Feeding Habits of Grape Phylloxera Using Electrical Penetration Graph

K.B. Kingston and K.S. Powell
Primary Industries Research Victoria
Department of Primary Industries
RMB 1145 Chiltern Valley Road
Rutherglen, Victoria 3685
Australia

P.D. Cooper
School of Botany and Zoology
Australian National University
Canberra, ACT 2600
Australia

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Abstract

Feeding by the radicicolae form of grape phylloxera (*Daktulosphaira vitifoliae*) leads to gall development and root decay on *Vitis vinifera* and may cause economic damage for the vineyard. This damage is managed by planting *V. vinifera* grafted to resistant rootstocks. The processes of grape phylloxera feeding and the mechanisms of rootstock resistance can be investigated with the application of the Electrical Penetration Graph (EPG) technique. EPG technology has been applied to investigate the feeding behaviour of aphids for over 40 years, but has not previously been reported on grape phylloxera. Deterrents for the application of EPG technology to grape phylloxera included the relative small size of the insect, the root-feeding habit and a lack of correlation studies involving non-vascular feeding insects. Here we report that EPG has successfully been applied to study the feeding behaviour of radicicolae grape phylloxera. EPG recordings have been obtained from both excised root and tissue culture plant recording systems, and from first instar to adult life stages of the insect. Feeding patterns have been recorded for up to eight hours and a number of EPG waveforms have been identified. To determine signature-feeding waveforms, initial studies involved recording insects in an active feeding position. In order to establish an understanding of the complete feeding behaviour of grape phylloxera (including stylet penetration and pathway to ingestion), insects in the probe initiation position were disrupted from feeding and relocated onto a new food source. Disturbed insects successfully re-established feeding.

INTRODUCTION

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch, Hemiptera: Phylloxeridae) is a worldwide pest of the European grapevine, *Vitis vinifera* L. The insect feeds on the leaves and roots of *V. vinifera* resulting in the development of galls at the feeding sites. The damage caused by grape phylloxera activity on the roots of *V. vinifera* can lead to an economic loss for the vineyard, and may result in the eventual death of the grapevine. Grape phylloxera that feed solely on the roots of grapevines are termed radicicolae. The grafting of *V. vinifera* to resistant rootstocks of American *Vitis* origin provide the main management option for continuing economic production when a vineyard becomes infested with radicicolae grape phylloxera (Granett et al., 2001).

Electrical Penetration Graph (EPG) is utilised to investigate plant-insect interactions. EPG amplifies the electrical signal that is created when the mouthparts of a piercing arthropod penetrate into a food substrate. The EPG amplifier detects changes in electrical conductivity within the stylet canal of the insect and the plant tissue of the food source (Tjallingii, 2000). A detailed description of the EPG system has been presented by Walker (2000), and is briefly outlined here (Fig. 1). The EPG amplifier consists of two electrical components, the voltage source and the input resistor. An electrical current travels from the voltage source through the output wire to the plant electrode, which is inserted into the moist soil of a potted plant. The insect is connected to an insect electrode, consisting of a short length of 8-20 μm gold wire, with conductive adhesive (silver paint). The insect electrode is connected via the input wire to the input resistor.

When the insect inserts its stylet into the plant, an electrical circuit is completed through the plant-insect biological connection. The biological connection introduces both variable resistance and variable voltage into the circuit. These signals are recorded by the EPG amplifier and are used to interpret insect feeding behaviour.

Since the development of the EPG concept by McLean and Kinsey, and further development by Tjallingii (Van Helden and Tjallingii, 2000), the EPG technique has become established as a methodology suitable for studying feeding behaviour for a range of piercing arthropods. EPG analysis can differentiate between the feeding activities of stylet penetration, stylet pathway to the food source, salivation and ingestion, and therefore assists with determining host plant resistance mechanisms and virus transmission. The majority of published research in the field involves Hemipteran taxa, including members of the families: Aphididae (Sandanayaka and Hale, 2003), Pemiphigidae (Cole et al., 1993), Miridae (Cline and Backus, 2002), Cicadellidae (Backus et al., 2005), Aleyrodidae (Jiang and Walker, 2003) and Phylloxeridae (Harrewijn et al., 1998). Studies have also been undertaken on other orders, including Thysanoptera: Terebrantia (Kindt et al., 2003) and Acarina: Tetranychidae (Guo and Zhao, 2000).

We planned to use the EPG technique to investigate grape phylloxera feeding behaviour on *V. vinifera*, with the intention of expanding the study in the future to include resistant rootstock varieties to understand host-plant resistance mechanisms. The aim of the research was to determine the feeding activity required by grape phylloxera for grapevine feeding establishment, gall initiation and population maintenance. EPG technology had previously been applied to only one member of the Phylloxeridae family, *Phylloxera coccinea* Heyden (Harrewijn et al., 1998), and there are only limited examples for the application of EPG to similar insects, including: similar sized whiteflies (Hemiptera: Aleyrodidae) (Lei et al., 1996); the root feeding lettuce root aphid (Homoptera: Pemphigidae) (Cole et al., 1993); and non-vascular feeding western flower thrips (Thysanoptera: Terebrantia) (Harrewijn et al., 1996).

This paper reports on the application of the EPG technique to grape phylloxera studies involving the susceptible grapevine, *V. vinifera*. Modifications to the EPG system described in Fig. 1 are detailed, current methodologies are discussed, and an initial analysis of the EPG data is presented. Potential further applications of the EPG technique to the grape phylloxera-grapevine model are stated.

MATERIALS AND METHODS

The DC (direct current) Giga-8 series EPG amplifier (Wageningen University, Laboratory of Entomology) used for all recordings was operated under an input resistance of 1 giga Ohm ($10^9\Omega$), 50 x amplification and an input bias current <1 pA. The number of channels utilised per recording varied from 1-6. All recordings were performed at room temperature within a Faraday cage covered with black cardboard to provide a dark environment for the radicicolae grape phylloxera. There was no standardisation for the timing of the recordings over the 24 hour/day period, and recording time length varied from 1-8 hours.

Insect Material

Radicicolae grape phylloxera were originally collected from infested vineyards in the King Valley, Victoria, Australia. Grape phylloxera from this region have been identified as a single genotypic class, G4 (Corrie et al., 2002). Populations were maintained at the Department of Primary Industries – Rutherglen Centre on excised *V. vinifera* root pieces (approximately 1 cm width x 10 cm length), prepared using a protocol slightly modified from Granett et al. (1985). The modifications were: (1) roots washed clean of attached soil with a soft brush under running water; (2) roots soaked for 5-minutes in Ridomil® Gold Plus systemic fungicide solution (~2.3 g/L); (3) roots triple rinsed with sterile distilled water prior to air drying; and (4) the size of the Petri dish was increased to 15 cm, with the increase in area reducing the level of water condensation within the chamber. Grape phylloxera populations were incubated in the dark at a constant temperature of 25 ±3°C prior to EPG recording.

Grape phylloxera were selected for EPG recording based on life stage and location on the excised root piece. Using a stereo microscope, insect life stage was determined by comparative increases in size, and the presence of nearby eggs indicated adult reproduction. If present, neighbouring moulted cuticles were counted to confirm the insects life stage. Insects feeding in an isolated location were preferentially connected to the EPG insect electrode in order to record individual grape phylloxera-grapevine interactions. However isolated insects were not exclusively used due to the group distribution of grape phylloxera on gall sites limiting their availability.

Plant Electrode and EPG Recording Systems

The plant electrode (modified from Fig. 1) consisted of a 1 cm brass pin which could be used with both the excised root and the tissue culture recording systems.

1. Excised Root. *V. vinifera* 'Sultana' root pieces were prepared as per Granett et al. (1985), with modifications as outlined above. Grape phylloxera populations were pre-established on the excised root pieces. The ends of the excised root pieces were wrapped in damp cotton wool, with the modified plant electrode placed between the root exterior surface and the cotton wool at one end (Fig. 3). Wetting the cotton wool provided a suitable environment for the movement of the electrical current for EPG recording.

2. Tissue Culture. *V. vinifera* 'Shiraz' micro-propagated grapevines were prepared as outlined by Kellow et al. (2002). Slight modifications included: (1) agar medium was not supplemented with benzyl aminopurine or napthaleneacetic acid; (2) plantlets were not transferred to a second agar medium prior to the perlite-based medium; and (3) after transfer to the perlite-based medium, tissue culture vines were not inoculated with grape phylloxera eggs. Once the tissue culture vines had established, they were removed from the perlite-based medium and positioned on a 10 cm Petri dish lined with filter paper. The modified plant electrode was placed in contact with the filter paper, which was kept wet throughout the recording (Fig. 3). Wetting the filter paper assisted in maintaining the structure of the tissue culture vine and provided a suitable environment for the movement of the electrical current.

Excised roots are the standard method for maintaining grape phylloxera insects in the laboratory, and provided the simplest method for data acquisition. The tissue culture method allowed for recording of a whole plant response to grape phylloxera feeding in the absence of a soil environment.

Insect Electrode and EPG Recording Positions

The EPG insect electrode consisted of 3-5 cm of 12.5 μm (0.0005 in) diameter gold wire (Sigmund Cohn, Mount Vernon, NY) connected with conductive silver paint (water-based) to the input wire. Using a stereo microscope, the end of the gold wire was repeatedly dipped into the silver paint until a droplet formed. The gold wire was held with self-closing forceps to prevent the wire from slipping. The silver paint droplet was then placed onto the abdomen of the insect and held for a few seconds until dry and the gold wire was fixed in place (Fig. 2). Due to the survival of grape phylloxera for up to eight days without feeding (Kingston et al., 2005), individuals used for EPG recording were not starved prior to set up.

Two insect recording positions were developed for grape phylloxera EPG recordings:

1. Active Feeding. Grape phylloxera were individually wired to the insect electrode without disturbance from the established excised root food source. The insect was then connected to the EPG amplifier for recording. Due to the sessile feeding nature of grape phylloxera, it was assumed that these insects were actively feeding at the time of data acquisition.

2. Probe Initiation. Grape phylloxera individuals were wired to the insect electrode and then gently disturbed from the established excised root food source with a sable-haired paintbrush. The insect was left hanging from the insect electrode for a short period of time until attached to the EPG amplifier and positioned onto a new food source. A stereo microscope was used to assist in this positioning. Probe initiation EPG recordings were performed on both excised root and tissue culture recording systems.

The active feeding position was to gain an initial understanding of grape phylloxera feeding waveforms as no published EPG data was available for comparison. The probe initiation position was used to understand the active feeding waveforms in relation to the complete feeding behaviour (stylet penetration and pathway to ingestion) required by grape phylloxera to survive on *V. vinifera*.

Waveform Analysis

EPG data was captured and analysed using PROBE 3.0 software (Wageningen University, Laboratory of Entomology). The first step of analysis was to identify common waveforms in the EPG recordings. Waveforms were characterised by amplitude (height of wave presented in mV), wave duration (width of wave in seconds), repetition rate (number of waves per second), voltage level (+ or – V) and a general description of the waveform shape (Fig. 4). The second stage of analysis was to determine the occurrence of these waveforms within the EPG recordings. PROBE 3.0 was used to monitor the incidence of each of the waveforms within each EPG recording. This information was then transferred to Microsoft® Excel to calculate the time spent in each waveform by each insect. Waveforms with a low occurrence (once during each recording) were treated as an undefined waveform and excluded from further analysis. Only adult and first instar ("crawler") life stages were used for the initial analysis presented in this paper. Data was grouped to calculate the mean percentage of total feeding time spent in each waveform by adult and crawler individuals. Due to the low sample number (n=3), statistical analysis was not attempted.

RESULTS

EPG recordings were successfully acquired from both the excised root and tissue culture recording systems. Wired grape phylloxera in the active feeding position remained stationary and appeared to continue feeding once connected to the EPG amplifier. Grape phylloxera in the probe initiation position were observed to move along the root surface once connected to the gold wire, although movement was restricted. Once insects had initiated feeding, there was no indication of disturbance to normal feeding behaviour caused by the EPG amplifier. EPG recordings have been successfully performed on all feeding life stages of radicicolae grape phylloxera; however results presented in this paper focus on the crawler and adult life stages.

Insect EPG Recording Positions

Combining data from both plant systems, the active feeding position obtained more successful EPG recordings than the probe initiation position (Table 1). Failed recordings were identified as receiving no EPG signal (a flat 0V line), a non-reactive test calibration pulse or a high voltage noise rhythm masking feeding waveforms. The active feeding insect position was only attempted on the excised root plant system, while the probe initiation insect position was attempted on both the excised root and tissue culture plant system (Table 2).

Due to the low sample number of EPG recordings in the probe initiation position (n=3), all waveform and data analysis has been performed on insects in the active feeding position. Comparing life stages within the active feeding position, 56% of crawler and 70% of adult recordings provided successful data acquisition.

Plant EPG Recording Systems

The tissue culture plant system had a lower success rate of EPG data acquisition than the excised root system (Table 1). The sample number obtained from tissue culture recordings was too low (n=2) for analysis, and details of the procedure are provided as a 'proof of concept' for the methodology only.

Within the excised root plant system, 42% of crawlers and 65% of adults resulted in successful EPG recordings. The majority of successful recordings were obtained from insects in the active feeding position (Table 2). Limiting EPG recordings for waveform and data analysis to the excised root system, with insects in the active feeding position, 15 crawler and 16 adult recordings were successful. However, as this work represented a major component of a PhD thesis, only an initial analysis is presented in this paper. Three recordings were selected from each life stage, based on the ease of interpretation of the recorded waveforms, as a means to validate the application of EPG to the grape phylloxera-grapevine model.

Waveform Analysis

Initial waveform characterisations are based on ~16 EPG recordings. During the analysis of the six (3 crawler, 3 adult) EPG recordings presented in this paper, four waveforms were reoccurring (Table 3). All waveforms occurred at a range of amplitudes, wave durations, repetition rates and voltage levels, and these values were not mutually exclusive between the different waveforms. The waveforms have been assigned random roman numerals, *v, vi, x, xi*, to avoid implying any biological function. The waveforms are assumed to represent feeding behaviour as the grape phylloxera individuals were actively feeding on the excised roots prior to connection to the EPG amplifier, and they maintained this position throughout the recording time. Correlation studies have not been performed to verify this assumption.

Waveform *v* was a monophasic pattern with a sharp positive peak and waves along the base of the pattern. Waveform *vi* was also monophasic in shape, although inverted with waves along the positive peak. The waveform was a complex pattern which was difficult to define and to calculate wave duration and repetition rate values (Table 3). Waveform *x* was biphasic in shape, with a sharp positive and negative peak. A silent period of varying length occurred between the peaks along the baseline. A silent period was defined as a temporary return to the baseline voltage level between waves, but for EPG analysis did not infer a change in waveform or the removal of the insect stylet from the food source. Waveform *xi* was also biphasic in shape, with a sharp positive and negative peak interrupted by a small and irregular silent period. Waveform *xi* was differentiated from waveform *x* by a higher repetition rate.

The percentage time each life stage spent in each of these waveforms indicated variation between crawler and adult insects (Fig. 5). The waveform distribution for crawlers identified two dominant waveforms, *v* and *x*. Waveform *xi* only occurred during 1% of the total recording time. Analysis of adult insect waveform distribution indicated three main waveforms, *v*, *x* and *xi*. An additional waveform, *vi*, was observed in adult recordings. Waveform *xi* was always preceded by waveform *x*, and waveform *v* preceded waveform *vi*.

DISCUSSION

The EPG data presented are a subset of what has been recorded to date. EPG recordings have been successfully performed on all feeding life stages of radicicolae grape phylloxera (second, third and fourth instars not presented). Data acquisition has been achieved on both the excised root and tissue culture recording systems, and with insects in both the active feeding and probe initiation positions. Analysis of additional data will further improve waveform characterisations and increase sample numbers to extend this research, however the EPG technique has already been validated as an application suitable for grape phylloxera feeding behaviour studies.

Anticipated difficulties with the application of the EPG technique to grape phylloxera were based on the relatively small size of the insect, the root feeding habit and the parenchyma cell food source, and how these factors may affect the strength of the EPG signal. Radicicolae grape phylloxera are relatively small insects, adults measure ~900 μm in length, and crawlers ~300 μm (Davidson and Nougaret, 1921). However, insect life stage (and size) did not impact on EPG recording success rate. It is important to obtain EPG recordings from all life stages of grape phylloxera that display biologically different associations with the grapevine food source. Crawlers are important for insect distribution, being active along the grapevine surface, before becoming sessile feeders once a feeding site is established. Feeding by crawlers initiates gall development which sustains the development of the other life stages. The adult life stage does not move during the process of parthenogenetic egg development (De Klerk, 1974), limiting available nutrition to the current feeding site.

The root-feeding habit of grape phylloxera presented challenges in the set up of the plant electrode system. Potted plants would be the optimal set up, but side-roots and bare soil cause the EPG signal to short-circuit in contact with the gold wire of the insect electrode (Cole et al., 1993). Although limitations have been reported to occur when using an excised plant system in place of a whole, intact plant (Van Helden and Tjallingii, 2000), the excised root and tissue culture plant electrode systems were the preferred options for grape phylloxera. The suitability of these two plant systems to sustain and provide suitable nutrition to grape phylloxera for feeding behaviour studies was supported by the use of excised grapevine roots and tissue culture plants for grapevine resistance experiments (Granett et al., 1985; Kellow et al., 2002). Both plant recording systems had successful data acquisition, and sufficient EPG signal strength for waveform analysis.

Grape phylloxera are sessile feeders, resulting in uncertainty that probe initiation insects would re-establish feeding after being disturbed from their original food source. The success rate (and sample number) for insects in the probe initiation position was reduced in comparison with insects maintained in an active feeding position, however successful data acquisition was obtained. EPG recordings of the complete insect feeding behaviour, acquired from the probe initiation position, are important for the interpretation of grape phylloxera-grapevine interactions. Probe initiation EPG recordings are required to understand the processes of site selection and feeding establishment, therefore the validation of this insect EPG recording position was an important advancement for the future application of EPG technology to investigating grape phylloxera feeding behaviour.

The excised root plant system was more successful in obtaining usable EPG data acquisition than the tissue culture plant system. However, this direct comparison was misleading as all insects used for the tissue culture recordings were in the probe initiation position. By comparing probe initiation recordings, there are similar success rates between the excised root and the tissue culture plant system, therefore validating both procedures for application to the grape phylloxera-grapevine model.

On the excised root plant system, the active feeding recording position obtained a higher percentage of successful data acquisitions than the probe initiation position. Insect tethering to the insect electrode is known to influence insect feeding behaviour, especially impacting upon an insects' ability to walk over the plant surface (Van Helden and Tjallingii, 2000). Insects in the active feeding position typically did not move during EPG recording, and were therefore less impeded by attachment to the insect electrode. Although grape phylloxera in the probe initiation position were observed to walk along the root surface, the impact of tethering to the insect electrode may have had an impact on the success rate of these EPG recordings.

The waveforms presented in Table 3 appear visually similar to waveforms that have been correlated to biological activity for the phloem-leaf feeding Aphididae (Reese et al., 2000). However EPG waveforms generated from grape phylloxera represent data based on a different insect family (Phylloxeridae), with a different food source (parenchyma cells) and at a different feeding location (roots). In a review of EPG experimental design (Van Helden and Tjallingii, 2000), it was stressed that correlation studies must be completed on each new taxon studied in order to be able to assign biological meaning to the EPG data. Previous research involving *Phylloxera coccinea* Heyden (Hemiptera: Phylloxeridae) does present similar EPG waveforms from a taxon closely related to grape phylloxera (Harrewijn et al., 1998), however no correlation studies were performed in this study to apply definition of biological meaning to the current EPG data.

Correlation studies relate insect activity (penetration, stylet pathway, ingestion, and oviposition) to the EPG waveforms (Walker, 2000). Specific correlation techniques used for aphids may not be applicable to grape phylloxera, and this requires further investigation before correlation studies can commence. Therefore, all waveform analysis presented in this paper was based on visual characterisation and not biological function.

Waveform *v* was observed in crawler and adult recordings, although waveform occurrence (mean percentage of total recording time) in crawlers was nearly twice that of the adult. The decrease in waveform *v* occurrence in adults was due to an increase in occurrence of waveform *xi*, and the additional pattern, waveform *vi*. Waveform *vi* was only observed in adult EPG recordings, suggesting that this pattern is unique to this life stage. The adult individuals used for analysis in this paper were observed to lay eggs during the recording time. Previous studies on whitefly (Walker and Janssen, 2000) have identified EPG waveforms that correlate with oviposition. The grape phylloxera egg surface was moist at the time of oviposition, providing electrical contact with the plant. The biological activity of egg oviposition does provide a possible source for the unique waveform *vi*, however further work is required in order to prove a correlation between the two events.

Waveform *x* appears visually similar to waveform *xi*, but occurs at a reduced repetition rate. These waveforms occurred in sequence and may be involved in the same biological function, with waveform *xi* occurring at a more rapid rate. Both waveforms were recorded when the EPG amplifier was adjusted to near 0V, where waveforms resulting from insect muscle contractions and fluid streaming potentials are detected (Harrewijn et al., 1998). Potentially, a change in the rate of insect ingestion could result in an increase in the frequency of either of these factors, leading to the increased repetition rate observed in waveform *xi*.

The differential occurrence of these four waveforms in the crawler and adult EPG recordings analysed to date suggests that there may be variable feeding requirements for these two life stages. The potential ability of EPG waveforms to differentiate between the feeding behaviour of life stages highlights the value of the EPG technique for studying grape phylloxera-grapevine interactions.

Although not presented here, initial trials have been conducted on grape phylloxera feeding behaviour on selected resistant rootstocks. EPG analysis can be used to identify the mechanical and chemical resistance response mechanisms displayed by plants during pest-insect feeding (Van Helden and Tjallingii, 2000). Resistant rootstocks are currently the only long-term management option for the viticulture industry, and knowledge of the feeding behaviour of grape phylloxera on rootstock varieties will improve our comprehension of resistance mechanisms. Grape phylloxera genetic variation is also a factor affecting rootstock resistance which can be addressed with the application of the EPG technique. The study presented in this paper was based on a single genotypic class, G4. Further studies involving a range of genotypic classes may provide insight into the observed differential rate of rootstock resistance by specific genotypic classes.

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Tables

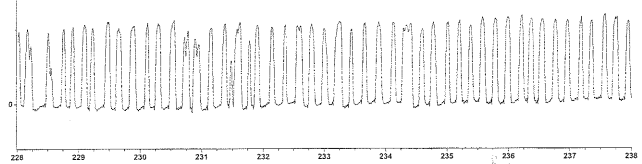
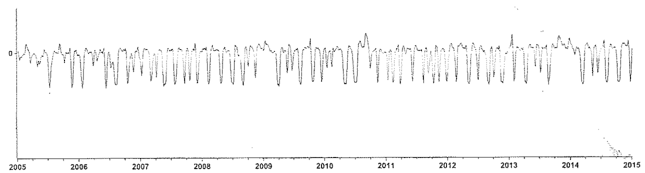
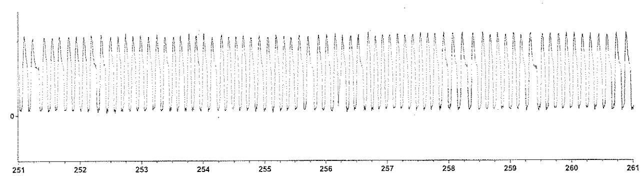
Table 1. Number of successful EPG recordings for experimental insect recording positions and plant recording systems, comparing crawler, adult and combined life stage data for radicicolae grape phylloxera. Total number of EPG recordings attempted in parenthesis.

Life stage	Plant EPG recording system		Insect EPG recording position	
	excised root	tissue culture	active feeding	probe initiation
crawler	15 (36)	1 (1)	15 (27)	1 (10)
adult	17 (26)	1 (6)	16 (23)	2 (9)
combined	32 (62)	2 (7)	31 (50)	3 (19)

Table 2. Number of successful EPG recordings for insect recording positions depending upon the plant recording system used, comparisons between crawler, adult and combined life stage data for radicicolae grape phylloxera. Total number of EPG recordings attempted in parenthesis.

Life stage	Active feeding		Probe initiation	
	excised root	tissue culture	excised root	tissue culture
crawler	15 (27)	NA (0)	0 (9)	1 (1)
adult	16 (23)	NA (0)	1 (3)	1 (6)
combined	31 (50)	NA (0)	1 (12)	2 (7)

Table 3. Characterisation and description of waveforms present in crawler and adult radicicolae grape phylloxera EPG recordings, voltage level is not included in the table as all waveforms occurred at + and – V. For other characteristics, a range of values are presented. Characteristics unable to be calculated are presented as not applicable (NA).

Wave form	waveform pattern 1000mV x 10 sec view	Amplitude (mV)	Duration (sec)	Repetition (sec)	Waveform description
v		250-1000	0.09-0.29	2-7	mono-phasic shape sharp positive peak waves in baseline
vi		250	NA	NA	mono-phasic shape waves in positive peak erratic, difficult to define
x		500-1000	0.11-0.26	2-5	bi-phasic shape sharp positive and negative peak silent period along base
xi		250-1000	0.07-0.22	5-8	bi-phasic shape sharp positive and negative peak irregular silent period

Figures

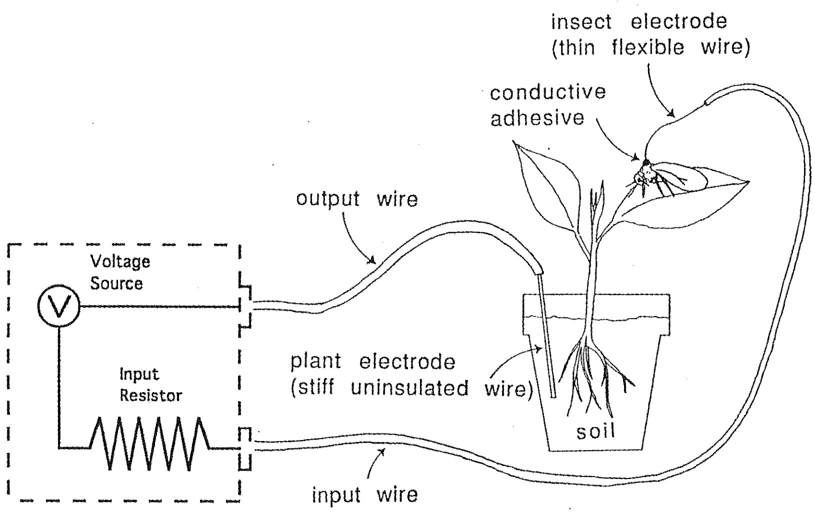


Fig. 1. Diagram of the EPG components, where the EPG amplifier (represented by the dashed box) is connected to the insect-plant system (from Walker, 2000). The main components of the system are labelled with arrows.

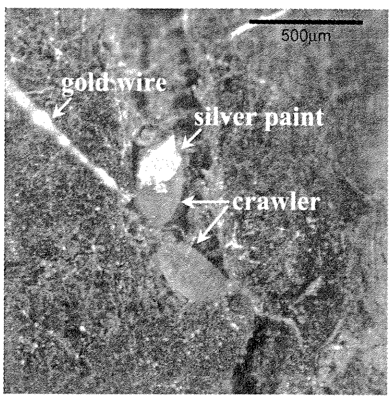


Fig. 2. Crawler radicicolae grape phylloxera connected to the insect electrode, consisting of 12.5 µm gold wire attached with conductive silver paint, scale 500 µm. The main features of the insect electrode are labelled with arrows.

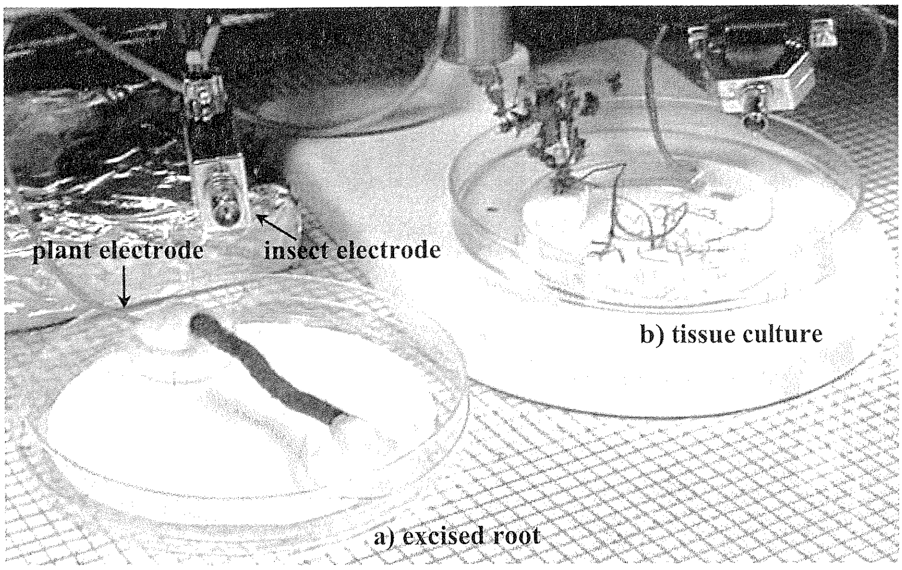


Fig. 3. The plant EPG recording system for the (a) excised root and (b) tissue culture protocols. The plant electrode (EPG output wire) and insect electrode (EPG input wire) are labelled on the excised root system.

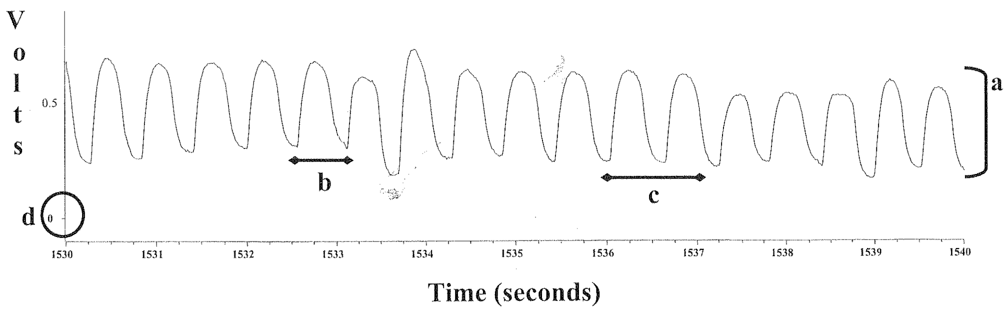


Fig. 4. Key points for waveform characterisation during EPG analysis include: (a) amplitude or height of wave presented in mV, (b) wave duration or width of wave in seconds, (c) repetition rate or wave number per second, (d) voltage level +/– in relation to 0V, and a general description of the wave shape.

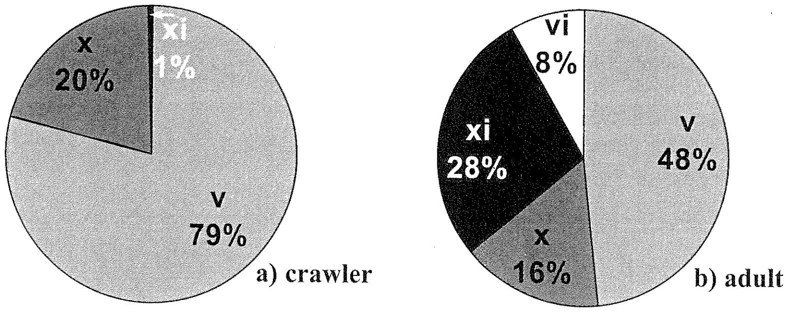


Fig. 5. Pie charts representing mean percentage of total recording time individual (a) crawler and (b) adult radicicolae grape phylloxera spent in waveforms *v, x, vi,* and *xi*.