Stylet Penetration Behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG Waveform Characterization and Quantification

RODRIGO P. P. ALMEIDA[1, 2] AND ELAINE A. BACKUS[3]

Ann. Entomol. Soc. Am. 97(4): 838–851 (2004)

ABSTRACT We analyzed the probing (stylet penetration) behaviors of the sharpshooter leafhopper *Graphocephala atropunctata* (Signoret) on grape with an alternating current (AC) electrical penetration graph (EPG) monitor. We characterized waveforms likely to represent stylet penetration pathway phase and xylem ingestion. The total probing duration of the cohort represented 68% of all 20-h monitoring periods for all insects, yet only a small proportion of that probing time was spent in high amplitude/pathway activities. Few changes in behavior occurred once a probe had started. This was shown by the low number of waveform events (i.e., uninterrupted occurrences of a behavior) per probe for each waveform type, which varied from a mean of 1–2.43. Conditional probability analysis supported that hypothesis, because insects usually terminated a probe and began a new one after ingestion-related events, rather than repeating in the same probe the previously performed waveforms. The size of grape leaves used for the assays directly influenced the amount of time insects ingested from xylem or performed other low-amplitude waveforms. Information from this work establishes benchmarks for future research addressing the mechanisms of *Xylella fastidiosa* Wells et al. transmission and sharpshooter ecology.

KEY WORDS blue-green sharpshooter, electrical penetration graph, electronic monitoring of insect feeding, probing, *Xylella fastidiosa*

ELECTRICAL PENETRATION GRAPH (EPG; electronic) monitoring of insect feeding (McLean and Weight 1968) is a technique developed to overcome technical limitations with feeding behavior studies of plant sap-sucking insects (McLean and Kinsey 1964). It allows real-time study of probing (stylet penetration) behaviors through analyses of electrical waveforms produced by insects on plants. The technique allows characterization of probing behavior (Hunter and Backus 1989, Walker and Perring 1994), investigation of plant resistance mechanisms (Montllor and Tjallingii 1989), and identification of behaviors necessary for plant pathogen transmission (Wayadande and Nault 1993, Prado and Tjallingii 1994, Martin et al. 1997). Waveforms are generated after a closed circuit is formed between the insect and plant by insertion of the stylets into the plant tissue. In the case of the alternating current (AC) system used here, waveforms are produced by changes in resistance in the circuit.

EPG has been used to study many hemipteran species, including several families in Sternorrhyncha (e.g., aphids, whiteflies) as well as a few families and subfamilies in Auchenorrhyncha (Backus 1994). Interest has focused mostly on aphids, and to a lesser extent, on leafhoppers, whiteflies, and thrips. These insects ingest preferentially from phloem or mesophyll cells and only occasionally ingesting xylem sap (Khan and Saxena 1984, Janssen et al. 1989, Spiller et al. 1990). Little attention has been given to species that (apparently) ingest preferentially from xylem. These include members of the leafhopper (Cicadellidae) subfamily Cicadellinae, known as sharpshooters (Houston et al. 1947), as well as spittlebugs (Cercopidae) (Horsfield 1978) and cicadas (Cicadidae) (White and Strehl 1978).

Two techniques are used to identify the tissues from which auchenorrhynchans ingest while probing. They are (1) histology of probed plant tissues, to identify the plant location of salivary sheaths and/or stylet tips, the latter through stylectomy (excision of stylets while the insect probes) (Fisher and Frame 1984); and (2) chemical analysis of honeydew (Andersen et al. 1989). Today, these methods are often coupled with EPG to provide a waveform correlation with plant cell type(s) penetrated (see "triangle of correlations" in Backus 1994). In the case of sharpshooters, both methods have been used to determine in which tissue ingestion occurred (Houston et al. 1947, Andersen et al. 1989). However, until now, there has been little interest in

[1] Department of Plant and Environmental Protection Sciences, University of Hawai'i, Manoa, HI 96822.

[2] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720.

[3] Corresponding author: USDA–ARS Exotic and Invasive Diseases and Pests Research, 9611 So. Riverbend Ave., Parlier, CA 93648 (e-mail: ebackus@fresno.ars.usda.gov).

using EPG to study sharpshooter probing behavior. Perhaps this is because it is difficult to rear most sharpshooter species in the laboratory; they have long life cycles (Severin 1949) and complex host preference patterns (Purcell 1976, Brodbeck et al. 1990), and they are not economically important as direct pests. However, xylem-sap ingesters are vectors of economically important plant pathogens.

Interest in sharpshooters has recently increased because of the current epidemic of Pierce's disease (PD) of grapevine in California and citrus variegated chlorosis in South America (Hopkins and Purcell 2002). These diseases are caused by the xylem-limited bacterium *Xylella fastidiosa* Wells et al., which is transmitted by sharpshooters and spittlebugs (Severin 1949, 1950). *Graphocephala atropunctata* (Signoret) is the most important native PD vector in coastal California (Purcell and Feil 2001) and is the species in which most experiments to characterize *X. fastidiosa* transmission have been performed (Severin 1949, Purcell and Finlay 1979, Hill and Purcell 1995, 1997). Although all xylem-sap ingesters may be vectors of *X. fastidiosa* (Purcell and Hopkins 1996), different cicadelline species transmit the bacterium with varying levels of efficiency (Severin 1949). *G. atropunctata* is a highly efficient vector of *X. fastidiosa* to grape (Severin 1949, Purcell and Finlay 1979) and thus is a good choice for studies of the vector transmission process. The lack of a latent period and loss of vector transmission after the vector molts imply that bacteria in the vector's foregut are essential for transmission (Purcell and Finlay 1979, Purcell et al. 1979).

No EPG studies of xylem-ingesting sharpshooters have been widely published to date. Crane (1970) was the first to electronically monitor the probing behavior of any leafhopper. He used the original AC version of the EPG monitor developed a few years earlier by McLean and Kinsey (1964) and McLean and Weight (1968) to record the cicadelline sharpshooter, *G. atropunctata* (identified in his dissertation as *Hordnia circellata* Baker) on mustard leaves, *Brassica juncea* L.

Because the electronics of EPG monitors and computerized waveform acquisition have made great strides in the 34 yr since Crane's work, it is now possible to record and analyze more detailed waveforms than before, with almost no background electrical noise. Our objective was to characterize and statistically describe waveforms during feeding of *G. atropunctata* probing on grapevines, using a modern AC EPG monitor, and to compare with, verify, and update the waveform correlations of the early work of Crane (1970). This background information is crucial for future research addressing the mechanisms of *X. fastidiosa* transmission and sharpshooter ecology.

Materials and Methods

Insects and Plants

Adult *G. atropunctata* were field-collected from roses and wild grape in Berkeley, CA, and placed in cages containing a combination of three host plants: grapevine, *Vitis vinifera* L. variety 'Cabernet sauvignon'; CA mugwort, *Artemisia douglasiana* (Besser); and sweet basil, *Ocimum basilicum* L. F1 eggs laid by these insects produced nymphs that were reared to adulthood on the same host plants in a greenhouse insectary at the University of California, Berkeley, CA. Young adults (1–2 wk old) were overnight shipped to the University of Missouri, Columbia, MO, where they were caged on mugwort in a growth chamber (25 ± 2°C, 14 L:10 D). Assays were conducted with insects feeding on 'Cabernet sauvignon' grape seedlings, which were grown at the same facility in Berkeley and previously overnight shipped to Columbia, MO. Plants were transferred from 4-in to 1-gal pots in Columbia at least 1 wk before monitoring.

Conditioning, Wiring, and Acclimating of Insects

Two groups of sharpshooters were used. For experiment 1, ≈25 insects were used for correlation studies, plus initial characterization of waveform types. For experiment 2, 29 other insects were used for 20-h recordings of descriptive statistics. Only adult females were used for the assays, and all insects were wired using the same protocol. Insects were removed from the colony and caged for 24 h on a grape plant (in growth chamber), providing the insects access to the entire plant. After this conditioning period, insects' wings were glued shut with electrically conducting silver print paint (Ladd Research Industries, Burlington, VT), and the insects were transferred for a few minutes to an empty petri dish while the paint dried. Females were held with an aspirator made from a glass Pasteur pipette that was flame-sealed to a size that would fit snugly around the insect's abdomen, while a drop of silver paint was placed on the pronotum and scutellum. One end of a 0.002-in (50 μm) diameter, 99.99% gold wire (Sigmund Cohn, Mount Vernon, NY) was placed in the drop, with a short section of the wire in contact with and bent parallel to the leafhopper's body. Before wiring the insect, the other end of the gold wire, ≈3 cm in length, was glued to a copper stub with silver paint. For experiment 2 insects, acclimation to the wire was accomplished by placing the insect on the abaxial surface of a grape leaf in the same room where the recordings were conducted for 1–2.5 h. At 1200 hours, the insects were starved for 1 h while kept dangling (i.e., no tarsal contact). There was no acclimation or starvation period for insects used in experiment 1.

EPG Equipment

All data acquisition was done at the Backus laboratory in Columbia at room temperature in a room with fluorescent lights and 12:12 (L:D) photoperiod. Insects were EPG-monitored within a Faraday cage. One AC EPG monitor (Backus and Bennett 1992, MO Monitor type 2.2; Electronic Instruments Laboratory, University of Missouri, Columbia, MO) with four available channels was used. The monitor was adjusted to a 100-mV and 1,000-Hz output signal, with

the plant electrode inserted into the soil of each test plant. Data output was digitized using a DI-720 analog-to-digital board (Dataq Instruments, Akron, OH) with a 16-bit resolution and 100-Hz sampling rate per channel. Data acquisition, storage, and real time display were performed using WinDaq/Pro+ software (Dataq Instruments).

Data Acquisition

Grape leaves, each on a separate plant, were taped (using transparent scotch tape) onto a Plexiglas support such that the abaxial surface was exposed. The copper stub with wired insect was placed into an alligator clip of a "helping hand" holder (van Sickle Electronics, St. Louis, MO) attached to a ring stand, so that the insect was suspended above the center of the leaf. For experiment 1, insects were monitored one at a time to observe the appearance of waveforms and perform correlation studies. Excretory droplets were observed, and plant samples were collected for later histological determination of salivary sheath location and termini. Insects used for correlations of waveforms with probing behavior were tested at any time of the day and were often disturbed to stop ongoing probes. Once waveform types had been identified, characterized, and partially correlated, experiment 2 was performed to measure durations and frequencies (i.e., number of times a waveform was performed) of probing behaviors and to develop descriptive statistics. For that study, a set daily protocol was used. First, four leaves (one each from four grape plants) were connected to the monitor (one per channel). The lengths along the mid-vein of each leaf were measured. The wired insects were lowered onto the leaves, and recordings were begun. Four insects, one per channel, were monitored daily. All recordings started at 1300 hours and continued for 20 h, until 900 hours of the following day. Twenty-eight females were monitored, but three pulled their wires off of the copper stubs before the end of the 20-h access period, and their data were discarded. Therefore, data from 25 recordings were used for a posteriori analysis. Of these, 7 insects terminated probing naturally, whereas 18 had final probes that were artificially terminated at the end of the 20-h access time.

Correlations of Waveforms with Unsectioned Salivary Sheaths

During experiment 1, when one of the desired waveforms was observed in real-time on the computerized Windaq display, the insect was disturbed from the probing site, and the area was marked with a black, fine-tipped Sharpie felt pen. The plant tissue was excised and processed according to Backus et al. (1988). Briefly, the exterior of the leaf was stained with a 1:1 (vol:vol) mixture of 0.5% aqueous safranin and CRAF III fixative (Berlyn and Mikshe 1976). This stained the salivary flange to allow easy localization; a 1-mm^2 area of tissue surrounding the flange was excised. The samples were left in McBride's solution overnight and stored in clearing solution (Backus et al. 1988). Each sample was autoclaved for 20 min in the clearing solution, and the unsectioned, whole salivary sheaths without stylets were photographed in situ using a digital camera (Micropublisher 3Mpix; Qimaging, Burnaby, British Columbia, Canada) attached to a compound light microscope (200×; Zeiss Axiophot, Thornwood, NY).

Waveform Designations and Measurements

We followed the terminology conventions of Backus (2000). A probe is defined as all behaviors occurring from start of stylet penetration into plant tissue until stylet withdrawal. After data acquisition, waveform categories were assigned based on stereotypical patterns. Frequencies, as well as relative and absolute amplitudes (defined below) were measured following the conventions described by van Helden and Tjallingii (2000). Duration measurements for each waveform event (i.e., a continuous, uninterrupted occurrence of one waveform type) were made with WinDaq/Pro+ Waveform Browser software (Serrano et al. 2000). These files were copied into a Microsoft Excel workbook developed for EPG analysis by van Giessen and Jackson (1998), slightly modified for *G. atropunctata.*

Descriptive and analytical statistics of waveform variables were performed as described by Serrano (1997), using Statistical Analysis Software (SAS, Cary, NC). In brief, descriptive statistics for feeding behaviors were compiled at the heuristic levels of waveform event, probe, insect, and cohort. Measurement variables are defined and described in more detail in Serrano (1997) and Serrano et al. (2000). Waveform duration per insect (WDI) and Waveform duration per event per insect (WDEI) were compared among waveform types using analysis of variance (ANOVA); subsequent pairwise comparisons were made using protected Fisher LSD (proc GLM; SAS). Differences were considered significant when $P < 0.05$. A sequential analysis of all waveform events within each probe was also performed. From these data, conditional probabilities were derived that determined the probability of occurrence for behavioral sequences.

Results

Waveform Categories: Phases, Families, and Types

EPG waveforms for *G. atropunctata* were hierarchically divided into waveform phases, families, and types, based primarily on biological correlations (see Discussion) and secondarily on their characteristic frequencies (if any), relative and absolute amplitudes (defined below), and shapes at various levels of resolution. These descriptions are preliminary and are subject to modification as future correlation studies are performed. Following the naming convention developed by Tjallingii (Reese et al. 2000, van Helden and Tjallingii 2000), we chose a hierarchical set of waveform categories, with the broadest termed the

Table 1. Summary of waveforms designated for *G. atropunctata* feeding on grapevines obtained with an AC electronic monitor

EPG waveform	Relative amplitude (%)	Shape[a]	Correlated activity
Nonprobing behavior			
Z	0–100	Various (v)	Baseline and nonprobing behavior
High amplitude/pathway phase			
A1	100	High amplitude with spikes (v)	Stylet penetration and salivation
A2	50–75	Undefined (v)	Pathway activities
A3	75	Undefined (v)	Pathway activities
A0	25–50	Transition out of A (v)	
B	50	Humps with small spike in between (u)	Unknown
Low amplitude/ingestion phase			
C1	25	Low absolute amplitude (u)	Ingestion
C2	25	Low absolute amplitude, superimposed on coarse structure or with frequent spikes (>1 spike/120 s) (u)	Ingestion
C0	25	Transition out of C (v)	
Medium amplitude phase			
D1	25–50	Wave-like with medium absolute amplitude (u)	Unknown
D0	25–50	Transition out of D1 (v)	
E1	25–50	Vertical spike or semi-wave shape (u)	Unknown
E0	25–50	Transition out of E1 (v)	
U	25–100	Undefined (v)	Undetermined and oviposition

[a] u, waveforms with uniform shape; v, those with variable shape.

"waveform phase." Phases were correlated with broad behavioral categories of stylet penetration, e.g., pathway versus ingestion activities that can also be distinguished at a coarse structure level of resolution (20–30 s/cm or Windaq compression of 25–35). When the compressed view in Windaq is spread out to a medium level of resolution (4–9.6 s/cm or Windaq compression of 5–12), further correlatable details can be seen. At this resolution, waveforms with related characteristics were designated alphabetically for different "waveform families" (e.g., A, B, C). With further spreading, more correlatable details can be seen at a fine structure of resolution (1.6–4 s/cm or Windaq compression of 2–5). At this resolution, waveform types or "subfamilies" were characterized (i.e., divided into describable types), based primarily on correlations from this study and Crane (1970) (see Discussion), and also on probable sequence of events during probing. Family letters were combined with numbers (e.g., A1, C1) to designate waveform types. Although the letters and numbers are sequential, the scheme does not imply sequential behaviors. Letters followed by "0" are waveform categories denoting an appearance that is transitional between two waveform types ("transition out"). Transitions were usually gradual changes in shape into a subsequent waveform type; sometimes they were events of undetermined shape between two clearly identifiable waveform events. Table 1 briefly summarizes present characterizations of each waveform type, with biological correlations, if known.

For this and future work, "relative amplitude" is defined as the approximate position of a waveform type relative to a "floor" of the on-plant baseline level (0%) and a "ceiling" of the highest peak (voltage level) during the probe (100%), estimated in percent. This is similar to the convention of Tjallingii (2001). "Absolute amplitude" is defined as the size of the waveform itself, i.e., measured from the lowest valley of the waveform to the highest peak of the waveform. This can be measured either as actual voltage or as relative percentage from floor to ceiling, as above. Thus, a waveform with very large absolute amplitude might span the full width, 0–100%, of the trace; therefore, it would also have a large relative amplitude. In contrast, a waveform with small absolute amplitude (e.g., 10% of the width of the trace) could "float" anywhere above the floor, e.g., a relative amplitude of 50% the width of the trace (Fig. 1).

The waveform phases designated herein are as follows: high amplitude/pathway, medium amplitude, and low amplitude/ingestion. In this case, "amplitude" refers to absolute amplitude, as defined above. Biological interpretations were included in the phase names only when strong correlational evidence was available (see Discussion).

Nonprobing Behavior: Z Waveform. This variable-frequency waveform, which also corresponds to the baseline, was correlated visually with walking, resting, oviposition, and labial dabbing. Relative amplitude was variable but usually low (0–25%). Labial dabbing was assumed to occur (E. A. B., unpublished observations) during successive brief increases in amplitude (but still distinctly lower than probing waveforms), with flat baseline in between spikes. When insects were observed ovipositing on plants, amplitude of signal was dramatically increased to a level that exceeded the maximum voltage level of the Windaq software.

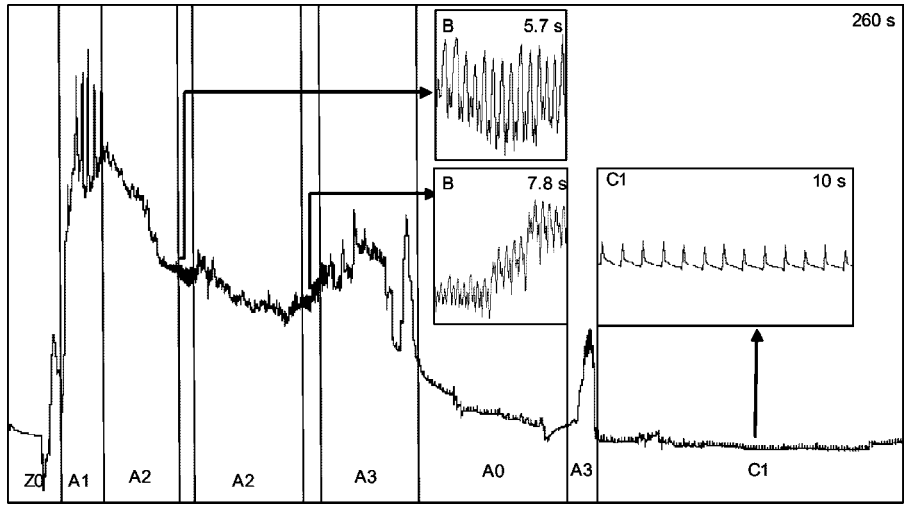

Fig. 1. High amplitude/pathway phase during *G. atropunctata* stylet penetration, followed by ingestion (C1), recorded with an AC EPG monitor. Boxes are expanded snapshots of respective waveforms. Z represents the baseline. This is a coarse-structure view at Windaq compression level 15, equivalent to 23 s/cm. Time on top right corner is total duration of each graphic segment.

High-Amplitude/Pathway Phase: A Waveforms. Waveform family A was characterized by a rapid, large increase in amplitude after Z, followed by a gradual decrease to a lower relative amplitude signal (Fig. 1). A1 was characterized by a sharp increase in amplitude immediately after Z, especially by high-amplitude spikes (the peaks representing 100% relative amplitude) often superimposed on the waveform base of 50–70% relative amplitude (Table 1). A1 was always followed by A2, which represented a gradual decline in relative amplitude, but with variable frequency and absolute amplitude and no clearly recognizable patterns. Longer (i.e., wider) spikes with intermediate, flatter absolute amplitude were often observed after A2 and were designated as A3. A3 also occurred as interruptions of lower amplitude, especially during ingestion waveforms, although less frequently and at lower relative amplitude level than during A2. The transitional waveform A0 had lower relative amplitude than A2, and it was frequently interspersed with A3 events. A0 often had low-amplitude C-like patterns (see below), but this was not always the case. In this work, both types of transition were labeled A0, although further division of A0 may be helpful for future work.

A1 always occurred at the beginning of a probe, immediately after Z. Visual observations through a dissecting microscope of a wired sharpshooter probing sideways into a vein consistently showed the A1 spikes immediately after labial contact with the typical probing posture (similar to the potato leafhopper, Ecale and Backus 1994). A2 always followed A1 when at the start of a probe.

High-Amplitude/Pathway Phase: B Waveform. The B waveform family was always observed after A2 and was followed by either A2 or A3. Its typical (although not exclusive and not revealed in Fig. 1), chevron-shaped phrase (Figs. 1 and 2A), plus constant relative and absolute amplitudes and frequency (Table 2) made it easily recognizable. In addition, more than one B phrase could occur in a single waveform event (defined below), and more than one event could occur in a single probe. We made no correlations or conclusions as to the biological meaning of this waveform. Also, at present, we have not further divided this family into waveform types.

Low-Amplitude/Ingestion Phase: C Waveforms. This waveform family was characterized by highly uniform frequency and low relative and absolute amplitude, with small range changes in amplitude. Two subfamilies or waveform types of C were designated. In C1, the relative amplitude of the signal did not change. In contrast, C2 was characterized by the C1 fine structure waveforms being superimposed on a variable-amplitude, low-frequency coarse structure. Alternatively, C waveforms with frequent (>1/120 s) spikes in amplitude (perhaps brief A3 events) were also categorized as C2 (Fig. 2B). In fine structure, the major wave-like portion of both C1 and C2 had uniform frequency and relative and absolute amplitudes (Table 2). However, the fine structure shape was variable, as recorded with maximum signal amplification allowed by our equipment (Fig. 3). C0 transitions into D were sometimes characterized by a gradual decrease in C1 or C2 frequency, followed by a slight increase in amplitude, and transition of waveform shape into D1. Other times, C0 was briefly followed by A3, and then D1 started immediately. Occasionally C0 appearances were not so stereotypical.

Two salivary sheaths, from separate insects, were correlated with performance of the C2 waveform and both terminated in vascular tissue (Fig. 4A). It was not possible, however, to determine whether they terminated in xylem or phloem. Production of copious, watery excretory droplets (≈1 drop/s) was observed

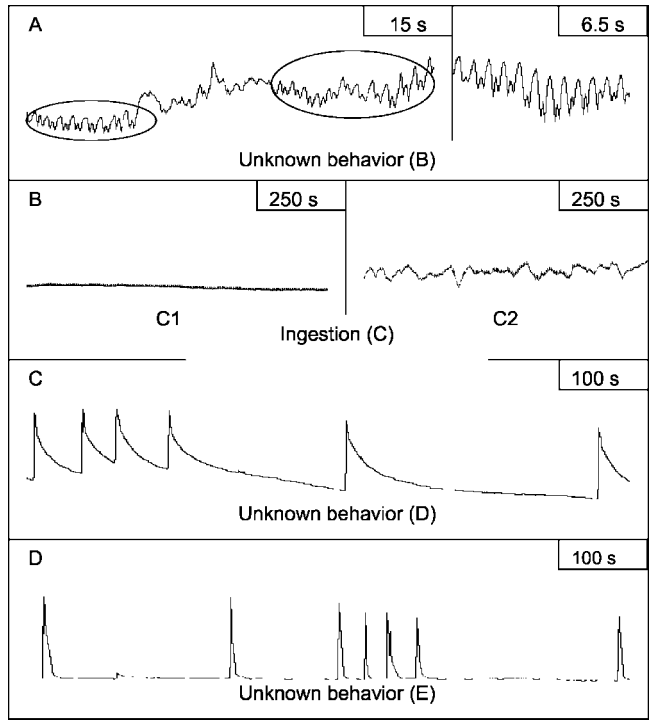

Fig. 2. Examples of AC waveforms generated by *G. atropunctata* probing on grapevines. (A) Fine structure view. (B–D) Coarse structure views. (A) Waveform B, unknown repetitive behavior. (B) Types of ingestion waveforms: (C1) no relative amplitude variation and (C2 − C1) waveform superimposed on more variable coarse structure. (C) Waveform D1, unknown behavior possibly occurring with stylet tips in mesophyll or parenchyma. (D) Unknown behavior. Top right corner shows total time of each graphic segment. Amplification level (y-axis scale) was held constant for best comparison among the waveforms.

in real-time during most C1 and C2 waveform events longer than 3–5 min. The pH of excretory drops produced during both waveforms, measured with pH paper, was ~6.5, as expected for xylem sap excretion (Walker 2000). We therefore considered that both the C1 and C2 waveforms indicate active xylem ingestion.

Medium-Amplitude Phase: D Waveforms. D1 waveform had a wave-like shape, with gradual decrease in amplitude, variable frequency, and medium absolute amplitude (Fig. 2C). Although the shape of D1 (Fig. 2C) resembled that of C1's fine structure (Fig. 3), the two waveforms were easily distinguishable by size. Absolute amplitude of C1 was quite small, often comprising <5% of the total height of the trace ("floor" to "ceiling") at a relative amplitude of ≈25%. In contrast, the absolute and relative amplitudes of D1 were much larger, commonly 40–50%. Five of seven salivary sheaths collected during this waveform were found to be incomplete salivary sheaths. Typically, they were composed of only salivary flanges that are an accumulation of sheath saliva on the surface of the plant (usually, but not always, a part of the salivary sheath that is secreted first, at the beginning of a probe). These incomplete sheaths were found in a leaf area with no vascular bundles. The tips of these sheaths were not found. The remaining two salivary sheaths appeared to be complete (i.e., with clearly defined flange, thick trunk, and thinner branch) and had the tip of the sheath ending in parenchyma cells (Fig. 4B). Eighteen other tissue samples were ana-

Table 2. Analysis of amplitude and frequency of waveforms B and C1 made by *G. atropunctata* on grapevines

Waveform	n^a	Relative amplitude [mean ± SE (range)]	Absolute amplitude [mean ± SE (range)]	Frequency [Hz mean; ± SE (range)]
B[b]	30	44.45 ± 2.22 (21.42–63.27)	3.52 ± 0.30 (1.22–7.14)	2.19 ± 0.07 (1.50–3.24)
C1[c]	30	23.68 ± 1.09a[e] (6.99–37.37)	1.02 ± 0.09a (0.46–2.76)	1.22 ± 0.05a (0.75–1.95)
C1[c,d]–ED	13	28.29 ± 3.57a (17.09–66.55)	1.07 ± 0.09a (0.62–1.54)	1.27 ± 0.09a (0.70–1.90)

[a] Number of waveform excerpts analyzed.
[b] Entire event measured.
[c] Measurement of 20-s sections of waveform from different insects.
[d] Events correlated with observation of excretory droplets (ED) at the time of waveform occurrence.
[e] Values within columns with same letter are not significantly different ($P > 0.05$, t-test, df = 41).

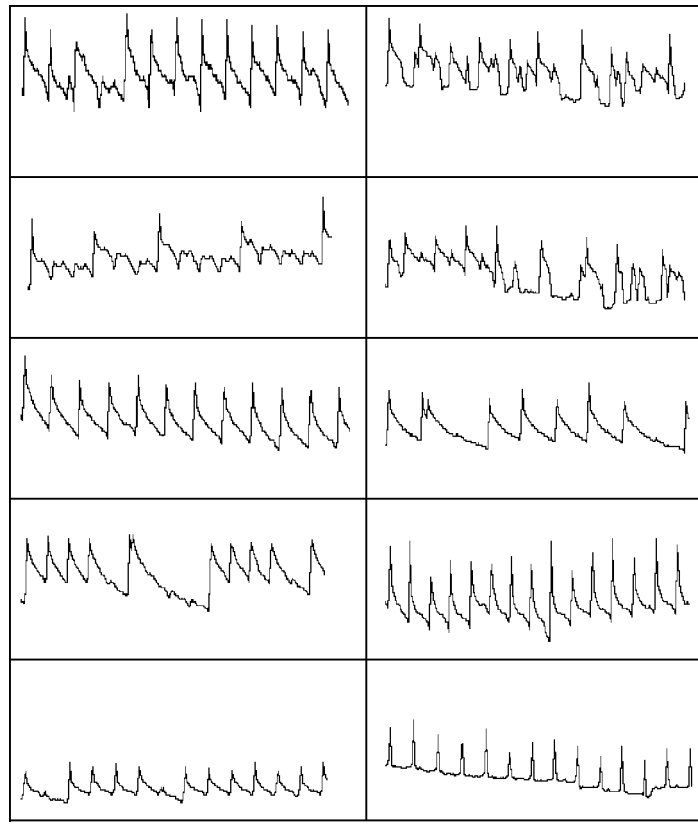

Fig. 3. Fine-structure views of C1 waveform shapes from *G. atropunctata* during events correlated with production of excretory droplets (≈1 drop/s, visual observations). Pictures were obtained with maximum amplification of waveforms recorded. Each square represents ingestion from different probes by both similar and different insects. Sections are 10 s each.

lyzed during D1, but no salivary sheath (or flange) was found in those samples. No honeydew excretion was observed in real-time when this waveform was generated. Transitions out of D1 (waveform D0) were variable, being followed by A3 events or another ingestion waveform.

Medium-Amplitude Phase: E Waveforms. The absolute amplitude of E1 was similar to that of D1, but instead of the spikes being followed by gradual decreases of amplitude, the decrease in amplitude was sudden (Fig. 2D). No correlations were obtained for this waveform or its respective transition out, E0.

U Waveform. This category included otherwise undefined waveforms, including very-high-amplitude events (perhaps oviposition) and other events with apparently random waveform characteristics. Few of the U events had repeats of frequency and shape for long periods of time. It occurred during both probing and nonprobing behaviors, but when clearly occurring during nonprobing behaviors, it was labeled Z instead of U.

Analysis of Waveform Measurements from Experiment 2

Cohort Level. The cohort level of waveform analysis shows the total behavioral repertoire of a sharpshooter population. The total probing duration (TPD) for this cohort was 335.7 h, which represents 68.5% of the total time the insects had access to plants. The remaining time was spent in nonprobing behaviors such as walking and oviposition. The total number of probes was 357, a relatively small number considering the long plant access periods (20 h) and the TPD. To understand this insect's behavior more completely, it is best to next subdivide the analysis into the smallest possible units, the waveform events, and then to return to the cohort level through the probe and insect levels.

Waveform Event Level. A waveform event is defined as a single, uninterrupted occurrence (beginning to end of one waveform type) of any given waveform. The total number of probing and nonprobing events (TNPE, i.e., events regardless of which waveform type) was 2,976; of these, 359 (or only 12%) were nonprobing events. All insects performed 10 of 12 waveform types; C0 and E0 (transition waveforms) were performed by fewer insects. A-family waveform events were performed by all insects; B was performed by all but one individual, and the other nontransition waveforms were performed by 21–24 individuals (see total number of insects by waveform [TNIw] in Table 3). However, the total number of waveform events (TNWE) varied greatly, from 12 (E0) to 616 (A2;

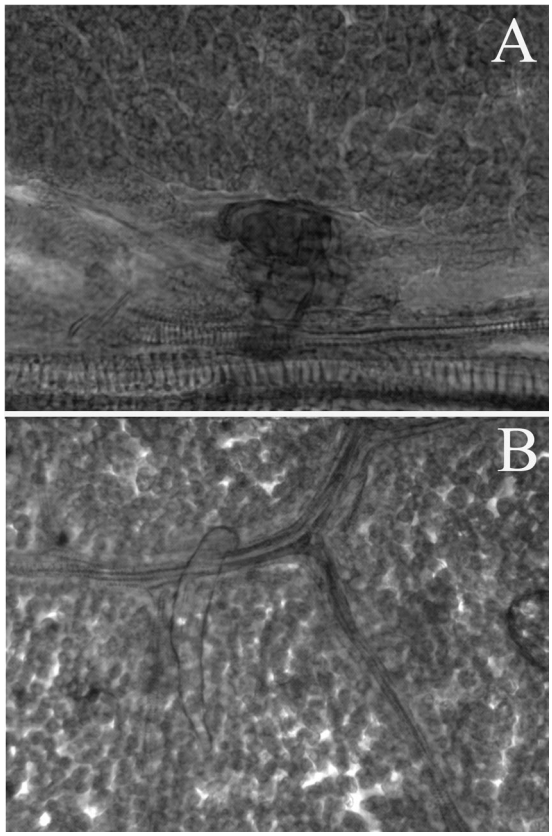

Fig. 4. Whole, unsectioned *G. atropunctata* salivary sheaths in cleared grapevine leaves. (A) Salivary sheath terminating in vascular tissue, produced during C2 waveform (200×). (B) Salivary sheath produced during D1 waveform (200×).

Table 3). Mean waveform duration per event (WDE), which calculates event duration for all insects pooled, is not discussed here. This is because of the large number of waveform events performed by these insects over the very long plant access time allowed, giving rise to less variation among individuals than in other studies with shorter access times (Serrano et al. 2000). Therefore, the values calculated for WDE were similar to those shown for the mean waveform duration per event per insect (WDEIa: "a" designating, herein and henceforth, that only those insects from whom a certain waveform was recorded were averaged) in Table 4, which are discussed below.

Probe Level. Data from the waveform event level can be combined at the probe level of analysis to reveal behaviorally relevant information that occurred during a probe. These data are different from those of the event

Table 3. Quantitative analysis of waveforms at the cohort level

Variable	A1	A2	A3	A0	B	C1	C2	C0	D1	D0	E1	E0	U	Z
TNIw	25	25	25	25	24	21	23	17	23	22	21	10	22	25
TNWE	378	616	600	321	266	195	327	57	206	72	84	12	63	382

Table 4. Quantitative analysis of waveforms at the insect level

Variable	A1	A2	A3	A0	B	C1	C2	C0	D1	D0	E1	E0	U	Z
NWEIa	15.1 ± 1.3[a]	24.6 ± 2.1	24.0 ± 2.1	12.8 ± 1.2	11.1 ± 1.3	9.2 ± 1.5	14.2 ± 2.1	3.3 ± 0.6	8.9 ± 1.1	3.2 ± 0.4	4.0 ± 0.7	1.2 ± 0.1	2.9 ± 0.4	15.2 ± 1.2
WDEIa (min)	13.5 ± 0.6[b]	41.9 ± 2.2[b]	30.1 ± 1.6[b]	1.8 ± 0.1	5.8 ± 0.2[b]	48.1 ± 12.2	10.7 ± 1.6	2.7 ± 0.8	25.4 ± 2.9	3.1 ± 0.5	36.9 ± 8.5	3.1 ± 0.6	12.1 ± 1.8	34.8 ± 8.0
WDIa (min)	3.3 ± 0.3	16.7 ± 1.6	12.1 ± 1.3	23.4 ± 2.7	1.1 ± 0.1	334.7 ± 48.6	125.3 ± 14.9	7.2 ± 1.4	224.9 ± 31.8	11.0 ± 2.3	142.2 ± 39.4	3.6 ± 0.6	37.1 ± 7.9	381.1 ± 47.5

[a] Mean ± SE.
[b] Values given in seconds.

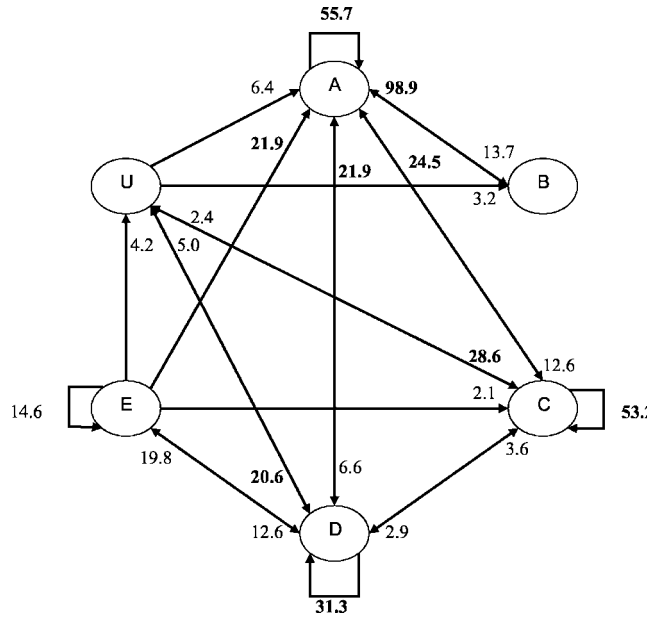

Fig. 5. Conditional probabilities of waveform events, giving likelihood of a certain waveform type being followed by any other waveform type. Probability values for each arrow direction are near the arrowhead. Probabilities <2% are not shown; those >20% have the values in bold format. Waveforms in the same family (A and C–E) were pooled to reduce number of possible combinations. Waveform B is not included because its duration was too short to diagram.

level because they can provide sequential information when more than one event of a certain waveform type occurs in a probe. The number of probes per insect (NPI) was 14.3 ± 1.2 (SE; range, 5–27 probes); thus, a moderate number of probes was made, with an average duration of 67.7 ± 7.6 min.

The mean number of waveform events per probe (NWEP) was low for all waveform types, ranging from 1.00 ± 0.00 to 2.43 ± 0.12, with under 2.0 per probe for each of the pathway phase waveforms (except A3), transition, E1, and U waveform types. In contrast, A3, C1, C2, and D1 occurred >2.0 times per probe. This shows the stereotypical behavioral pattern of most blue-green sharpshooter probes monitored, i.e., that most probes had only one major pathway phase, at the beginning; a minority of probes also had a second pathway phase, at the end. The early pathway phase usually progressed into more than one ingestion or medium-amplitude phases, often with A3 interspersed among those events.

This behavioral pattern, plus the relative rareness of D and E events, is further shown by conditional probabilities among the families. There was a 55.7% probability that an A-type event would be followed by another A event, yet a much lower probability that it would be followed by a B or C event (13.7 or 12.6%, respectively; Fig. 5). That B represents a pathway/preingestion behavior is also shown. Once a B event occurred, it was virtually always (98.9%) followed by a return to A. Only 6.6% of the time was A followed by D; even less by E or U. Once any medium- or low-amplitude events occurred, they were frequently followed by additional events from the same waveform family (e.g., 53.2% of the time, C followed C; 31.3% of the time, D followed D). However, for all of these ingestion waveforms, such activity was followed by a return to A in 22–25% of cases (Fig. 5). *G. atropunctata* finished probes by abruptly withdrawing the stylets from the plant during a low- or medium-amplitude waveform or by transitioning out of an event, followed by A3, and finally stylet withdrawal.

C1 and C2 were different in medium-structure waveform appearance. There also was a significant difference in the mean WDEIa of each waveform ($P < 0.0001$; Table 4), with C1 events being much longer than C2 events. The maximum duration of an individual C1 event was 281.8 min, the longest single probing event recorded. None of the WDEIa values for any of the "transition out" waveforms (A0, C0, D0, and E0) were significantly different (smallest significance value obtained was $P = 0.841$). Thus, in our recordings, insects took approximately the same time to change from one behavior to another, regardless of waveform types.

Insect Level. This level of analysis deals with the behaviors of each individual insect, as well as those of an "average" insect in the cohort. Figure 6 shows the differences and similarities among individuals in the cohort for probing durations and the waveform durations within them, for each individual insect (PDi and WDi, respectively). All but one leafhopper performed waveform C1 or C2 (probable xylem ingestion) during the recordings (Fig. 6). This one leafhopper (no. 25) was also one of two individuals to spend >50% (83.5%) of its plant access time in nonprobing behaviors; the other individual (no. 24) used 60.5% of its plant access

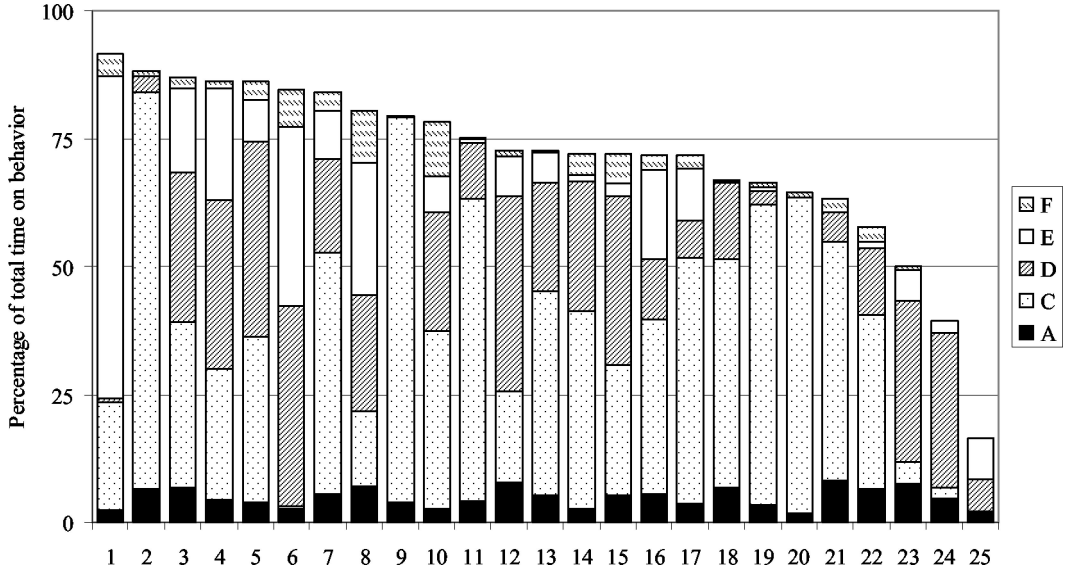

Fig. 6. Variability in waveform and probing durations by *G. atropunctata*. Each bar contains the behavior durations by an individual insect (probing duration by insect [PDi]); stacked bar divisions within represent the waveform duration by insect (WDi). Waveforms were clumped in families associated with behaviors discussed. Results were normalized for each individual; nonprobing behavior (Z) is represented by the difference between 100% and the top of each bar.

on such behaviors. Figure 6 shows again a similarity among all individuals: pathway phase (in this case represented by waveform family A) was always a relatively small proportion of an insect's PDi. However, all other waveform durations varied greatly among individuals. Pathway phase waveforms were shorter (WDIa; Table 4) but significantly more frequent (NWEI) compared with any other waveforms ($P <$ 0.0001 for both A2 and A3 compared with other waveforms).

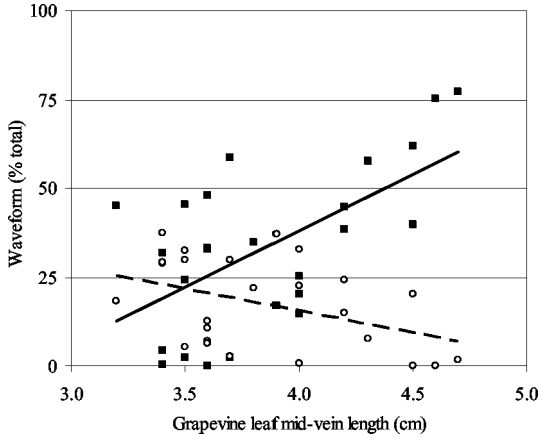

Fig. 7. Percentage of time per *G. atropunctata* spent in ingestion C1 + C2 or D1 waveforms; correlated with length of grapevine leaf mid-vein. Solid squares and empty circles represent data points for C1 + C2 and D1, respectively. Solid line is linear regression for C1 + C2 ($y = 31.729x - 88.95$, $r^2 = 0.355$, $P = 0.002$); broken line is the regression for D1 ($y = -9.433x + 67.09$, $r^2 = 0.177$, $P = 0.045$).

There was a significant correlation between length of the leaf mid-vein and the combined duration per insect (percent of TPD) of the waveforms (C1, C2, and D1; Fig. 7). Insects on larger leaves (thus, larger mid-vein lengths) spent more time in C1 or C2, whereas insects on smaller leaves spent more time in D1. There was no significant correlation between leaf size and nonprobing behaviors. WDEIa values for A- and B-type waveforms were not statistically different from each other, but both were shorter than all other waveforms considered individually (C1, C2, D1, and E1; $P = 0.0017$, was the largest P value observed for possible combinations). Insects also spent more time in C1 than any other waveform ($P < 0.001$), except Z (nonprobing; $P = 0.160$; Table 4).

Discussion

Relationship of Our Work to Previous Studies

The first EPG study of any leafhopper (Hemiptera: Auchenorrhyncha) was that of Crane (1970), in which he used the same species as our study, the blue-green sharpshooter [cited as *Hordnia circellata* (Baker) therein]. Although Crane used the earliest design of the original AC system (McLean and Weight, 1968), his waveform images are clear and interpretable by today's standards (Backus et al. 2000). Also, his correlation observations were thorough and meticulously documented.

In the 20 yr after the work of Crane (1970), 28 more EPG studies of 13 other auchenorrhynchan species were published, using all of the monitor designs that evolved during that time (Backus 1994). Several more studies have been published since 1990 (e.g., Way-

adande and Nault 1993, 1996). Almost all of them used AC EPG monitors, but a few recent papers have used the DC standard monitor (Kimmins and Bosque-Perez 1996). However, xylem-ingesting sharpshooters were not studied in any of them. Our present work updates Crane (1970) and provides a bridge for his detailed correlation results to be applied to modern waveform analysis. The result is a clearer picture of sharpshooter feeding activities.

Comparison of Our Results with Those of Crane (1970)

Overall Waveform Resolution. As described by Tjallingii (2000), very early AC EPG studies in the 1960s to 1970s, such as Crane (1970), typically employed strip chart recorders, with slow response times and pen drag, that were run rather slowly to conserve expensive paper. Thus, most waveform traces from early papers are quite compressed by today's standards, typically presented at 30 s/cm. To provide an appropriate basis of comparison, we presented Fig. 1 waveforms at 23 s/cm. Nonetheless, waveforms from early studies can be instructive, as long as it is realized that they represent a broad, coarse structure resolution of behavior comparable with our waveform phase.

Unlike other early AC studies, Crane (1970) depicts waveforms in some detail, with more spread (10 s/cm) and extra amplification of very-low-level waveforms. Applying the same level of compression to our waveforms (data not shown) reveals many similarities between ours and Crane's recordings (e.g., Figs. 19 and 21 of Crane 1970), which will be discussed for each waveform. The main difference is that Crane's waveforms are slightly rounded, often lacking the small-to-medium–sized waveform peaks that are most rapid and spike-like. This effect is probably because of a combination of slow response time of the strip-chart recorder and differences in monitor signal processing (Backus et al. 2000). Otherwise, the waveforms of Crane (1970) are very similar to ours.

High-Amplitude/Pathway Phase. The rapid rise in voltage to near or at 100% relative amplitude is characteristic of the start of a probe in every auchenorrychan species that has been monitored by AC EPG. The subsequent high-amplitude (both absolute and relative), irregular waveforms in the first few seconds to minutes of a probe have been repeatedly correlated with secretion of saliva in several species. Specifically, this is sheath saliva in the case of sheath-feeders like sharpshooters (Crane 1970) or deltocephaline leafhoppers (Triplehorn et al. 1984), or watery saliva as in lacerate-and-flush feeders like *Empoasca* spp. (Kabrick and Backus 1990).

Crane (1970) elegantly correlated the formation of the salivary sheath with the high-amplitude signals in the early stages of a probe through observation of stylet activities of 32 insects feeding through a Parafilm membrane into plant extracts or artificial diet. The substrate also contained suspended chloroplasts used as markers. He termed the waveform "SSF" or "salivation and stylet sheath formation." Crane believed that watery, as well as sheath, saliva was produced during this waveform, because he observed chloroplasts moving away from the stylets at times when no sheath saliva was being secreted. At no time did Crane see ingestion (indicated by chloroplasts moving inward) during his SSF waveform. Also, extensive histological studies showed that salivary sheaths were secreted into plant tissues during the same SSF waveform.

Crane's figures show many subpatterns within the SSF waveform quite similar to the waveform families and types we have designated. It is, therefore, likely that Crane could have further subdivided his SSF waveform, but chose not to do so. Our A1 waveform usually has a "hump" or "waveform base" from which the tall A1 spikes arise; this "hump" and some hint of spikes are clearly visible in Fig. 19 of Crane (1970). The later humps, peaks, and valleys of Crane's SSF waveform (his Figs. 13, 19, and 20) strongly resemble those of our A2 and A3 waveforms. There are also some chevron-like, dense, downward-curving waveform segments that resemble a compressed view of our B waveform.

Thus, evidence supports that our high-amplitude/pathway phase is identical to Crane's SSF waveform and that both are correlated with salivary sheath formation. These behaviors seem to be analogous to aphid "pathway" activities (Prado and Tjallingii 1994); hence, our assignment of the pathway term to this phase. However, this assignment is provisional until we complete further sheath correlations currently underway. These studies will pinpoint which parts of sheath formation, and probably other behaviors that Crane could not detect, correspond to each of our waveform families and types.

Low-Amplitude/Ingestion Phase. Crane (1970) characterized two low-amplitude waveforms and correlated them with ingestion. He termed these "TI" (for "trial ingestion") and "SI" (for "sustained ingestion"). TI and SI had the same, very flat appearance. However, when Crane substantially increased the gain, wave-like details similar to our waveform C were revealed. Therefore, our waveform family C is likely to be the same as Crane's TI and SI, but revealed in more detail and clarity. Crane (1970) distinguished TI from SI because TI events were shorter in duration (usually $<$20 s, rarely 2–3 min) and interspersed among events of SSF. We saw similar short durations of C, but chose not to assign waveform types by duration or "purpose" (i.e., trial or nontrial ingestion). Instead, we combined the categories based on position and correlation with excretory droplets.

Crane clearly correlated TI and SI with ingestion on both artificial diets and plants. During diet observations, he visually correlated both waveforms with stylets being still, no further sheath saliva secretion or outward flow of chloroplasts, but rapid and continuous inward flow of chloroplasts. In one case, he was able to observe a chloroplast within the food canal of the

nearly transparent stylets of a teneral adult and follow it as it moved up into the head.

In experiments with mustard plants, Crane correlated ingestion with excretory droplets and determined the plant tissues in which behaviors occurred. Twelve (nonstylectomized) insects were given 45 min of access time. For the nine SI-containing probes they made, excretion data were compiled, and tissues were excised for salivary sheaths. Only six probes produced excretory droplets, on average 5.28 min after the start of SI. Droplets continued at an average rate of 20–45 droplets/min (range, 10–60 droplets/min), for an average duration of 14.78 min of ingestion. We also correlated our C family waves with excretory droplets and found a similar rate (≈1/s) on grape.

Sixty-seven other insects were stylectomized 5–10 min into SI, before excretion, and their probed plant tissue was excised. Only 12 of these salivary sheaths had stylets intact after preparation. After sectioning and examining the plant tissues from both these experiments, Crane found that all 21 SI probes left full salivary sheaths identical to those seen in diets, presumably during the SSF waveform preceding SI. Our work also showed salivary sheaths in plant tissues excised during waveform C, presumably secreted during preceding A and/or B waveforms.

Seven of Crane's nine nonstylectomized, SI probes terminated in xylem, one in phloem, and one in mesophyll/parenchyma. Four probes (three into xylem, one into phloem) produced the first excretory droplet at ≈5 (average, 5.38) min after the start of SI. However, one xylem probe's first drop occurred at 0.58 min, and the mesophyll/parenchyma probe's first drop was at 9.62 min after the start of SI. For the stylectomized SI probes, 11 of the 12 salivary sheaths terminated in the xylem and 1 in mesophyll/parenchyma.

Crane (1970) also performed chemical analysis of the pooled excreta from another set of 12 probes that reached SI. Four to five microcapillary tubes full of excreta were collected from each insect during SI, which lasted, on average, 45.73 min. Excretory droplets were analyzed with gas-liquid chromatography (GLC; sugars) and paper chromatography (amino acids). Plant tissues were sectioned to determine salivary sheath termini. Free amino acid analysis detected only glutamic acid and asparagine at low concentrations similar to those known at the time for xylem fluid. Interestingly, Crane also found sucrose in much higher concentrations. Although Crane (1970) does not explain the presence of sucrose, we suspect it was because 2 of the 12 pooled SI probes' salivary sheaths terminated in the phloem; the remaining 10 terminated in xylem. Both phloem probes were long enough (33.83 and 52.35 min in duration) to ingest significant quantities of sucrose.

Thus, evidence supports that our waveform family C is identical to Crane's TI and SI waveforms and that all are correlated with ingestion, primarily (but not exclusively) from xylem. Eighty-five percent of Crane's sectioned salivary sheaths terminated in xylem, 9% in phloem, and 6% in mesophyll/parenchyma. In our study, we found that the fine structure of the C1 and C2 waveform types varied. These differences may reflect changes in resistance associated with plant cell type, cell size, or with muscle activities during ingestion. The peak frequencies observed for C1 and C2 were similar to the frequency of contractions of the cibarial pump muscles (Purcell et al. 1979) observed (but not measured) for sharpshooter nymphs feeding on artificial diets (unpublished observations). Direct measures of both variables (waveforms and muscle contraction frequencies) in real-time must be performed to elucidate their relationship and function.

Medium-Amplitude Phase. Crane (1970) did not display or discuss any waveforms like our D or E waveform families. We suspect that these waveforms were not made, or were made very seldomly, on mustard (the plant used by Crane) compared with their greater performance on grape in our tests. Alternatively, D and E waveforms may have occurred during Crane's observations, but they were much less spike-like in their appearance (perhaps because of strip chart recorder problems like pen drag) and may have been mistaken for SSF or I waves. Based on the similarities between our systems for the other waveforms, however, this seems less likely. At present, we have almost no information on the biological meaning of these waveforms, although our tentative findings suggest that D may represent a type of mesophyll ingestion without a salivary sheath. This hypothesis is being tested during further correlation experiments underway.

Further Considerations and Conclusions

We used long data access times (20 h), in general longer than those used for other leafhoppers (Crane 1970, Serrano et al. 2000). Extensive recordings may be desirable for EPG studies involving sharpshooter leafhoppers, because *G. atropunctata* (considered here as a representative species of the tribe Cicadellini) made relatively few probes per unit time, and ingestion events were long (C1 had an average of 48 min of WDEIa) compared with *Empoasca* (Serrano et al. 2000).

Because of technical limitations, the age of insects used in our recordings was variable, and our insects had survived possible shipping and acclimation stresses. These factors may have influenced insects at the individual level. However, probing behaviors were apparently more affected by the size of leaves they probed. Because we had limited numbers of plants available, we could not standardize the leaf size for this study. This allowed us, however, to show the leaf size effect on feeding. This meshes with the findings of Crane (1970), who noted that unwired *G. atropunctata* feeding on mustard leaves preferred secondary veins (50% of the time) to tertiary (28%) and quaternary (2.5%) veins.

In conclusion, we have applied modern EPG recording and analysis methods to update the classical work of Crane (1970) by characterizing waveforms in more detail and with updated terminology. We have shown that our high-amplitude phase is correlated

with salivary sheath formation and other pathway activities and is the same as Crane's SSF waveform. We have also shown that our C family waveforms during low-amplitude phase are correlated with active ingestion and are the same as Crane's TI and SI waveforms. Further experiments with sharpshooters are underway and will build on this information for more waveform correlations and understanding of the mechanism of *X. fastidiosa* transmission.

Acknowledgments

We thank C. Montllor and A. H. Purcell for manuscript suggestions and revision. R.P.P.A. received a scholarship from the CNPq (Brazil). This work was partially supported by United States Department of Agriculture Grant 2001-3442-10406 to A.H.P.. This work is part of R.P.P.A.'s dissertation presented to the University of California, Berkeley, CA.

References Cited

Andersen, P. C., B. V. Brodbeck, and R. F. Mizel III. 1989. Metabolism of amino acids, organic acids and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata.* Entomol. Exp. Appl. 50: 149–160.

Backus, E. A. 1994. History, development and applications of the AC electronic insect feeding monitor, pp. 1–51. *In* M. E. Ellsbury, D. E. Ullman, and E. A. Backus (eds.), History, development and application of the AC electronic insect feeding monitor. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.

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

Backus, E. A., M. J. Devaney, et al. 2000. Comparison of signal processing circuits among seven AC electronic monitoring systems for their effects on the emf and R components of aphid (Homoptera: Aphididae) waveforms, pp 102–143. *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. Entomological Society of America, Lanham, MD.

Backus, E. A., and W. H. Bennett. 1992. A new AC electronic insect feeding monitor for fine-structure analysis of waveforms. Ann. Entomol. Soc. Am. 85: 437–444.

Backus, E. A., W. B. Hunter, and C. N. Arne. 1988. Technique for staining leafhopper (Homoptera: Cicadellidae) salivary sheaths and eggs within unsectioned plant tissue. J. Econ. Entomol. 81: 1819–1823.

Berlyn, L. C., and J. P. Mikshe. 1976. Botanical microtechnique and cytochemistry. Iowa State Univesity Press, Ames, IA.

Brodbeck, B. V., R. F. Mizell, III, W. J. French, P. C. Andersen, and J. H. Aldrich. 1990. Amino acids as determinants of host preference for the xylem feeding leafhopper, *Homalodisca coagulata* (Homoptera: Cicadellidae). Oecologia (Berl.). 83: 338–345.

Crane, P. S. 1970. The feeding of the blue-green sharpshooter *Hordnia circellata* (Baker) (Homoptera: Cicadellidae). Ph.D. dissertation, University of California, Davis, CA.

Ecale, C. L., and E. A. Backus. 1994. A characteristic body posture of the potato leafhopper is correlated with probing. J. Entomol. Sci. 29(4): 490–495.

Fisher, D. B., and J. M. Frame. 1984. A guide to the use of the exuding-stylet technique in phloem physiology. Planta. 161: 385–393.

Horsfield, D. 1978. Evidence for xylem feeding by *Philaenus spumarius* (L.) (Homoptera: Cercopidae). Entomol. Exp. Appl. 24: 95–99.

Hill, B. L., and A. H. Purcell. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata.* Phytopathology. 85: 209–212.

Hill, B. L., and A. H. Purcell. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology. 87: 1197–1201.

Hopkins, D. L., and A. H. Purcell. 2002. *Xylella fastidiosa:* cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86: 1056–1066.

Houston, B. R., K. Esau, and W. B. Hewitt. 1947. The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. Phytopathology. 37: 247–253.

Hunter, W. B., and E. A. Backus. 1989. Mesophyll-feeding by the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae): results from electronic monitoring and thin-layer chromatography. Environ. Entomol. 18: 465–472.

Janssen, J.A.M., W. F. Tjallingii, and J. C. van Lenteren. 1989. Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. Entomol. Exp. Appl. 52: 69–81.

Kabrick, L. R., and E. A. Backus. 1990. Salivary deposits and plant damage associated with specific probing behaviors of the potato leafhopper, *Empoasca fabae,* on alfalfa stems. Entomol. Exp. Appl. 56: 287–304.

Khan, Z. R., and R. C. Saxena. 1984. Electronically recorded waveforms associated with the feeding behavior of *Sogatella furcifera* (Homoptera: Delphacidae) on susceptible and resistant rice varieties. J. Econ. Entomol. 77: 1479–1482.

Kimmins, F. M., and N. A. Bosque-Perez. 1996. Electrical penetration graphs from *Cicadulina* spp and the inoculation of a persistent virus into maize. Entomol. Exp. Appl. 80: 46–49.

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 R. B. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. Nature (Lond.). 202: 1358–1359.

McLean, D. L., and W. A. Weight Jr. 1968. An electronic measuring system to record aphid salivation and ingestion. Ann. Entomol. Soc. Am. 61: 180–185.

Montllor, C. M., and W. F. Tjallingii. 1989. Stylet penetration by two aphid species on susceptible and resistance lettuce. Entomol. Exp. Appl. 52: 103–111.

Prado, E., and W. F. Tjallingii. 1994. Aphid activities during sieve element punctures. Entomol. Exp. Appl. 72: 157–165.

Purcell, A. H. 1976. Seasonal changes in host plant preference of the blue-green sharpshooter *Hordnia circellata* (Homoptera: Cicadellidae). Pan-Pac. Entomol. 52: 33–37.

Purcell, A. H., and A. H. Finlay. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology. 69: 393–395.

Purcell, A. H., and D. L. Hopkins. 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34: 131–151.

Purcell, A. H., and H. Feil. 2001. Glassy-winged sharpshooter. Pesticide Outlook. 12: 199–203.

Purcell, A. H., A. H. Finlay, and D. L. McLean. 1979. Pierce's disease bacterium: mechanism of transmission by leafhopper vectors. Science 206: 839–841.

Reese, J. C., W. F. Tjallingii, M. van Helden, and E. Prado. 2000. Waveform comparisons among AC and DC electronic monitoring systems for aphid (Homoptera: Aphididae) feeding behavior, pp. 70–101. *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.

Serrano, M. S. 1997. Probing behaviors of *Empoasca kraemeri* Ross & Moore (Homoptera: Cicadellidae) on common bean genotypes and the use of AC electronic feeding monitors to characterize tolerance. Ph.D. Dissertation. University of Missouri, Columbia, MO.

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

Severin, H.H.P. 1949. Transmission of the virus of Pierce's disease of grapevines by leafhoppers. Hilgardia. 19: 190–206.

Severin, H.H.P. 1950. Spittle-insects vectors of Pierce's disease virus II. Life history and virus transmission. Hilgardia. 19: 357–381.

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

Tjallingii, W. F. 2000. Comparison of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans, pp. 41–69. *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.

Tjallingii, W. F. 2001. Analysis of circulative transmission by electrical penetration graphs, pp. 69–85. *In* K. F. Harris, O. P. Smith, and J. E. Duffus (eds.), Virus-insect-plant interactions. Academic. New York.

Triplehorn, B. W., L. R. Nault, and D. J. Horn. 1984. Feeding behavior of *Graminella nigrifrons* (Forbes). Ann. Entomol. Soc. Am. 77: 102–107.

van Giessen, W. A., and D. M. Jackson. 1998. Rapid analysis of electronically monitored homopteran feeding behavior. Ann. Entomol. Soc. Am. 91: 145–154.

van Helden, M., and W. F. Tjallingii. 2000. Experimental design and analysis in EPG experiments with emphasis on plant resistance research, pp. 144–171. *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.

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.

Walker, G. P., and T. M. Perring. 1994. Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) on young and mature lemon leaves. Ann. Entomol. Soc. Am. 87: 363–374.

Wayadande, A. C., and L. R. Nault. 1993. Leafhopper probing behavior associated with maize chlorotic dwarf virus transmission to maize. Phytopathology. 83: 522–526.

Wayadande, A. C., and L. R. Nault. 1996. Leafhoppers on leaves: an analysis of feeding behavior using conditional probabilities. J. Insect Behav. 9: 3–22.

White, J., and C. E. Strehl. 1978. Xylem feeding by periodical cicada nymphs on tree roots. Ecol. Entomol. 3: 323–327.

Received 12 May 2003; accepted 6 March 2004.