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Characterization of the Probing and Feeding Behavior of *Planococcus citri* (Hemiptera: Pseudococcidae) on Grapevine

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ABSTRACT The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), is a vector of Grapevine leafroll-associated virus 3 (GLRaV-3), which causes severe damage to grapevines (*Vitis* spp.) worldwide. We studied the feeding behavior of *P. citri* on grapevine leaves and whole plants infected with GLRaV-3 and on artificial feeding membranes using DC-electrical penetration graphs (EPGs). *P. citri* ingested from phloem sieve elements, but it also spent long intervals in the xylem. Waveforms, not described before for mealybugs, were characterized, some of them resembling those of aphids: 1) one new pattern occurring within the phloem phase, named E23, correlated with honeydew excretion and positive ninhydrine reaction and therefore was associated with sap ingestion from the phloem sieve elements; and 2) an extracellular waveform, named G, also possibly associated with ingestion in artificial membranes, which probably represented xylem ingestion. The potential drops (pd) of *P. citri* showed two distinct phases (pd1 and pd2). The occurrence of pds was, on average, less frequent than in aphids (0.14/min), but they lasted much longer (32.5 s). The temporal analysis of 20 EPG recordings on detached leaves lasting 20 h showed great variability among individuals. Only 11/20 mealybugs reached the phloem phase, and ingestion from the phloem sieve elements (E23) was the predominant phloem-related activity. However, the G pattern was even more frequent, and most insects (16/20) showed xylem ingestion activities with an average duration of 8.7 h. This work represents the first step to identify specific stylet activities associated with the acquisition and inoculation of GLRaV-3 by *P. citri*.

KEY WORDS *Planococcus citri*, citrus mealybug, feeding behavior, DC-electrical penetration graph, grapevine

The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), is a polyphagous insect known to occur on 175 genera belonging to 74 families (Ben-Dov 2007). It is cited as a pest of several fruit trees and shrubs, such as citrus (Bodenheimer 1951), cocoa (Acknor 2002), coffee (Staver et al. 2001), and grapevine (*Vitis* spp.) (Ruiz Castro 1965, Lucas Espadas 2002, Cid et al. 2006) as well as a large number of horticultural and ornamental crops mainly grown in greenhouses (Brødsgaard and Albajes 2000, Laffin and Parrella 2004). Detection and control of citrus mealybug is difficult, as for other mealybugs, due to its particular cryptic behavior and to its wax cover that protects these insects from pesticide applications (Walton and Pringle 2004, Daane et al. 2006).

Although mealybugs are not as well known as vectors of virus diseases as other hemipterans (e.g., aphids and whiteflies) (Nault 1997), they are able to transmit at least three different genera of plant viruses: Badnavirus (Lockhart and Olszewski 1994), Vitivirus (Adams et al. 2004), and Ampelovirus (Martelli et al.

2002). *P. citri* and related mealybug species are able to transmit many of these viruses to a wide range of economically important crops (Roivainen 1980, Cabaleiro and Segura 1997b, Lockhart et al. 1997, Phillips et al. 1999, Kubiriba et al. 2001). Low populations of mealybugs causing no direct economic damage have been able to rapidly spread Grapevine leafroll-associated virus 3 (GLRaV-3) to grapevines grown in California's Napa Valley (Golino et al. 2002) as well as in northwestern Spain (Cabaleiro and Segura 1997b, 2006; Cabaleiro et al. 2008). Studying virus transmission mechanisms with mealybugs is difficult, because of their low transmission efficacy and their cryptic feeding behavior. *P. citri* is one of the vector species, transmitting GLRaV-3 which was reported several years ago to be transmitted in a semipersistent manner based on retention time studies (Cabaleiro and Segura 1997a). A later work showed that the virus was present in the salivary glands of *P. citri*, and a circulative mode of transmission was suggested (Cid et al. 2007). A recent work (Tsai et al. 2008) analyzed the transmission parameters of GLRaV-3 by a related species, *Planococcus ficus* (Signoret), and showed evidence that

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the transmission occurred in a typical semipersistent manner.

Several techniques have provided new information on the mechanisms of transmission and interactions among viruses, vectors and their host plants. One such technology is electronic monitoring of insect feeding behavior. This technique, initially developed by McLean and Kinsey (1964), has been modified and improved over the past 30 yr and is generally known as the electrical penetration graph (EPG) technique (Tjallingii 1978, Walker 2000). It gives detailed information on the position of the tip of the stylets and the activities carried out by sap-sucking insects in real time. Only two works have been published reporting the feeding behavior of mealybugs (Calatayud et al. 1994, Calatayud et al. 2001). These papers analyzed the effect of host plant resistance and parasitism on the feeding behavior of the cassava mealybugs *Phenacoccus herreni* Cox & Williams and *Phenacoccus manihoti* Matile-Ferrero, by using a DC-EPG system. Another study, still unpublished, analyzes the behavior and the specific EPG waveform patterns of *Planococcus minor* (Maskell) on coffee and on pineapple plants (Santa-Cecilia 2003). These three studies have provided some insight on mealybug EPG signals and have described correlations, based on light microscopy and on similarities with known patterns of aphids and whiteflies, between waveforms and specific behavioral events, such as sap phloem ingestion. However, a complete characterization and analysis of EPG signals of mealybugs, such as *P. citri*, and their correlation with specific probing or feeding behavioral events has never been published. This information is critical for a better understanding of the mechanism of transmission of mealybug-borne viruses.

The present work aimed to characterize the EPG waveforms patterns observed when *P. citri* feeds on grapevine plants and on artificial feeding membrane systems and to provide a tool for further studies on the transmission mechanisms of plant viruses by mealybugs. Moreover, our study aims to measure sequential and nonsequential feeding behavior variables produced by *P. citri* when feeding on grapevine plants infected with GLRaV-3. This basic information could be used in the future to determine which specific stylet activities are associated with the acquisition and inoculation of GLRaV-3 by *P. citri* and to give insight on the discrepancies observed between its high rate of acquisition and its low rate of transmission (Cabaleiro and Segura 1997a).

Materials and Methods

Insects and Plants. The insects used in this study were from a colony of citrus mealybug collected in 2004 in a vineyard at Bueu, northwestern Spain, where severe epidemics of GLRaV-3 were reported in the past (Cabaleiro and Segura 1997a,b). The mealybug colony was maintained on sprouted potato (*Solanum L.*) tubers placed on a layer of vermiculite inside a plastic cage with a cover made of filter paper to allow

ventilation. The cages were kept in semidarkness at laboratory temperature (20–24°C).

The plants used for EPG recording were 8-yr-old GLRaV-3-infected 'Cabernet franc' grapevines. They were grown in pots from cuttings taken from the grapevine collection kept at the Escuela Politécnica Superior de Lugo, Lugo, Spain. The plants were checked for virus infection by enzyme-linked immunosorbent assay performed with commercial anti-GLRaV-3 antibodies (Bioreba, Reinach, Switzerland). Prior the experiments the grapevines were maintained in a greenhouse at Centro de Ciencias Medioambientales, Madrid, Spain, at 18–25°C with supplemental artificial light.

Electrical Monitoring of Feeding Behavior of *P. citri* on Grapevine Leaves. Groups of immature adult females were collected with a paintbrush from the rearing cage, avoiding those that were in a feeding position. The dorsal wax was partially removed with a paintbrush wetted in an aqueous solution of 25% ethanol and 1% Triton-X100 to facilitate connection to the gold wire. The partially wax-free mealybugs were left standing inside a plastic cage until the ethanol evaporated and were used for experiments thereafter.

The grapevine mature leaves used for EPG recording were detached from the plants immediately before the experiments began and were maintained with the petiole inserted in a plastic vial full of water through a hole in the lid. The plant electrode was inserted in the same plastic vial through another hole, and both the leaf petiole and the electrode were sealed with Parafilm to prevent leaks. The vial was maintained in a horizontal position, to ensure the immersion of the petiole in the water, and with the abaxial side of the leaf facing upward to facilitate the manipulation of the mealybugs, which normally feed on the abaxial surface.

Groups of 10–15 mealybugs were placed on the abaxial side of the leaves. The mealybugs that stopped walking on the leaf and selected a feeding site close to the principal leaf veins, the usual feeding place of these mealybugs, were chosen for connection to the EPG device. A small drop of water-based silver conductive paint was then placed on the insect dorsum and connected to a thin gold wire (10 μm in diameter by 1.5 cm in length). Before attaching the gold wire to the insect, the opposite end of the gold wire was attached with silver paint to a 2-cm-long thin copper wire, which was soldered to a brass nail. The nail was introduced into the BNC connector of the EPG probe. Experiments were carried out inside a Faraday cage in the laboratory at a temperature of 20–25°C. The recordings were started at mid-morning under continuous laboratory lighting. The EPG recordings were discarded if the electrode was glued—circuit completed—when the insect was already probing.

The EPG recordings were acquired at 100 Hz per channel through a four-channel Giga-99 DC-amplifier (Department of Entomology, Wageningen Agricultural University, Wageningen, The Netherlands). This 1 Giga-ohm input resistance DC-amplifier system has its own AD converter, which allows direct recording

of the EPG signals onto the PC hard disk at the time that the EPG waveforms are displayed on the computer monitor. Data acquisition and screen display were controlled by Stylet 3.0 software, and data analysis was performed with MacStylet version 2.0 b10 software (Febvay et al. 1996). Additional EPG analysis was conducted with Probe 3.0 software (Department of Entomology, Wageningen Agricultural University) after Stylet 3.0 data conversion for determining the predominant frequency of the recorded EPG signals. The 20 EPG recordings used for characterization and analysis of the EPG signals lasted 20 h starting from the beginning of the first probe. Each one was performed on a different leaf obtained from three clonal plants.

The electrical origin (R, emf or both) of the waveforms was determined switching between positive and negative system voltage. EPG signals were characterized and labeled according to their duration, voltage level (extracellular or intracellular), relative amplitude, frequency and electrical origin and were compared with those characterized previously for aphids and other hemipterans. In addition to the above-mentioned characteristics, peaks and waves were distinguished in some specific patterns. Waveforms were labeled following aphid and whitefly standards (A, C, E, G, etc.), and subpatterns (1, 2, and 3) were similarly labeled to correspond to previous studies with whiteflies and mealybugs (Janssen et al. 1989, Calatayud et al. 1994).

Fourteen supplementary EPG recordings were carried out on whole plants to ensure the data obtained on detached leaves were not an artifact. The recordings were made in the same way as on detached leaves. The data processing and analysis were substantially the same, but potential drop (pd) waveforms were excluded. The comparison of the mean values of the EPG variables obtained on detached leaves and whole plants was performed with a nonparametric Mann-Whitney *U* test (Statview 4.02, Abacus Concepts, Berkeley, CA).

For characterization of the potential drops and comparison of AC and DC EPG signals, a series of four additional EPG recordings was carried out using an AC/DC EPG device that allows concurrent recording of AC and DC signals (Kindt et al. 2006).

In total, 36 sequential and nonsequential EPG variables (VanHelden and Tjallingii 2000) was considered for analysis of the feeding behavior of *P. citri* on grapevine leaves. The calculation of these variables was performed by means of a modified version of the Excel (Microsoft, Redmond, WA) worksheet (Sarria et al. 2009), which allows automatic calculation of up to 114 EPG variables.

Electrical Monitoring of Feeding Behavior of *P. citri* on Artificial Feeding Membranes. Parafilm was stretched and placed over the opening of a 1.5-ml Eppendorf tube partially filled with 30% sucrose aqueous solution to make an artificial feeding system. The substrate electrode, a thin brass wire, was introduced through a lateral hole in the Eppendorf and sealed with instant glue. The insect electrode was glued to the partially dewaxed mealybug dorsum with water-

based silver paint under a stereomicroscope. Then, the Eppendorf was placed in a horizontal position, and the mealybug was placed on the surface of the membrane. EPG signals were recorded for a period of 2 h, and 10 insects in total were used for this study.

Recording of Honeydew Excretions. A honeydew clock was made to record excretion of honeydew droplets during *P. citri* feeding. The frequency of honeydew excretions was measured on a 24-h clock whose rotating drum was covered by a strip of Whatman paper (Whatman, Maidstone, United Kingdom) sprayed previously with a 2 mg/ml ninhydrine solution on 96% ethanol to detect the presence of free amino acids. The deposition of honeydew droplets was revealed as violet spots on the paper strip—as a reaction with free amino acids—that are often associated with phloem sap ingestion (Lei et al. 1995).

Simultaneous recordings of the honeydew clock and the DC-EPG device were carried out to correlate the occurrence of specific EPG patterns with honeydew excretions. However, the correlation between honeydew excretion and waveform patterns was not possible during the EPG recordings, because the feeding position of mealybugs made it difficult to place the honeydew clock in an optimal position for collecting excretions.

Two simultaneous DC-EPG and honeydew recordings were performed to confirm the expected correlation between pattern E23 and phloem ingestion. Two mealybugs, which remained at the same feeding position for 3 d, were connected to the DC-EPG device by gluing an insect electrode to the partially dewaxed dorsum. The honeydew clock was placed \approx 5 mm over the mealybug dorsum to ensure the collection of excretions when an E23 waveform was observed. No excretions of honeydew were collected under EPG patterns other than E23. The distance between the honeydew spots allowed us to calculate the excretion frequency (Lei et al. 1995).

Results

Characterization of the EPG Waveforms of *P. citri* on Grapevine Leaves. Six main distinct EPG waveforms or patterns were characterized for *P. citri* when feeding on grapevine leaves (Table 1). They were labeled as A, C, pd, G, E, and H on the basis of their resemblance with the aphid and whitefly waveforms and according with the nomenclature used for other mealybug species (Calatayud et al. 1994, 2001).

Waveform A. Waveform A was always the first one observed at the beginning of stylet penetration (Fig. 1A). This waveform was extracellular and was characterized by a high amplitude and very irregular frequency.

Waveform C. Waveform C was always the second pattern detected within a probe. The transition between waveforms A and C was gradual, and sometimes the transition pattern was similar to the waveform B of aphids, which has been correlated with salivary sheath formation (Fig. 1A). Waveform C was a complex pattern composed by the rhythmical superposition of

Table 1. Characteristics of the EPG waveforms recorded during the feeding of *P. citri* on detached grapevine leaves and comparison with the activities carried out by aphids during similar waveforms

Waveform	Subpattern	Element	Characteristic				Activities assigned for similar waveforms in aphids
			Cellular level	Electrical origin (emf/R)	Frequency (Hz) median (min-max)	Duration	
A			Extracellular	R	Irregular	≈15 s	First electrical contact
C			Extracellular	R/emf	Varied	6 s–57 min	Intercellular stylet penetration and sheath salivation
pd	pd1	Peak	Intracellular	nd	1.5 (1–2)	6–127 s	Short cell puncture; in aphids 5–15 s
		Wave		emf	10.5 (5–16)		
G		Wave	Extracellular	emf	5 (2–7.5)	0.8–30 s	Active ingestion of xylem sap
		Peak		R/emf	2 (0.7–4.5)		
E	E1	Wave	Intracellular	nd	4 (1.4–9.5)	10 s–17.8 h	Sieve element salivation
		Peak			1.5 (0.4–2.5)		
H	E21	Wave		nd	10 (7.5–13)	2–65 min	
		Peak			1.5 (0.5–4)		
	E22	Wave		nd	4.75 (3–10)	4–18 h	Sieve element ingestion (E2)
		Peak			6 (3–9.5)		
E23	Wave		nd	R	2 (1–4.5)	Possibly similar to Ele	
	Peak				emf		3 (1.8–7)
		Wave			Irregular	1.6–6.4 h ^a	
		Wave			11 (10–12)		

R, resistance; emf, electromotive force; nd, not determined.

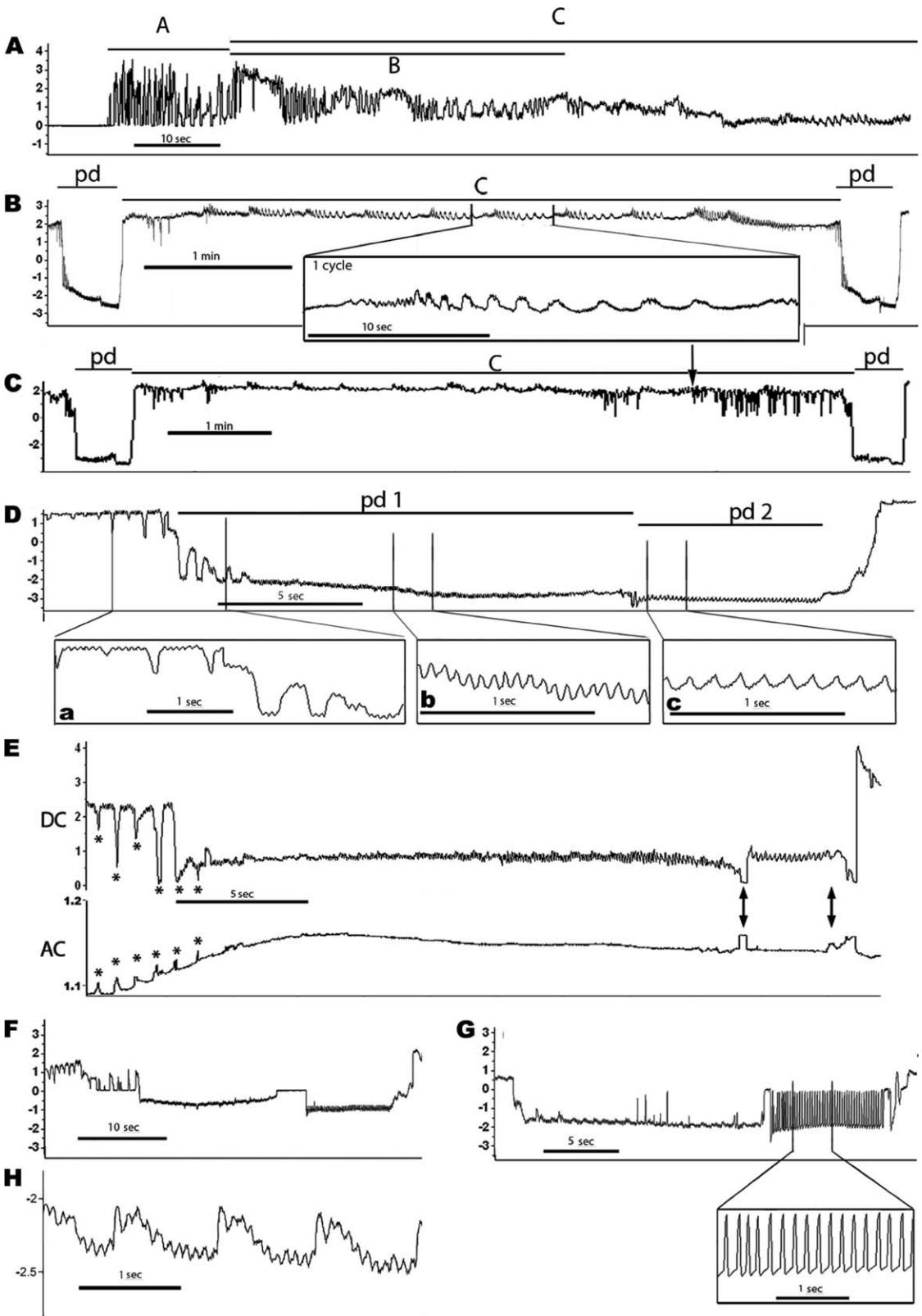
^aData obtained from 14 20-h recordings on whole plants because the recordings carried out on detached leaves had only one H period.

several kinds of waves of variable duration (Fig. 1B). The electrical origin of the C pattern of mealybugs was based mainly on the resistance (R) origin component, because the pattern changed its amplitude and sign most of the time during voltage adjustments. The duration of the C waveform was very variable. A variation of the C waveform with greater amplitude sometimes appeared before a potential drop (Fig. 1C). During the C waveform several pds occurred (Fig. 1B) similar to the ones produced by aphids, although they occurred at a much lower frequency. The mean duration of C between consecutive pds was 5.3 min, and the mean number of pds per hour was 8.7. During C there was another kind of short potential drops (Fig. 2A), which we named pseudo-pds.

Potential Drops. The pattern observed just before a potential drop (pre-pd) was characterized by the presence of waves of very low amplitude and high frequency (Fig. 1D). These waves looked very similar to the waves observed during the first phase of the pd. During the pre-pd, short falls of potential arose gradually, and these intensified to reach the 0 level (Fig. 1D) and finally became negative when the stylet penetrated the cell membrane (Fig. 1D). The short falls of potential of the pre-pd (Fig. 1E) and the strong drop at the beginning of the pd (Fig. 1E), just after piercing the cell membrane, had an emf origin. These drops on the DC signal were simultaneous with the peaks on the AC signal (Fig. 1E), implying that an increase of the emf component, of negative value, had a stronger effect on the EPG signal than the decrease in R. Two phases (pd1 and pd2) were easily distinguished in the standard pds of mealybugs (Fig. 1D). The first phase, pd1, was characterized by a pattern with low amplitude waves at a high frequency. The frequency of pd1 was ≈11–12 waves per s at the beginning of the probe and lowered down to ≈6–7 waves per s when the

probe progressed in time. This pd1 pattern remained the same after voltage adjustments, so its main electrical component was emf and could be associated with intracellular salivation as reported for aphids. In addition to the wave pattern, sometimes a sequence of peaks appeared superimposed on the waves with a frequency of 1–1.5 peaks per s (Fig. 1H). Rarely, the voltage level showed continuous oscillations without a defined pattern, and it was difficult to discern the typical waveform. The mean duration of the pd1 pattern was 23.7 s., with a fluctuation range between 6 and 127 s.

The second phase of the potential drop, pd2, was more uniform than pd1 (Fig. 1D). It showed a baseline completely flat, with waves easily recognizable due to their uniform shape. These were similar to the pattern of subphase II of the long pds reported for whiteflies (Jiang et al. 1999). The frequency of the pd2 pattern varied from six waves per s at the start of the probe and slows to four waves per second several hours later. This wave, as that of the pd1, remained invariable during voltage adjustments, and so its origin was mainly emf. The mean duration of pd2 was 8.8 s., with a fluctuation range between 0.8 and 30 s. The pd2 pattern was not present in 19% of the pds, although this value was variable among individuals. Between the first and the second pd phase a slight but sudden change in the potential level frequently appeared (Fig. 1D). This change varied both in intensity as in sign, although it was normally a small drop. At the end of the pd2 phase the potential usually recovered to the level of pd1 (Fig. 1D) before reaching the extracellular voltage level. The recordings with the dual AC/DC-EPG system (Fig. 1E) showed a gradual increase in the voltage level of the AC signals, indicating a gradual decrease in resistance, in the pre-pd pattern. There was also a sudden rise in the voltage level of the



AC signals both at the beginning and at the end of the pd2 phase, which corresponded to small drops in the potential level in the simultaneously recorded DC signals. Positive spikes shown in the AC signals indicated sharp declines in resistance of the circuit, whereas negative spikes in the DC signal indicated drops in the electrical potential due to strong electromotive forces (Fig. 1E).

Another particular pre-pd pattern was sometimes observed just before the cell membrane was pierced and was characterized by relatively long periods of 0 voltage (Fig. 1F). These periods were caused by strong increases of resistance that were so strong that the EPG signal remained at 0 V even when the substrate voltage was adjusted. Furthermore, these high electrical resistance periods (HERPs) also were observed between the pd1 and pd2 phases (Fig. 1F), and less frequently at the end of the second phase. Another uncommon variant to the standard pds was the pd comb (Fig. 1G). During these particular pds, the waves at the pd2 phase increased their amplitude, and the signal rose to reach the 0 level on the crests but dropped to the standard pd2 level on the troughs.

Pseudo-pds. The pseudo-pd (Fig. 2A) was similar to a standard pd of ≈ 25 s in duration. It started with a drop in potential of lower magnitude than a pd, and then it remained with a positive sign. Its low frequency precluded to carry out voltage settings to determine the electrical origin of the potential drop and the waves. The waves after the potential drops were rather similar to the pd2 waveform, but their frequency was irregular. At the end of the pseudo-pd, the potential rose to the potential level of the C waveform in an exponential-like curve shape. In some rare occasions the pseudo-pd was an isolated event, but most frequently two or three pseudo-pds appeared consecutively. In such cases the time between pseudo-pds was only few seconds.

Unknown Activity Periods. Unknown activity periods (Fig. 2B) always appeared during stylet pathway (C waveform) and just before the insect started an active ingestion period or stylet withdrawal. It was a characteristic pattern that started with a potential drop to the 0 voltage level followed by a series of cycles of positive peaks that declined progressively in amplitude. These cycles of peaks of R origin (Fig. 2C) were separated by periods of 0 voltage level. In each

cycle, the intensity of the peaks increased to reach a maximum level and then decreased until the end of the cycle. The amplitude of the cycles decreased gradually to become nil, and the voltage remained at 0 level for several seconds or minutes.

Waveform G. A waveform (Fig. 2D) very similar to the waveform G correlated with active xylem ingestion in aphids was detected in most of the EPG recordings of *P. citri*. This G waveform was characterized by the presence of peaks with a frequency of 1–4.5/s. Its relative amplitude was variable: sometimes reaching similar values to that of the A waveform and sometimes decreasing to lower values. The electrical origin of peaks was mainly emf, because it remained constant during voltage adjustments. The superimposed waves had a higher frequency, and the effect of voltage settings was variable: sometimes the effect was inconspicuous, but other times the modification was great, suggesting that both R and emf components were involved. The duration of the waveform G produced by *P. citri* on detached grapevine leaves was very variable, ranging from several seconds to >15 h. The pattern was normally homogeneous, but sometimes short periods (several seconds) of low amplitude, without peaks, occurred between longer periods of high amplitude. In a few cases the pattern lost its typical shape, but its frequency remained constant (Fig. 2E).

Waveform E. Waveform E groups had four different subpatterns: E1, E21, E22 and E23. All of them had an intracellular origin and small amplitude with similarities to phloem-related activities reported for other hemipterans. The E1 waveform (Fig. 2F) was always the first one observed immediately after the potential drop. Its mean duration was ≈ 19 s and was very similar in shape, frequency and amplitude to the first phase of potential drops (phase pd1) as described above. When the E1 waveform ended, the frequency sharply declined and was always followed by the E2 pattern with alternation of two different subpatterns: E21 and E22 (Fig. 2G), following the nomenclature proposed by Calatayud et al. (1994). The subpattern E21 showed clear positive peaks superimposed on a baseline composed by waves. The subpattern E22 was composed only of waves. Both E21 and E22 subpatterns alternated in time but not always in a cyclical manner as reported by Calatayud et al. (1994) for *Phenacoccus*

Fig. 1. (Opposite) DC-EPG waveforms recorded for *P. citri* feeding on grapevine leaves. (A) Start of a probe showing waveform A followed by waveform C (stylet pathway). The beginning of waveform C starts with a brief pattern that resembles waveform B of aphids. (B) Intercellular stylet pathway (C) between two intracellular punctures (pd) showing the repetitive cycles of waveform C. The box shows details of one of the repetitive cycles. (C) A variant of waveform C with sharp voltage drops occurring just before a pd that starts at the arrow. (D) Intracellular stylet puncture or pd. Two distinct phases of the pd can be distinguished (pd1 and pd2). The boxes show details of (a) pre-pd with its characteristic waves and peaks followed by the start of the pd1 phase, (b) standard pattern of the pd1 phase, and (c) standard pattern of the pd2 phase. (E) Simultaneous recording of a pd with the AC/DC EPG device. Signal of DC (top) and AC (bottom) are shown. Asterisks (*) indicate sharp declines in resistance of the circuit (displayed as positive spikes in the AC signal) and simultaneous strong changes in electrical potential due to electromotive forces (displayed as negative spikes in the DC signal) at the beginning of the pd. Double arrows (2) indicate the same phenomenon at the start and end of the pd2 phase. (F) Anomalous pd with high electrical resistance periods: one intermittent in the pre-pd phase and another continuous between the two pd phases. (G) Potential drop showing a comb-like pattern during the pd2 phase characterized by positive spikes of high amplitude and frequency of 7–8 Hz. (H) Variation of pd1 waves with superimposed peaks. (All the y-axes are expressed on V).

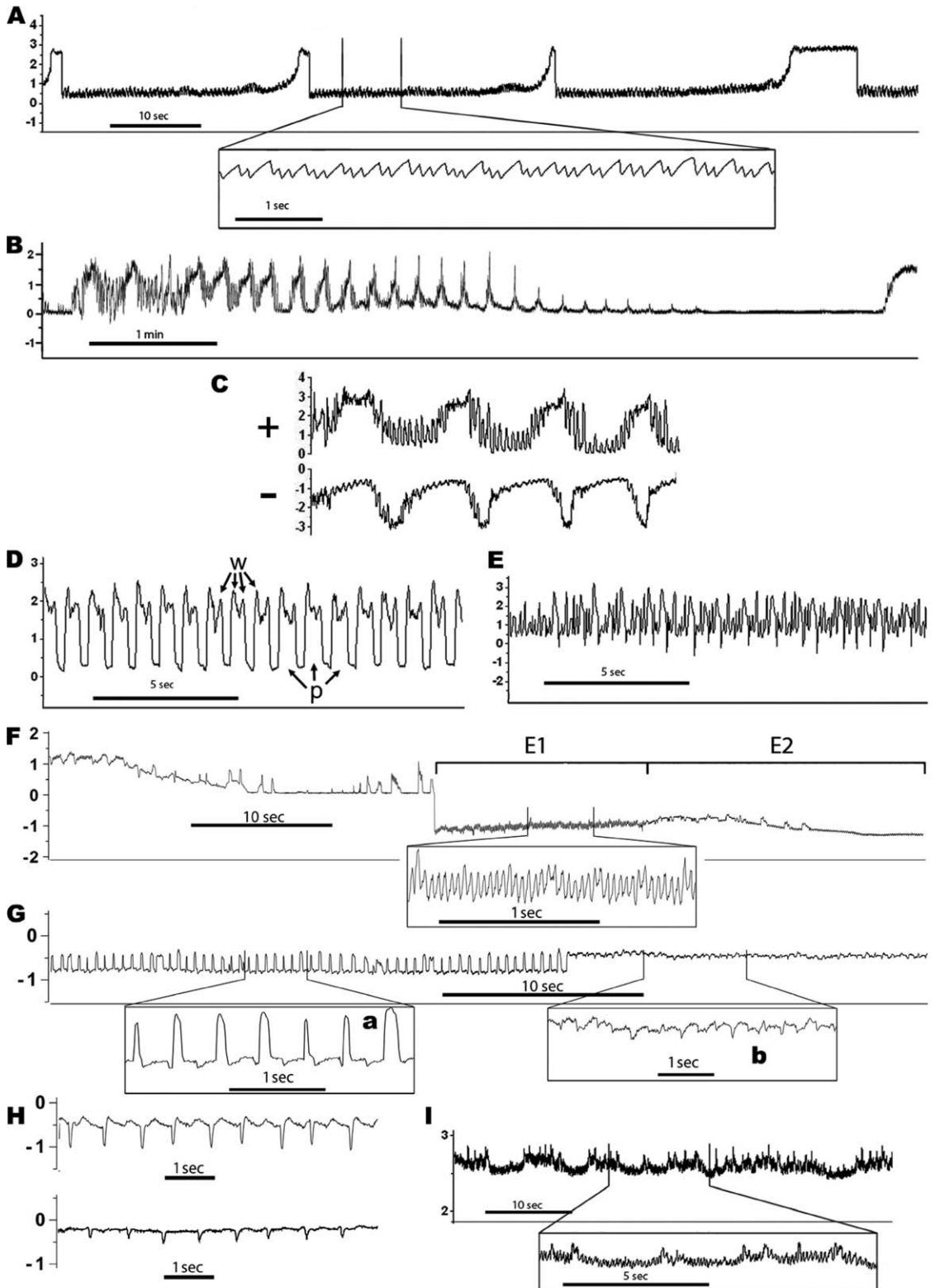


Fig. 2. DC-EPG waveforms recorded for the citrus mealybug, *P. citri* feeding on grapevine leaves. (A) Sequence of repetitive pseudo-pods showing details of one of them. (B) Unknown activity period. (C) Voltage adjustments of the unknown activity period reveal the resistance origin of the peaks. (D) Typical waveform G, similar to the waveform G of aphids which

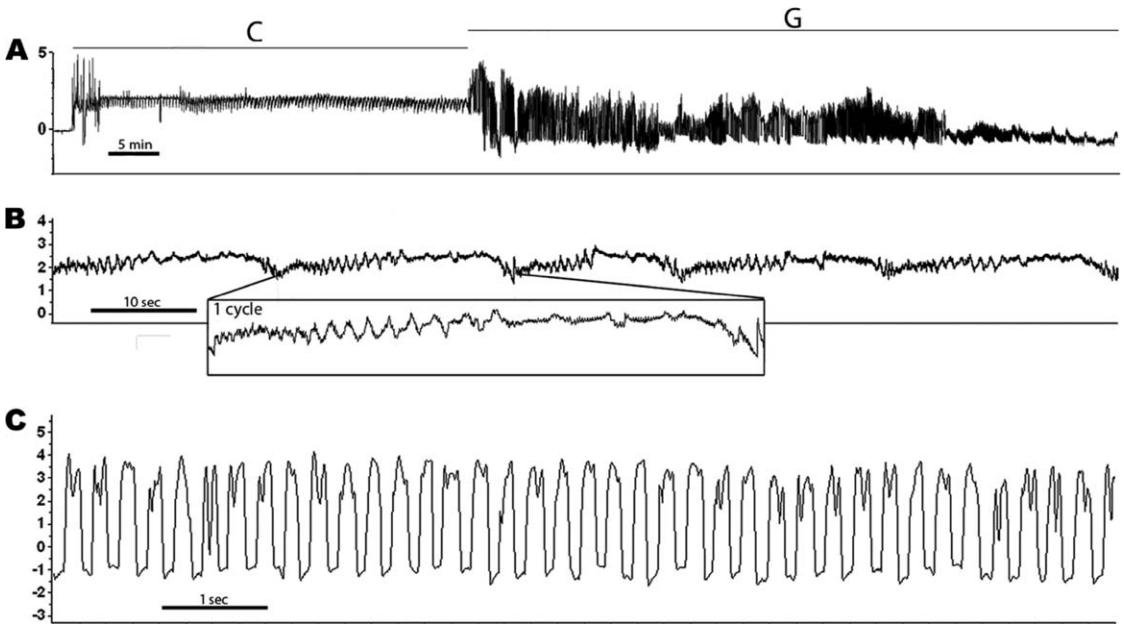


Fig. 3. Waveforms recorded during feeding of *P. citri* in artificial diet. (A) Typical EPG signals from a 2-h recording. (B) A period of waveform C showing its irregular pattern similar to waveform C on grapevine leaves (see Fig. 1B). (C) Period of waveform G showing very similar characteristics to pattern G recorded in grapevine leaves. (All the y-axes are expressed on V).

manihoti Matile-Ferrero. The mean duration of the E21-E22 subpatterns was 18 min, although it was quite variable ranging from 2 to 65 min (Table 1). The third phloem-related pattern showed a waveform (E23) (Fig. 2H) that had not been reported previously for other mealybug species. The E23 pattern had very similar peaks and waves and almost identical frequency and electrical origin as the E2 waveform reported for aphids. Its duration was normally very long, from several hours to days, and it was always preceded by the E21-E22 waveforms. Waveform E23 was composed of negative peaks superimposed on a wave baseline. The peaks had R as main electrical component, and they showed variable amplitude and frequency. The waves that appeared in the E23 pattern sometimes varied in frequency and amplitude.

Waveform H. This extracellular uncommon pattern had a baseline of waves with high frequency (10–12 waves per s) and irregular peaks (Fig. 2I). It was present in just one of the 20 EPG recordings. This pattern resembled pattern H described for *Phenacoccus herreni* Cox & Williams by Calatayud et al. (2001).

Electrical Monitoring of Feeding Behavior of *P. citri* on Artificial Diet. The standard EPG waveforms recorded when *P. citri* adults fed on artificial diet through stretched Parafilm membranes were shown in Fig. 3A. The main pattern recorded during stylet path-

way activities through the membrane was composed of a cyclic sequence of signals (Fig. 3B) with a strong resemblance to waveform C observed for mealybugs feeding on grapevine leaves (Fig. 1B). The average duration of the stylet pathway was 20.7 ± 6.1 min (ranging from 3.5 to 59.8 min; $n = 10$). During the stylet pathway, no drops in potential were observed due to the lack of living cells in the feeding media. This C-like pattern was usually interrupted by long periods of EPG signals (Fig. 3C) with a pattern almost identical to the G waveform observed when feeding on grapevine leaves. The G pattern observed in artificial media suggested an active ingestion period, and its average duration in the 2-h recording period was 32.9 ± 18.4 min ($n = 10$). The percentage of probing time occupied by waveform G varied between 1 and 70%. Waveform G, observed in artificial diet, was very similar in shape, frequency, amplitude and electrical origin to the one observed for mealybugs feeding on grapevine leaves (Fig. 2D).

Correlation of EPG Waveforms With Honeydew Excretions. The collection of honeydew excretions during EPG recordings was attempted in 20 occasions, but we were unsuccessful collecting secretions. The two successful simultaneous EPG recordings-honeydew collections were done for 20 h with two different individuals that were allowed to feed before recording

Fig. 2. (Continued) is correlated with xylem ingestion. (E) Variation of G waveform with the same frequency but different shape. (F) Phloem-related activities: E1 waveform is followed by E2 waveform. (G) E2 waveform showing two distinct and subpatterns: E21 (a) and E22 (b) with its characteristic peaks and waves. (H) Two variations of waveform E23 with morphology resembling waveform E2 from aphids. Waves may become conspicuous (top) or be less apparent (bottom). (I) Waveform H showing its characteristic peaks and frequency. (All the y-axes are expressed on V).

Table 2. Parameters of the feeding behavior of *P. citri* on detached grapevine leaves and whole plants calculated from 20 and 14 recordings, respectively, of 20 h of duration each

Parameter	Detached leaves		Whole plant	
	Mean \pm SE	(min-max)	Mean \pm SE	(min-max)
Time from the recording start				
to the first pd	4 \pm 0.59	(0.71-10.32)		
to the first E1	374.72 \pm 87.4	(77.71-1019.35)	874.45 \pm 76.02	(494.73-1168.43)
to the first E23	394.39 \pm 88.52	(118.63-883)	901.17 \pm 148.94	(656.14-1170.37)
to the first G	234.81 \pm 81.93	(25.07-1185.04)	387.30 \pm 79.08	(163.97-927.79)
Time from the beginning of the probe until				
the first pd	5.59 \pm 0.64	(0.72-10.32)		
the first E1	310.11 \pm 334.90	(77.71-709.16)	383.85 \pm 91.72	(93.09-791.58)
the first E23	334.90 \pm 66.57	(118.63-779.96)	277.74 \pm 18.14	(254.50-313.49)
the first G	221.23 \pm 79.41	(38.17-1126.52)	290.27 \pm 60.73	(127.31-745.79)
No. probes	1.8 \pm 0.23	(1-4)	3.64 \pm 0.73*	(1.00-9.00)
No. nonprobes	0.85 \pm 0.22	(0-3)	2.71 \pm 0.81*	(0.00-8.00)
No. pds	58.6 \pm 8.5	(6-131)		
No. G	3.65 \pm 0.85	(0-13)	2.57 \pm 0.77	(0.00-7.00)
No. E1 followed by E21-E22	0.4 \pm 0.15	(0-2)	0.43 \pm 0.21	(0.00-1.00)
No. E1 followed by E21-E22 and E23	0.45 \pm 0.11	(0-1)	0.29 \pm 0.35	(0.00-2.00)
Mean of pds no. per probe	43.5 \pm 7.77	(3-121)		
Mean of G no. per probe	2.3 \pm 0.46	(0-6.5)	1.52 \pm 0.59	(0.00-5.00)
Mean duration of probes	800.52 \pm 85.52	(280.31-1199.00)	552.21 \pm 116.91	(114.92-1200.00)
Mean duration of C between pds	6.32 \pm 0.39	(3.68-9.52)		
Mean duration of pds	32.58 \pm 1.46†	(21.11-49.11)†		
Mean duration of pd1	23.66 \pm 1.27†	(17.01-40.54)†		
Mean duration of pd2	8.84 \pm 0.56†	(3.87-15.22)†		
Mean duration of E1	30.52 \pm 4.25†	(10.01-48.62)†	45.74 \pm 9.78†	(15.54-86.33)†
Mean duration of E21-E22	29.39 \pm 9.25	(2.71-98.23)	6.94 \pm 3.14	(1.27-25.50)
Mean duration of E23	668.04 \pm 109.89	(270.64-1080.37)	242.38 \pm 154.93	(29.62-543.86)
% whole phloem phase spent in E1	1.1 \pm 0.73	(0.02-7.6)	23.57 \pm 11.26	(0.32-100.00)
% whole phloem phase spent in E21-E22	22.2 \pm 11.01	(0.31-96.32)	50.68 \pm 14.41	(1.56-98.63)
% whole phloem phase spent in E21-E22 and E23	93.74 \pm 2.91	(71.74-99.66)	94.13 \pm 2.22	(90.43-98.11)
% time spent in nonprobing	1.7 \pm 0.5	(0-6.49)	7.67 \pm 2.26*	(0.00-23.89)
% time spent probing	98.3 \pm 0.5	(93.51-100)	92.33 \pm 2.00*	(76.11-100.00)
% time spent in C	33.11 \pm 4.23	(5.8-61.41)	71.69 \pm 4.12*	(47.59-91.84)
% time spent in pd	2.74 \pm 0.42	(0.18-6.08)		-
% time spent in G	34.96 \pm 7.45	(0-92.49)	9.30 \pm 2.77	(0.00-33.12)
% time spent in E1	0.02 \pm 0.01	(0-0.07)	0.05 \pm 0.02	(0.00-0.15)
% time spent in E21-E22	1.35 \pm 0.5	(0-8.19)	0.45 \pm 0.30	(0.00-2.62)
% time spent in E23	24.67 \pm 7.45	(0-90.11)	5.24 \pm 7.73	(0.00-45.32)
% time spent in E	26.04 \pm 7.68	(0-93.52)	5.74 \pm 4.61	(0.00-46.19)

The temporal parameters are expressed in minutes except for those marked with †, which are expressed in seconds.

* Indicate statistical differences between the data obtained on detached leaves and on whole plants ($P < 0.05$; Mann-Whitney).

started in the same feeding position for a period of 3 d. Both individuals were producing a very long and extended E23 pattern when the honeydew excretion was collected. The excretion frequency was very variable within individuals (4.8–8.8 drops per h) and between individuals (3.8–5.8 drops per h). In some occasions, two consecutive droplets were excreted almost immediately one after the other.

Probing and Feeding Activities of *P. citri* in Grapevine Leaves During a 20-h Period. More than half the time (11/20), *P. citri* made a single probe that lasted the entire 20-h recording period. The mean number of probes per EPG recording was 1.8, and the mean duration was 13.3 h (Table 2). The variation in the predominant feeding behavior events among individual insects was large (Fig. 4). Approximately half (11/20) of the mealybugs reached the phloem, but only nine individuals exhibited a sustained phloem ingestion activity with a long (>4 h) E23 phase. Just six individuals remained in E23 at the end of the 20-h recording period. A single E23 period per recording was observed, and it had a mean duration of 10.9 h (Table 2). The mean time to reach the

phloem from the beginning of the 20-h recording was 6.2 h, ranging from 1.3 to 17 h, although not all insects were able to reach the phloem in the 20-h period. Most mealybugs (16/20) spent some time in xylem ingestion activities: four individuals spent <2 h and six >10 h (Fig. 4). The mean duration of xylem ingestion was 8.7 h distributed in 3.6 periods of 3.7 h of mean duration. Among the three individuals that finished phloem ingestion activities before the end of the recording, two of them fed from the xylem for 14 min and 4.4 h, respectively, at a later time.

The probing and feeding behavior observed in whole plants differed little from those on detached leaves (Table 2). The main differences were detected in the number of probes made by the mealybugs and in the time spent on nonprobing activities, as well as in the percentage of time spent on stylet pathway phase activities (waveform C), which was greater on whole plants than on detached leaves.

Figure 5 shows, on an hour-by-hour basis, the analysis of the predominant activities of *P. citri* on grapevine leaves. Stylet pathway activities (waveform C)

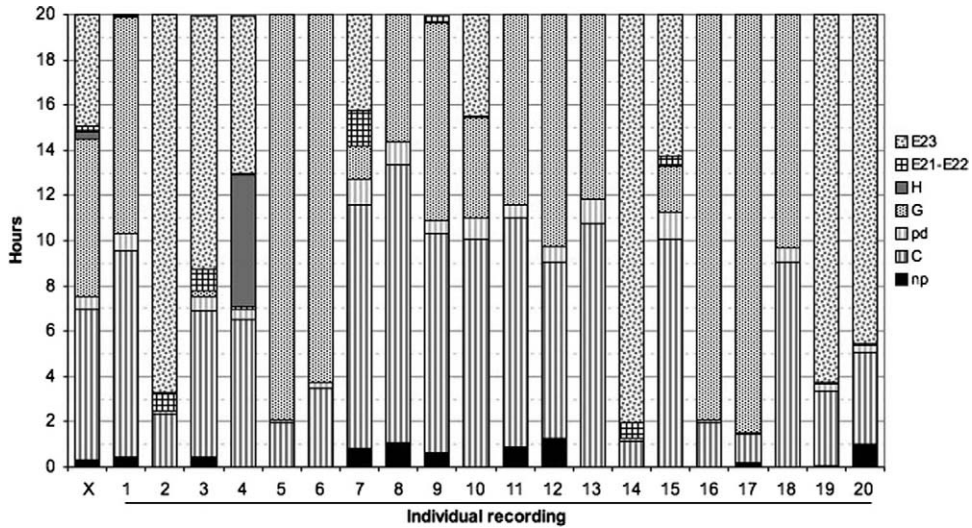


Fig. 4. Time spent in the different activities or waveform obtained from the 20 EPG-recordings of each individual *P. citri* feeding on grapevine leaves. The first column (X) represents the mean duration of each waveform for all 20 individual recordings. The other columns, numbered from 1 to 20, represent the total time spent in each waveform for each individual mealybug. The sequence of waveforms within columns does not represent the real order of occurrence of waveforms.

were the only ones present at the start of the probe and were the most frequent in the first 3 h. Later, stylet pathway activities fluctuated between 20 and 45% of the recording time. Approximately one third of the mealybugs ingested from xylem vessels (waveform G) after the second hour of recording, and between 25 and 45% did for the remainder of the recording period. After the sixth hour of recording, the occurrence of stylet activities in the phloem ranged between 25 and 35%, with E23 (phloem sap ingestion) as the main phloem-related behavior. E21-E22 appeared several

times, and E1, due to its very short duration and low occurrence, was never computed in this hour-by-hour analysis. After the sixth hour of recording, the waveform distribution was relatively stable with $\approx 30\%$ of insects in C, 40% in G and 30% in E. The nonprobing periods and the H waveform appeared erratically.

The succession of behavioral events of *P. citri* in grapevine leaves during the 20 h recording period is shown in Fig. 6. Nonprobing was always followed by stylet pathway activities (Waveform C). Potential drops embedded within waveform C were frequently

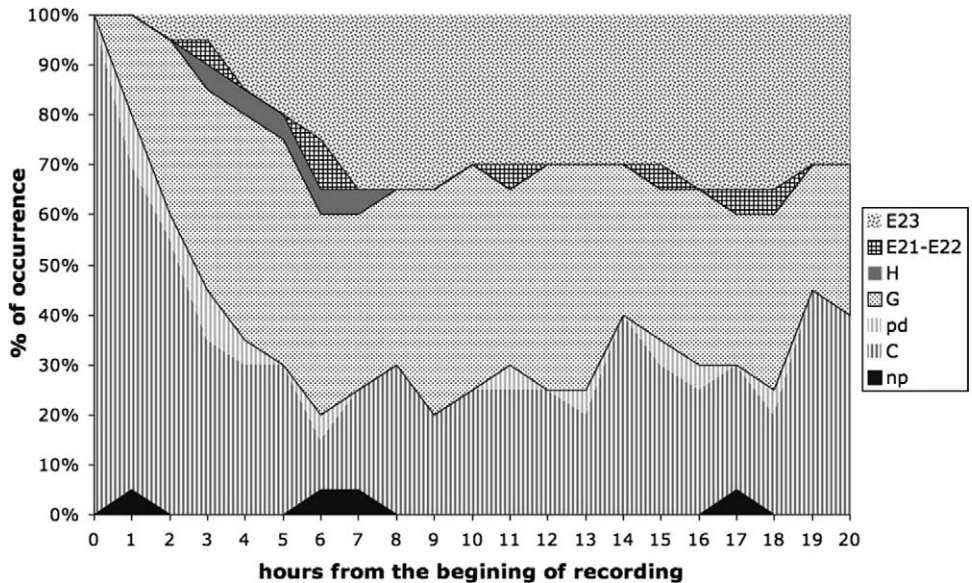


Fig. 5. Temporal evolution of the different waveforms of *P. citri* feeding on grapevine leaves. The graph represents the percentage of individuals in each waveform performed by *P. citri* at the end of each hour of the 20-h recording period.

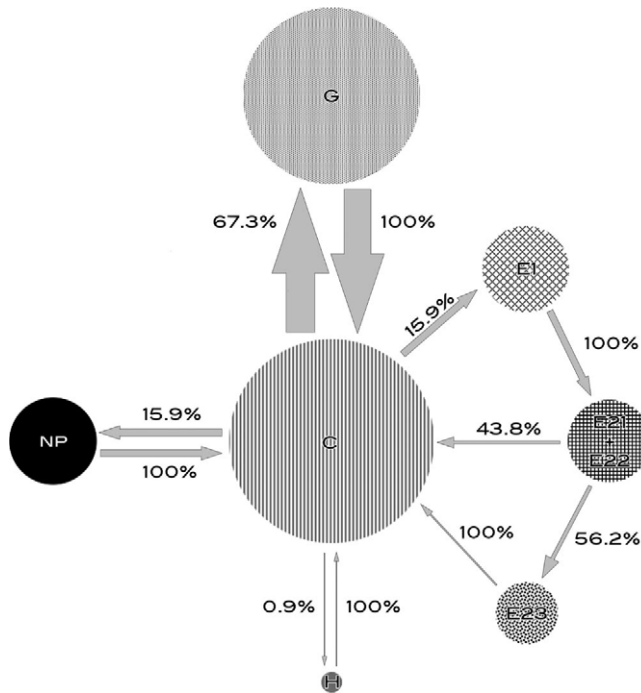


Fig. 6. Succession of behavioral events of *P. citri* in grapevine leaves during the 20-h recording period. Numeric values indicate the occurrence (%) of transitions from one waveform to the next. The number of occurrences of each specific waveform is represented by the size of each circle. The thickness of the arrows represents the frequency of each change of waveform in relation to the total changes produced in the 20 recordings. The changes C-pd and pd-C are not represented because their great number would overtake other changes between waveforms.

recorded up to a total of 1,169 times in the 400-h recording period (20 insects \times 20 h per insect). Pattern C was frequently (67.3%) followed by xylem ingestion activities (waveform G). Phloem activities occurred in 15.9% of the cases following the stylet pathway phase. Pattern E1 was always followed by patterns E21-E22, and these patterns were sometimes followed by E23 (phloem sap ingestion) (56.2% of the cases) or returned back to stylet pathway activities (43.8% of the cases).

Discussion

P. citri has been reported as being a phloem feeder (like aphids and whiteflies) but has a more sedentary way of life (except for whitefly nymphs), and this is reflected in *P. citri*'s feeding behavior. *P. citri* produce a much lower number of probes of longer duration than those produced by aphids (Collar et al. 1997). The sequence of feeding behavior events reported for mealybugs is quite similar to that reported for aphids: insertion of the stylet in the plant, intercellular stylet pathway with a series of intracellular stylet punctures into the mesophyll and, immersed on the intercellular pathway, periods of xylem ingestion and phloem-related activities (presumably salivation followed by long periods of phloem sap ingestion). This is not surprising as aphids (Aphidoidea) and mealybugs (Coccoidea) are the most closely related superfamily

lies within the Sternorrhyncha suborder according to molecular phylogenetic studies (von Dohlen and Moran 1995).

We also found that the EPG waveforms of *P. citri* on grapevine leaves are quite similar to those produced by cassava mealybugs (*Phenacoccus* spp.) (Calatayud et al. 1994, 2001) and those observed for *Planococcus minor* (Maskell) when feeding on coffee plants (Santa-Cecilia 2003). In naming the EPG waveforms, we tried to follow the nomenclature established by Calatayud et al. (1994, 2001), which labels the waveforms in a similar way as those assigned to aphids. Some waveforms produced by *P. citri* resemble those produced by aphids, although some differences were also found, such as in the phloem-related patterns. The main differences between the nomenclature assigned to *P. citri* and those assigned by Calatayud et al. (1994, 2001) for *Phenacoccus* spp. refer to waveforms C, E and G. The G-like pattern was not clearly observed in the recordings of *Phenacoccus* spp. but was frequently observed in *P. minor* (Santa-Cecilia 2003).

Calatayud et al. (1994) distinguished two subpatterns, CI and CII, that alternate in sequence, and a third more erratic, CIII, that is characterized by a greater amplitude. We decided not to differentiate between C subpatterns because of the absence of correlation studies with specific activities, as suggested by previous studies on whitefly feeding behavior (Janssen et al. 1989, Jiang et al. 1999). Pattern C in

P. citri has cyclical variations that are probably related to stylet pathway activities as in aphids, mainly piercing and salivary sheath formation (secretion of gelling saliva, mechanical work of stylets and probably other activities, such as apoplast tasting). The several C-like waveforms found for *P. citri* are similar to the C sub-patterns of whiteflies (Janssen et al. 1989, Jiang et al. 1999, Jiang and Walker 2003), but their continuous modification and lack of a standard frequency or amplitude complicates their comparison. The third sub-pattern described by Calatayud et al. (1994) (CIII) is a modification of the standard C pattern and is quite similar to the variation of waveform C reported by Jiang and Walker (2003) for whiteflies just before the beginning of a potential drop. We observed a similar pattern for *P. citri* just before the occurrence of pds (Fig. 1C), which shows strong voltage drops, nearly reaching the 0 level but without becoming negative.

The potential drops (pds) of *P. citri* are composed of two phases (Fig. 2B) and are very similar to the pds reported for other mealybugs species (Calatayud et al. 1994, Santa-Cecilia 2003) and to the long pds described for whiteflies (Jiang et al. 1999). The two phases (pd1 and pd2) are very distinct, but their biological meaning remains unclear. Calatayud et al. (1994) suggested that the first phase could be related to intracellular salivation and the second phase to ingestion because of its resemblance to the II-1 and II-3 pd subphases of aphids. However, this assumption is difficult to test, because mealybugs do not transmit viruses in a nonpersistent manner, and experiments, such as the experiments conducted by Martin et al. (1997) or Powell (2005) by using viruses as markers of intracellular salivation/ingestion, are not feasible. One important difference between potential drops of *P. citri* and those of aphids is that they occur much less frequently (at an average rate of 0.14/min) but are much longer (mean duration of 32.5 s). Potential drops in aphids occur at an average rate of 1/min and last \approx 5–15 s (Tjallingii 1985). The pds of *P. citri* show recurrent HERPs both at the start of the pd, as well as between the two phases, and to a lesser extent at the end of the pd (Fig. 1F).

The phloem punctures of *P. citri* present many similarities with those described for *Phenacoccus* spp. and *P. minor*, although some differences also were observed. Phloem activities of *P. citri* in a complete feeding puncture into a sieve element can be separated in four different patterns: a first waveform (E1), which is almost identical to the first phase of a potential drop (pd1); two alternate patterns (E21 and E22) similar to those described for *Phenacoccus* spp. by Calatayud et al. (1994); and a fourth pattern (E23), very similar to the E2 waveform of aphids (Prado and Tjallingii 1994) and the phloem ingestion waveform of *P. minor* (Santa-Cecilia 2003). The E23 waveform was frequently present in *P. citri* but never described for *Phenacoccus* spp. It was the only E-like waveform that lasted for several hours to days and was correlated with the excretion of honeydew and a positive ninhydrine reaction. Therefore, we can state that E23 is clearly associated to phloem sap ingestion. The behavioral

events performed by *P. citri* during the two first E phases are unknown, but their similarity to other waveforms of aphids may provide some indications of its function. The first phase, E1, could represent salivation into the sieve element as reported for the E1 waveform, which has been linked to salivary secretions in virus transmission studies (Prado and Tjallingii 1994). The insect activity occurring during E21-E22 waveforms is unclear, but Calatayud et al. (1994) suggested that alternations of salivation (E21) and ingestion (E22) could take place, but there is no experimental evidence to support this. In our work, we decided to maintain the nomenclature used by Calatayud et al. (1994) for phloem-related waveforms (E1, E21, E22). However, further studies will be needed to confirm whether waveforms E of mealybugs represent the same activities as those reported for the E-waveforms of aphids. We decided to use the term E23 for referring to the phloem ingestion activity with the same meaning as the "E2" of aphids.

Waveform G of *P. citri* was detected during artificial membrane feeding experiments, suggesting that diet ingestion may occur during this phase. The presence of the same waveform during plant feeding also could indicate that active ingestion of nonpressurized fluids, such as those existing in the xylem, is likely to occur. Also, the resemblance of the waveform G of *P. citri* with the waveform G of aphids (Spiller et al. 1990) and psyllids (Bonani et al. 2010), which was correlated with xylem sap ingestion, suggests that the same behavioral event is taking place. Nevertheless, histological studies should be used to confirm the interpretation of all of the waveform patterns described for mealybugs or any other new taxon.

The waveform called H in *P. citri* is similar to the H waveform described by Calatayud et al. (2001). They associated this waveform with feeding activities occurring in places far from the leaf veins and with feeding activities of parasitized mealybugs. They suggested that H waveform could be related to the E(c) = E1e pattern of aphids because of its extracellular nature and waveform morphology, although the activity performed by aphids during this waveform was never probed.

No waveforms similar to the pseudo-pds and to the unknown activity periods of *P. citri* have been described for other hemipterans. The activity carried out by the mealybug during these waveforms is unknown.

The feeding behavior of *P. citri* is quite similar to other phloem-feeding species. The main difference we observed in our study was the predominance of xylem ingestion activities over the rest of the recorded patterns. The predominance of the G waveform in *P. citri* may be related to the removal of wax during the connection of the electrode to the insect body before EPG recording. This might have forced the mealybugs to feed from the xylem to rehydrate their body and prevent water loss. An increase in frequency and duration of xylem ingestion has been related to a rehydration mechanism in hemipterans (Spiller et al. 1990, Bonani et al. 2010). Prolonged xylem ingestion may play a similar role in mealybugs. The long duration of

the xylem ingestion observed for *P. citri* may be explained by its high capacity to support changes in body volume as well as to prevent desiccation or promote hemolymph excretion through the cephalic and caudal ostioles (Gullan and Kosztarab 1997). The G pattern was not clearly observed in the work of Calatayud et al. (1994, 2001), although short G-like patterns were occasionally detected. However, a long G-like pattern was observed in *P. minor* in 90% of the EPG recordings lasting 11 h on average (Santa-Cecilia 2003). The EPG recordings of *P. citri* and *P. minor* were made with adult females, whereas the experiments conducted with *Phenacoccus* spp. (Calatayud et al. 1994, 2001) were made with third-instar females; so, the life stage of the insect may explain, in part at least, the differences observed.

The other main difference observed for *P. citri* was the long time needed to reach the phloem, which on average was ≈ 6 h. Only half of the mealybugs were able to ingest from the phloem (occurrence of E23 waveform), and the time elapsed from the start of the first probe to the first phloem contact was very variable. However, we observed in additional observations that sustained phloem ingestion could last for several days once the insect reached the E23 waveform (data not shown).

There were only small differences in the recorded EPG signals between the assays performed on detached leaves and on whole plant. Nonsignificant differences were detected for the time spent in the phloem or xylem, but both parameters had an overall lower duration on whole plants than on detached leaves. The only significant differences were related with the nonprobing activity, both in duration as in number, and the stylet pathway activities (C pattern), which were longer on whole plants than on detached leaves. This difference may be an indirect effect of the shorter time spent on phloem and xylem activities altogether.

The information obtained on the stylet penetration activities and the characteristic waveforms of *P. citri* when feeding on grapevine leaves provided a foundation for further study of the transmission mechanisms of GLRaV-3 or any other mealybug-transmitted virus disease. All viruses transmitted by *P. citri* are transmitted in a semipersistent or in a circulative non-replicative manner (Lockhart and Olszewski 1994, La Notte et al. 1997, Cid et al. 2007); therefore, their transmission mechanisms will be affected by phloem-related events (E1, E21-E22, and E23), because these viruses are all restricted to the phloem tissues in natural infections.

Our study provides an excellent framework for future work on the behavioral events leading to the acquisition and inoculation of mealybug-transmitted viruses, and the gathered information could be used to improve current management strategies for disease control. One approach would be to design systemic chemicals able to move through the xylem vessels and produce a repellent or knockdown effect to mealybugs. Such chemicals could be used to avoid the in-

oculation or acquisition of GLRaV-3 and other phloem-restricted viruses.

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References Cited

- Ackonor, J. B. 2002. Current levels of incidence of parasitism and predation in *Planococcus citri* Risso (Homoptera: Pseudococcidae) in Ghanaian cocoa (*Theobroma cacao* L.) farms. *Insect Sci. Appl.* 22: 105–112.
- Adams, M. J., J. F. Antoniw, M. Bar-Joseph, A. A. Brunt, T. Candresse, G. D. Foster, G. P. Martelli, R. G. Milne, and C. M. Fauquet. 2004. The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation. *Arch. Virol.* 149: 1045–1060.
- Ben-Dov, Y. 2007. ScaleNet. Pseudococcidae. (<http://www.set.barc.usda.gov/SCALENET/SCALENET.HTM>).
- Bodenheimer, F. S. 1951. *Citrus entomology in the Middle East*. W. Junk, The Hague, The Netherlands.
- Bonani, J. P., A. Fereres, E. Garzo, M. P. Miranda, B. Appezzato-Da-Gloria, and J.R.S. Lopes. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. *Entomol. Exp. Appl.* 134: 35–49.
- Bødsgaard, H. F., and R. Albajes. 2000. Insect and mite pests, pp. 48–60. *In* R. Albajes [ed.], *Integrated pest and disease management in greenhouse crops*. Kluwer Academic Publishers, Hingham, MA.
- Cabaleiro, C., and A. Segura. 1997a. Some characteristics of the transmission of *grapevine leafroll associated virus 3* by *Planococcus citri* Risso. *Eur. J. Plant Pathol.* 103: 373–378.
- Cabaleiro, C., and A. Segura. 1997b. Field transmission of *grapevine leafroll associated virus 3* (GLRaV-3) by the mealybug *Planococcus citri*. *Plant Dis.* 81: 283–287.
- Cabaleiro, C., and A. Segura. 2006. Temporal analysis of *grapevine leafroll associated virus 3* epidemics. *Eur. J. Plant Pathol.* 114: 441–446.
- Cabaleiro, C., C. Couceiro, S. Pereira, M. Cid, M. Barrasa, and A. Segura. 2008. Spatial analysis of epidemics of *Grapevine leafroll associated virus-3*. *Eur. J. Plant Pathol.* 121: 121–130.
- Calatayud, P. A., C. D. Seligmann, M. A. Polania, and A. C. Bellotti. 2001. Influence of parasitism by encyrtid parasitoids on the feeding behaviour of the cassava mealybug *Phenacoccus herreni*. *Entomol. Exp. Appl.* 98: 271–278.
- Calatayud, P. A., Y. Rahbe, W. F. Tjallingii, M. Tertuliano, and B. Leru. 1994. Electrically recorded feeding-behavior of cassava mealybug on host and nonhost plants. *Entomol. Exp. Appl.* 72: 219–232.
- Cid, M., S. Pereira, A. Segura, and C. Cabaleiro. 2006. Seguimiento de la población de *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) en un viñedo de las Rías Baixas (Galicia). *Bol. San. Veg. Plagas* 32: 339–344.
- Cid, M., S. Pereira, C. Cabaleiro, F. Faoro, and A. Segura. 2007. Presence of *Grapevine leafroll-associated virus 3* in primary salivary glands of the mealybug vector *Planococcus citri* suggests a circulative transmission mechanism. *Eur. J. Plant Pathol.* 118: 23–30.

- Collar, J. L., C. Avilla, and A. Fereres. 1997. New correlations between aphid stylet paths and nonpersistent virus transmission. *Environ. Entomol.* 26: 537–544.
- Daane, K. M., W. Bentley, V. M. Walton, R. Malakar-Kuennen, J. A. Millar, C. A. Ingels, E. A. Weber, and C. Gispert. 2006. New controls investigated for vine mealybug. *Calif. Agric.* 60: 31–38.
- Febvay, G., Y. Rahbé, and M. van Helden. 1996. MacStylet, software to analyse electrical penetration graph on the Macintosh. *Entomol. Exp. Appl.* 80: 105–108.
- Golino, D. A., S. T. Sim, R. Gill, and A. Rowhani. 2002. California mealybugs can spread grapevine leafroll disease. *Calif. Agric.* 56: 196–201.
- Gullan, P. J., and M. Kosztarab. 1997. Adaptations in scale insects. *Annu. Rev. Entomol.* 42: 23–50.
- Janssen, J.A.M., W. F. Tjallingii, and J. C. Lenteren. 1989. Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomol. Exp. Appl.* 52: 69–81.
- Jiang, Y. X., and G. P. Walker. 2003. Electrical penetration graphs of the nymphal stage of *Bemisia argentifolii*. *Entomol. Exp. Appl.* 109: 101–111.
- Jiang, Y. X., H. Lei, J. L. Collar, B. Martin, M. Muniz, and A. Fereres. 1999. Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. *J. Econ. Entomol.* 92: 357–366.
- Kindt, F., N. N. Joosten, and W. F. Tjallingii. 2006. Electrical penetration graphs of thrips revised: combining DC- and AC-EPG signals. *J. Insect Physiol.* 52: 1–10.
- Kubiriba, J., J. P. Legg, W. Tushemereirwe, and E. Adipala. 2001. Vector transmission of *Banana streak virus* in the greenhouse in Uganda. *Ann. Appl. Biol.* 139: 37–43.
- La Notte, P., N. Buzkan, E. Choueiri, A. Minafra, and G. P. Martelli. 1997. Acquisition and transmission of *grapevine virus A* by the mealybug *Pseudococcus longispinus*. *J. Plant Pathol.* 79: 79–85.
- Lafin, H. M., and M. P. Parrella. 2004. Developmental biology of citrus mealybug under conditions typical of California rose production. *Ann. Entomol. Soc. Am.* 97: 982–988.
- Lei, H., W. F. Tjallingii, and J. C. van Lenteren. 1995. Recording of EPGs and honeydew excretion by the greenhouse whitefly, pp. 53–68. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia 1995: taxonomy, biology, damage control and management*. Intercept, Andover, United Kingdom.
- Lockhart, B. E., and N. E. Olszewski. 1994. Badnavirus group pp. 516. *In* R. G. Webster and A. Granoff [eds.], *Encyclopedia of Virology*. Academic, New York.
- Lockhart, B.E.L., K. KiratiyaAngul, P. Jones, L. Eng, P. DeSilva, N. E. Olszewski, N. Lockhart, N. Deema, and J. Sangalang. 1997. Identification of *Piper yellow mottle virus*, a mealybug-transmitted badnavirus infecting *Piper* spp. in Southeast Asia. *Eur. J. Plant Pathol.* 103: 303–311.
- Lucas Espadas, A. 2002. Comportamiento de melazo (*Pseudococcus citri* Risso) en uva de mesa en la Región de Murcia. *Alternativas de control biológico*. Phytoma-España. 138: 28–36.
- Martelli, G. P., A. A. Agranovsky, M. Bar-Joseph, D. Boscia, T. Candresse, R.H.A. Coutts, V. V. Dolja, B. W. Falk, D. Gonsalves, W. Jelkmann, et al. 2002. The family Closteroviridae revised. *Arch. Virol.* 147: 2039–2044.
- Martin, B., J. L. Collar, W. F. Tjallingii, and A. Fereres. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *J. Gen. Virol.* 78: 2701–2705.
- McLean, D. L., and M. G. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. *Nature (Lond.)* 202: 1358–1359.
- Nault, L. R. 1997. Arthropod transmission of plant viruses: new synthesis. *Ann. Entomol. Soc. Am.* 90: 521–541.
- Phillips, S., R. W. Briddon, A. A. Brunt, and R. Hull. 1999. The partial characterization of a badnavirus infecting the Greater Asiatic or water yam (*Dioscorea alata*). *J. Phytopathol.* 147: 265–269.
- Powell, G. 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. *J. Gen. Virol.* 86: 469–472.
- Prado, E., and W. F. Tjallingii. 1994. Aphid activities during sieve element punctures. *Entomol. Exp. Appl.* 72: 157–166.
- Roivainen, O. 1980. Mealybugs, pp. 15–38. *In* K. F. Harris and K. Maramorosch [eds.], *Vectors of plant pathogens*. Academic, New York.
- Ruiz Castro, A. 1965. *Plagas y enfermedades de la vid*. Instituto Nacional de Investigaciones Agronomicas, Madrid, Spain.
- Santa-Cecilia, L.V.C. 2003. Interação coconilha (Pseudococcidae)-planta avaliada mediante estudos biológicos e da técnica de “Electrical Penetration Graphs” (EPG). Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.
- Sarria, E., M. Cid, E. Garzo, and A. Fereres. 2009. Excel Workbook for automatic parameter calculation of EPG data. *Comput. Electron. Agric.* 67: 35–42.
- Spiller, N. J., L. Koenders, and W. F. Tjallingii. 1990. Xylem ingestion by aphids—a strategy for maintaining water balance. *Entomol. Exp. Appl.* 55: 101–104.
- Staver, C., F. Guharay, D. Monterroso, and R. Muschler. 2001. Designing pest-suppressive multistrata perennial crop systems: shade-grown coffee in Central America. *Agrofor. Syst.* 53: 151–170.
- Tjallingii, W. F. 1978. Electronic recording of penetration behaviour by aphids. *Entomol. Exp. Appl.* 24: 521–530.
- Tjallingii, W. F. 1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomol. Exp. Appl.* 38: 187–193.
- Tsai, C., J. Chan, L. Fernandez, D. Bosco, K. M. Daane, and R. P. Rodrigo. 2005. Transmission of *Grapevine leafroll-associated virus 3* by the vine mealybug (*Planococcus ficus*). *Phytopathology* 98: S159–S159.
- VanHelden, M., and W. Tjallingii. 2000. Use of electrical penetration graphs in plant resistance research, pp. 144–172. *In* G. P. Walker and E. A. Backus [eds.], *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Thomas Say Publications in Entomology/Entomological Society of America, Lanham, MD.
- von Dohlen, C. D., and N. A. Moran. 1995. Molecular phylogeny of the Homoptera: a paraphyletic taxon. *J. Mol. Evol.* 41: 211–223.
- Walker, G. P. 2000. A beginner’s guide to electronic monitoring of homopteran probing behavior, pp. 14–40. *In* G. P. Walker and E. A. Backus [eds.], *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Thomas Say Publications in Entomology/Entomological Society of America, Lanham, MD.
- Walton, V. M., and K. L. Pringle. 2004. A survey of mealybugs and associated natural enemies in vineyards in the Western Cape province, South Africa. *S. Afr. J. Enol. Vitic.* 25: 23–25.

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