Novel Methods of Monitoring the Feeding Behavior of *Homalodisca coagulata* (Say) (Hemiptera; Cicadellidae)

PETR DOLEŽAL,[1] BLAKE R. BEXTINE,[2, 3] ROMANA DOLEŽALOVÁ,[1] AND THOMAS A. MILLER[2]

Department of Entomology, University of California, Riverside, CA 92521

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ABSTRACT The glassy-winged sharpshooter, *Homalodisca coagulata* (Say), is an important agricultural pest because it is an effective vector of *Xylella fastidiosa*, the pathogen that causes Pierce's disease in grapevines. Knowledge of the feeding behavior of *H. coagulata* is important in understanding pathogen transmission, and this knowledge is important in developing innovative pathogen control strategies. Ingestion of fluid by sharpshooters was monitored as movement of fluid from reservoirs connected to short stems of plant tissue. We quantified the amount of fluid processed while the insects were freely moving on the plants stems offered for feeding. Females fed longer than males, and both ingested large amounts of plant fluid and both excreted large amounts of fluid. Excreta droplets were often actively flung from the body by flicking the abdomen. While actively ingesting, the abdomen made exaggerated movements that stopped during excretion. These movements only appeared after mouthparts penetrated the plant tissues. The abdominal movements were correlated with ingestion of plant fluids as monitored by fluid uptake from the reservoir.

SEVERAL STRAINS OF *Xylella fastidiosa* Wells exist that cause scorch-type diseases in a number of perennial plants (Purcell and Saunders 1999a). One strain of *X. fastidiosa*, which has been present in California for >100 yr, causes Pierce's disease of grapevine (Freitag 1951, Davis et al. 1978). The most prevalent vectors of *X. fastidiosa* are the leafhoppers known as sharpshooters (Hemiptera: Cicadellidae), which acquire *X. fastidiosa* during ingestion of fluid from xylem vessels of infected plants (Purcell and Hopkins 1996). The bacterium attaches inside the foregut of the insect and is transmitted to new hosts when the insect probes the xylem of naive plants. *X. fastidiosa* is transmitted by the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Costa et al. 2000, Almeida and Purcell 2003, Blua and Morgan 2003), which was introduced into southern California in the mid-1980s (Blua et al. 1999, Purcell and Saunders 1999b). This polyphagous insect has become an epidemic pest, mainly because it is an efficient vector of *X. fastidiosa.* Transmission of *X. fastidiosa* by several vectors, including *H. coagulata*, has been well characterized (Purcell et al. 1979, Brlansky and Timmer 1982, Mizell and French 1987, Hill and Purcell 1995, Purcell and Hopkins 1996, Brlansky et al. 2002, Almeida and Purcell 2003). *H. coagulata* has a wide host range, is an efficient vector, and is a strong flier as an adult (Costa et al. 2000, Blua and Morgan 2003, Hoddle et al. 2003). These factors increase the opportunity for *H. coagulata* to acquire and disperse *X. fastidiosa* over long distances and across plant taxa. The combination of recently introduced *H. coagulata* and this native strain of *X. fastidiosa* has caused great concern, because it has affected table grape and wine grape production in several regions of southern California and threatens grape production throughout the state. In addition to pathogen transmission, the habit of feeding by large aggregations of *H. coagulata* causes significant water loss from host plants (Brodbeck et al. 1995, Blua et al. 1999).

Hemipteran feeding activities, from test probing to active ingestion, have been well characterized for many important agronomic pests (McLean and Kinsey 1984, Backus 1985, 1988, Tjallingii 2000, Walker 2000). Two systems of electrical penetration graphs (EPG) have been used to study the feeding behavior of homopterans, an alternating current (AC) system (McLean and Kinsey 1964) and a direct current (DC) system (Tjallingii 1988). By connecting a piercing-sucking insect into an electrical circuit, either system monitors the stylet penetration of different plant tissues (phloem, xylem, or mesophyll), as fluctuations in resistance caused by the conductivity characteristic of these different plant tissues (Walker 2000). EPG wave forms are correlated with different feeding behaviors (i.e., probing, salivation, and ingestion). Information on feeding behavior has been used to understand the damage caused by hemipteran feeding (Kabrick and Backus 1990, Ecale and Backus 1995), to identify insect behavior associated with transmission of plant pathogens (Martin et al. 1997, Jiang et al. 2000, John-

[1] Physiology Department, Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, České Budějovice, Czech Republic.

[2] Department of Entomology, University of California, Riverside, CA 92521.

[3] E-mail: Blake.Bextine@ucr.edu.

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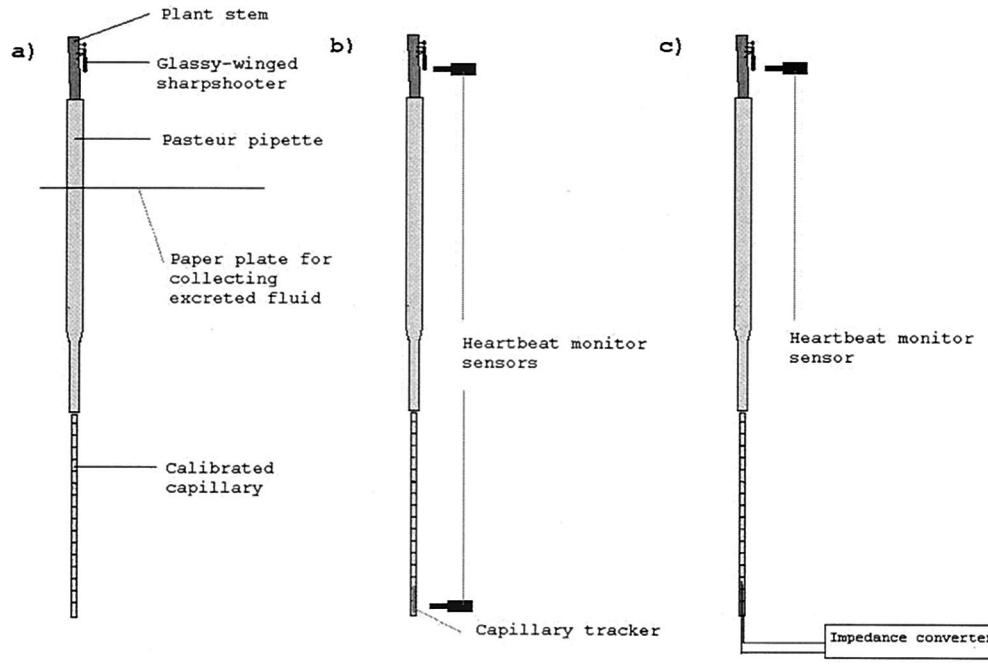

Fig. 1. Materials used for (a) quantifying the amount of ingested liquid, (b) quantifying the amount of ingested liquid and monitoring abdominal movements and meniscus fluctuations with heartbeat monitor, and (c) same as b, but with impedance converter.

son et al. 2002, Palacios et al. 2002, Kindt et al. 2003), and to assess host plant resistance to hemipteran feeding (Auclair and Baldos 1982, Khan and Saxena 1988, Kimmins 1989).

While monitoring of feeding behavior through EPG is a powerful tool, certain aspects of feeding are not monitored directly, and the attachment of test insects to wires could potentially interfere with normal behavior (Tjallingii 1986). While Annan et al. (1997) found that tethering aphids had no significant affects on stylet penetration behavior, larger insects, such as *H. coagulata*, present challenges to EPG. In this study, we describe an alternative method for measuring fluid consumption by *H. coagulata* that could be used in conjunction with EPG methods. We have developed a simple, noninvasive monitoring method that can be used to characterize the feeding behavior of any sucking insect.

Materials and Methods

Maintenance of Plants and Stem Preparation. *Chrysanthemum grandiflora* cultivar 'White Diamond' were grown in UC mix number three soil in 20-cm pots from cuttings in an insect-free greenhouse on the University of California campus, Riverside, CA (natural light at 27°C and 70% humidity). Chrysanthemum stems were cut in 3-cm sections from 10-wk-old plants with stem diameters ranging from 0.3 to 0.4 cm.

Collection and Maintenance of Glassy-Winged Sharpshooters. *Homalodisca coagulata* adults were collected from orange trees (*Citrus sinensis* Osbeck) on the University of California, Riverside campus. Test insects were transported to the laboratory in 30 by 30 by 30-cm collapsible, field collecting chiffon-screened cages (Bioquip Products, Gardena, CA) and maintained on chrysanthemum plants (12 L:12 D, at 27°C and 70% RH). In experiments requiring freshly molted adults, fifth-instar *H. coagulata* were collected in the field as above and maintained in rearing cages on chrysanthemum until they molted to adults.

Quantification of Ingestion. Leaves were removed from chrysanthemum stem sections, and the top of the stem section was sealed with parafilm (Pechiney; Plastic Packaging, Menasha, WI) to prevent evaporation and desiccation (Bextine et al. 2003). The lower part of the stem was sealed in a Pasteur pipette with parafilm, so that at least a 1-cm-long area of bare stem was exposed above the pipette (Fig. 1). A calibrated capillary tube was sealed to the bottom of the Pasteur pipette with parafilm. The Pasteur pipette and capillary were filled with sterile deionized water containing a food coloring (0.5 ml dye/15 ml H_2O; McCormick & Co., Hunt Valley, MD). Glassy-winged sharpshooters previously fed on whole chrysanthemum plants excreted clear liquid, whereas excrement of *H. coagulata* that fed on the artificial system was blue because of the added dye. Changes in the fluid level were noted during feeding (Fig. 1a). Water consumption of the system alone without insects was measured in 5-min intervals for 1 h to obtain a baseline for transpiration for each stem. Then, a single adult glassy-winged sharpshooter was placed on the bare part of the stem, and the ingested volume of liquid was

measured visually as before. Once placed there by hand, the adults stayed in place even though they were not restrained and usually started feeding soon after. Variables such as sex, body position on stem (head up or head down), behavior during feeding (leg movements, abdominal movements), and excretion rate were recorded. The rate of excretion and volume of excreted liquid was estimated by collecting fecal fluid on paper plates coated with parafilm (20 cm diameter) that were fixed to the top of Pasteur pipette (Fig. 1a). Excreted liquid was collected, and its volume was measured.

Fluid Ingestion (Determined by Heartbeat Monitor) and Abdominal Movements. Opto-electronic heartbeat monitors designed and built by Dr. Karel Slama (Institute of Entomology, Prague, Czech Republic) have been used for measuring the heartbeat mainly in immobile life stages of insects, such as pupae (Slama and Miller 2001). The principle of this monitor is to reflect a laser beam pulsed at 1-kHz frequency off a convenient tissue from the outside of the insect. Any movement changing the light reflectance can then be recorded, such as heartbeat shadows. The drawback of this approach is the lack of calibration and arbitrary units of measurement; however, the advantage of monitoring the movement of an organ at some distance away from the test insect is advantageous. This instrument has two channels. The first channel was used to measure the abdominal movements of the glassy-winged sharpshooter during experiments. A second channel recorded fluctuations of liquid meniscus in the capillary tube during ingestion of xylem fluid, which was interpreted as active fluid ingestion by the glassy-winged sharpshooter. The light beam of the heartbeat monitor covered only a small surface of the capillary so a thin piece of capillary was painted red and placed inside the larger capillary to calibrate and serve as a marker of fluid movement. This technique allowed measurement of the liquid uptake and movements, reflecting the ingestion pumping motion concurrently (Fig. 1b).

Fluid Ingestion (Determined by Impedance Converter) and Abdominal Movement. Ingestion and abdominal movements were compared, and capillary liquid movements were recorded by the impedance converter (UFI, Moro Bay, CA) (Fig. 1c). Briefly, this instrument measures changes in impedance in the liquid between two thin wires in the capillary (Slama 2000). One bare wire recorded the movement of liquid and one covered wire served as a reference electrode. All of our measurements were taken in AC system using the built-in filter selectable for long or short waveforms of the AC system. All measurements with the impedance converter were taken in the AC mode, because the range of the recorder did not allow the measurements in DC because of intense fluid fluctuations. Outputs from both the impedance converter and the heartbeat monitor were connected to an analog recorder (Linseis L120E). All means that are reported herein are followed by the SD.

Results

When adult insects were placed by hand onto the test stem, they all settled down quickly and began feeding. When adult males finished a feeding bout, they tended to remove their stylets and wander off, walking around on the clamps and rods holding the stem and capillary tube arrangement. The females also exhibited feeding periods but rarely removed the stylets at the end of a feeding bout and remained in place on the stem with stylets attached. Because of these differences between the sexes in feeding behavior, we used adult females exclusively for making the recordings reported here.

Quantification of Ingestion. Before insect contact with the test stem (Fig. 2, first 34 min), a baseline was set for movement of fluid without insect ingestion. This constant fluid uptake was attributed to normal transpiration of the plant stem. Thereafter, major fluctuations in fluid movement were attributed to insect ingestion. As soon as *H. coagulata* were placed on the experimental stem (34 min in Fig. 2), water consumption from the connected reservoir substantially increased. The experimental bout reflected in Fig. 2 depicts a sample of adult female feeding over 2.5 h.

The amount of xylem fluid ingested by glassy-winged sharpshooters varied greatly. The average volume ingested was 0.088 ± 0.03 μl/min for males ($n = 25$) and 0.122 ± 0.07 μl/min ($n = 25$) for females. During periods of intense feeding, glassy-winged sharpshooters ingested >5.5 μl/min. Three types of feeding bouts were observed. *H. coagulata* did not actively ingest, ingested at a moderate rate, or ingested at a high rate. The moderate and high feeding periods are shown in Fig. 2, with the increased level of fluid consumption starting at 144 min and continuing until the end of the observations.

We marked excretion events during the monitoring of xylem fluid consumption (dots on Fig. 2 from 95 to ~144 min). From all of the bouts we recorded, the average volume of excreted fluid was 2 μl per excretion and occurred in regular cycles of 3 ± 0.43 excretions/2 min ($n = 27$). There is a lag period between the onset of feeding and the onset of excretion bouts. One such discrete bout is indicated on Fig. 2. *H. coagulata* excreted mainly in the head down orientation. The average length of time for processing of the ingested liquid was easy to recognize, because of the blue color of the excrement. The average length of time for processing of the ingested the artificial fluid was 93 ± 30.03 min ($n = 27$). The periods of intense sucking showed greatly increased amounts of xylem fluid processed (Fig. 2, 144–166 min) and lasted an average of 67 ± 41.5 min in males ($n = 25$) and 111 ± 16.5 min in females ($n = 25$).

Fluid Ingestion (Determined by Heartbeat Monitor) and Abdominal Movements. Regular abdominal movements were observed and measured in all glassy-winged sharpshooters while stylets were inserted into the stem (Fig. 3). These regular abdominal movements originated at the tip of the abdomen, deviating up and down from the antero-posterior body axis. The

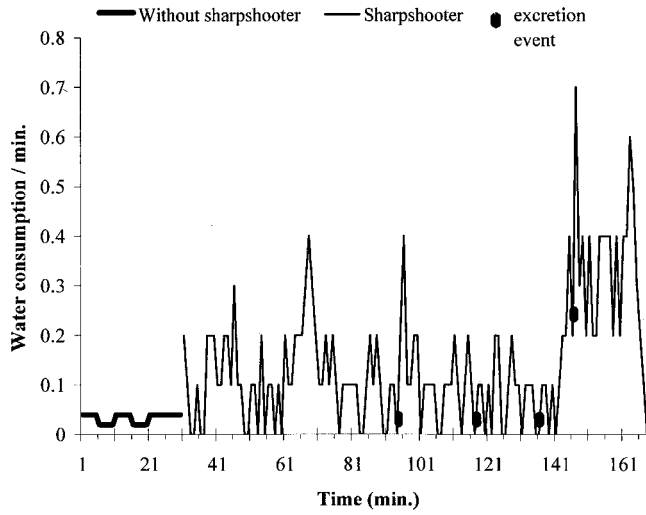

Fig. 2. The volume of ingested liquid measured in 1-min intervals. The volume consumed by plant is constant, whereas the volume consumed by glassy-winged sharpshooter varies rapidly. Dots mark the excretion bouts consisting of two to three 2-μl excretions.

average frequency of this movement was 16 ± 0/min (n = 200; Fig. 3), but these were not likely directly associated with ingestion, because frequency and intensity did not differ between inactivity and ingestion of fluid. Interestingly, this characteristic movement was only associated with the sharpshooter stylets being inserted into the plant tissues. Once the stylets were withdrawn, these regular abdominal movements did not occur. The regular abdominal movements ceased during excretion except when a droplet was flicked away (Fig. 3).

Fluid Ingestion (Determined by Impedance Converter) and Abdominal Movement. Fluctuations in water level caused by active ingestion of glassy-winged sharpshooter were measured both with the impedance converter and heartbeat monitor. The results measured with the heartbeat monitor and impedance converter showed intense peaks with the same frequency in both waveforms, suggesting a correlation between abdominal movements and liquid fluctuations (Fig. 4). The average frequency of this fluctuation was 3/min, although the length of intervals changed (Fig. 4). Both the results from the impedance converter and heartbeat monitor also showed differences between periods of intense ingestion and resting periods (Fig. 5).

Discussion

A novel method of monitoring the fluid ingestion patterns and abdominal movements has been developed. These two behaviors were well correlated in *H. coagulata;* fluid ingestion was nearly always followed by a significant abdominal movement. Slight, yet consistent, abdominal movements (16/min) were detected only when stylets were inserted. These mild abdominal movements may be related to probing behaviors; however, this correlation was not determined in this study. Stylet insertion was not always followed immediately by feeding bouts. Often *H. coagulata* remained on the stem with sylets inserted but without detectable ingestion. These periods of relative quiescence were often followed by a bout of excretion of fluid, although several exceptions were observed.

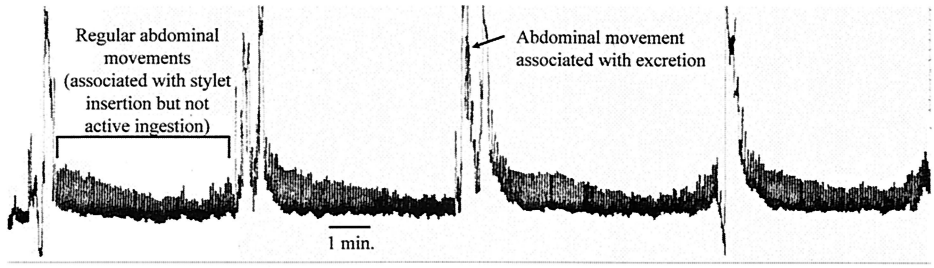

Fig. 3. Abdominal movements of adult female *H. coagulata* recorded by an opto-electronic device aimed vertically at the tip of the abdomen. The smaller, regular movements of the abdomen occurred on average at 16/min during nonfeeding bouts. The female had stylets inserted into the stem, but fluid ingested was not detected. The large deviations occurred when the abdomen moved in relation to excretion.

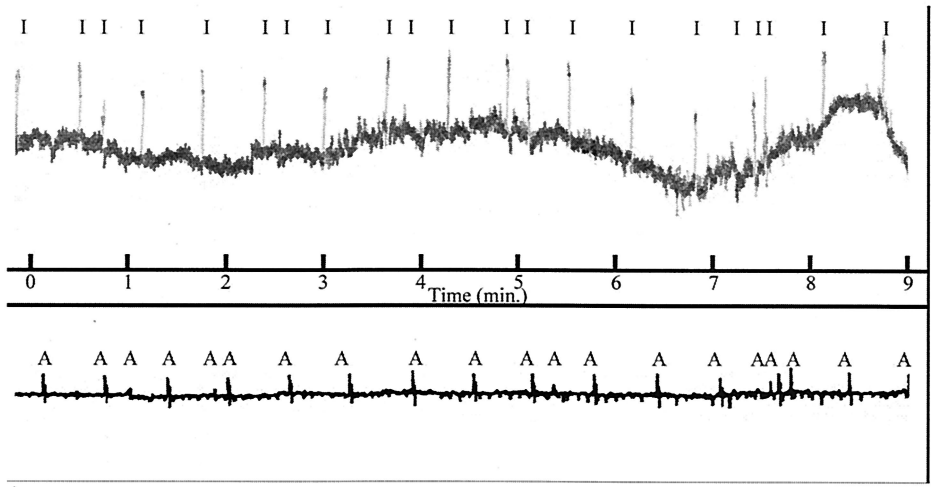

Fig. 4. Top line shows opto-electronic recording of fluid level as indicated by changes in the meniscus in the xylem fluid capillary tube. Bottom line shows opto-electronic recording of movement of the tip of the abdomen at reduced sensitivity compared with Fig. 3. Pulses are present with average frequency of 3/min. Note the abdominal movements (A) of the lower trace align with pulses (I) in the top line. Because the recorder pens are fixed off-set, the pulses in the top line coincide exactly with those in the bottom line.

Also, a bout of excretion often proceeded periods of intense ingestion, seemingly to clear the alimentary canal of previously ingested fluid to make room for the large volume of fluid about to be consumed. Long periods without ingestion with mouth parts inserted in the plant were typical for female glassy-winged sharpshooters, whereas male adults typically left the stem after finishing a feeding bout. Female glassy-winged sharpshooters often excreted a small drop of liquid and placed it on the tarsi of the hind legs. This behavior occurred independently on the feeding phase and was not observed in male glassy-winged sharpshooters. This finding was consistent with anointing behavior (Rakitov 1999, 2000).

Regular abdominal movements (with a frequency of 16/min) probably had no relation to ingestion, although they were present only when mouth parts were inserted into the plant tissues. Although we speculate that these movements could be related to probing, the abdominal movements may be associated with breathing or postmidgut digestion. By contrast, the intense abdominal movements (with a frequency of 3/min) may have been closely associated with fluid ingestion, because the data show direct correlation between abdominal movement and fluctuations of water level. The intense spikes during periods of ingestion were interpreted as actual drawing of the liquid by sucking, because xylem feeding insects need to suck against the negative pressure created by the plant to move the xylem fluid.

Parallel measurements of ingested liquid volume and graphs constructed from these results confirmed all electronically recorded data. Waveforms from both the impedance converter and heartbeat monitor showed intense fluid ingestion caused by insect sucking. However, low fluid consumption corresponded with waveforms that showed no fluid ingestion, suggesting that this consumption was caused by the plant itself. To determine the mechanism of *H. coagulata* fluid ingestion monitored in this study, future studies will employ the EPG feeding monitoring system along with our system. Whereas our system was useful for determining fluid consumption behaviors and abdominal movements related to ingestion, EPG may offer data on specific insect behaviors and plant/insect interactions that relate to fluid consumption.

We concede that this method for monitoring ingestion of fluid is artificial, but we nonetheless believe the method provides valuable data on feeding behaviors of *H. coagulata.* Chrysanthemum stems were used in this study, because they are an acceptable host for the insect being tested, often being used to maintain laboratory-reared colonies, and amenable to the cut stem method. *H. coagulata* has a wide host range, and whereas differences in feeding behavior might exist on other plants, chrysanthemum was a good experimental plant. In most cases, probing began shortly after an insect was placed on a cut stem and active ingestion could be monitored. Because the stem was cut and all leaves were removed, the normal physiological movements of fluid within vascular tissues were probably altered. However, fluid consumption by the stem alone without insect involvement was detected. *H. coagulata* excreted less fluid while feeding in these studies (2 μl/excretion with 10–20 excretions/h, resulting in $\approx$0.02–0.04 ml/h) than has been previously reported on other host plants, e.g., 0.265 $\pm$ 0.086 ml/h when fed on *Lagerstroemia indica* L. but 0.090 $\pm$ 0.031 ml/h when fed on *Prunus persica* L. (Andersen et al. 1989). In these previous studies, excretion rates were taken at hourly intervals and not associated with individual excretion events. The lack of excretion during the first 90 min of Fig. 2 seems unusual, but it could be because of the use of a "starved" sharpshooter that had

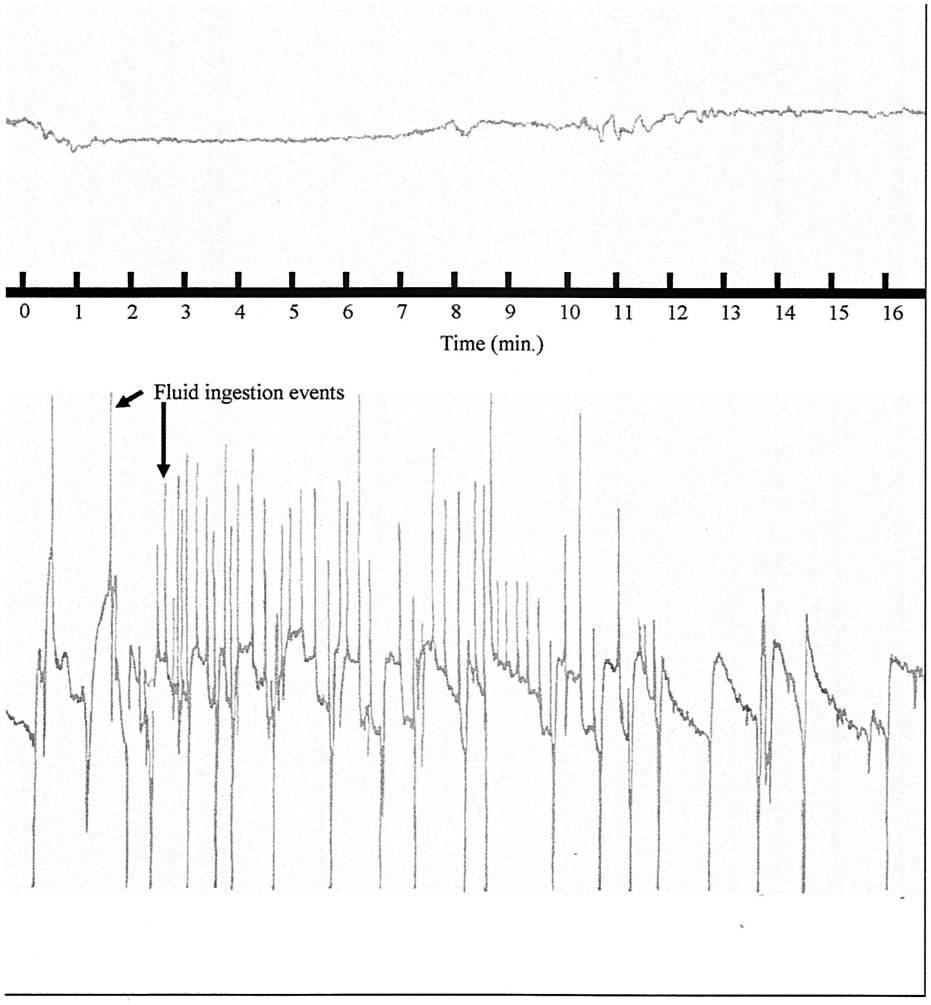

Fig. 5. (Top) Impedance converter record with electrodes in capillary fluid. Adult female *H. coagulata* is not feeding, but stylets penetrate to the xylem of the stem. Fluid ingestion is absent. (Bottom) Similar recording as top line, but here the female is intensely ingesting. The major drops result from the active ingestion. This record with impedance conversion is comparable with the record of Fig. 5 using the opto-electronic recording method.

not yet processed fluid for excretion. Additionally, excretion can fluctuate throughout a 24-h period based on many factors attributed to the physiology of the plant. Lower rates of excretion in this study were evident; therefore, interpretation of these data must be made relative to the test plant and ingestion source.

The methods developed in this study allow fluid ingestion and related abdominal movements to be monitored in a noninvasive manner. Although recordings were made while insects were feeding from an artificial system, the data collected on the feeding behavior of *H. coagulata* are valuable. To avoid the drawbacks of the artificial system, monitoring of abdominal movements could be taken while the insect was on an intact plant. The correlation between abdominal movements and fluid uptake allows inferences on feeding behavior and ingestion.

Ingestion monitoring methods are applicable to all insect species that feed from plant vascular systems, not just xylophages. Securing the plant stem to the pasture pipette water reservoir did not limit connection of only the xylem vessels. In fact, phloem vessels were also attached; therefore, ingestion behaviors of phloem feeders could be monitored in the same way. The advantage of this method is direct explanation and confirmation of recorded waveforms by parallel measurements of liquid consumption. Moreover, all parts of the method are noninvasive, do not require direct contact with the surface of the insect, and thus do not influence its feeding behavior. The most complete information on feeding behavior of glassy-winged sharpshooter would be ensured by a combination of EPG and the methods described above. A combination of the techniques will lead to better understanding of

H. coagulata feeding behavior as it relates to host plant damage and pathogen transmission, which in turn may lead to enhanced methods to improve plant health.

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