Journal of Economic Entomology, 110(3), 2017, 893–902
doi: 10.1093/jee/tox090
Advance Access Publication Date: 16 March 2017
Research article

Characterization of Direct Current-Electrical Penetration Graph Waveforms and Correlation With the Probing Behavior of *Matsumuratettix hiroglyphicus* (Hemiptera: Cicadellidae), the Insect Vector of Sugarcane White Leaf Phytoplasma

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Subject Editor: John Trumble

Received 25 December 2016; Editorial decision 22 February 2017

Abstract

The leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) (Hemiptera: Cicadellidae) is an important vector of phytoplasma causing white leaf disease in sugarcane. Thus, the aim of our study was to understand and describe the stylet-probing activities of this vector while feeding on sugarcane plants, by using direct current (DC) electrical penetration graph (EPG) monitoring. The EPG signals were classified into six distinct waveforms, according to amplitude, frequency, voltage level, and electrical origin of the observed traces during stylet penetration into the host plant tissues (probing). These six EPG waveforms of probing behavior comprise no stylet penetration (NP); stylet pathway through epidermis, mesophyll, and parenchymal cells (waveform A); contact at the bundle sheath layer (waveform B); salivation into phloem sieve elements (waveform C); phloem sap ingestion (waveform D); and short ingestion time of xylem sap (waveform E). The above waveform patterns were correlated with histological data of salivary sheath termini in plant tissue generated from insect stylet tips. The key findings of this study were that *M. hiroglyphicus* ingests the phloem sap at a relatively higher rate and for longer duration from any other cell type, suggesting that *M. hiroglyphicus* is mainly a phloem-feeder. Quantitative comparison of probing behavior revealed that females typically probe more frequently and longer in the phloem than males. Thus, females may acquire and inoculate greater amounts of phytoplasma than males, enhancing the efficiency of phytoplasma transmission and potentially exacerbating disease spreading. Overall, our study provides basic information on the probing behavior and transmission mechanism of *M. hiroglyphicus*.

Key words: electronic monitoring, electropenetrography, sap-sucking insect, leafhopper, phytoplasma

The leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) (Hemiptera: Cicadellidae) is a major insect vector of sugarcane white leaf (SCWL) disease. Sugarcane white leaf disease is one of the most destructive diseases of sugarcane in Southeast Asia that causes severe losses to sugarcane production. The typical symptoms of SCWL include leaf chlorosis, proliferation, and stunting. A severe infection by the SCWL phytoplasma during the early growth may lead to plant death or can hinder the production of millable or ratoon cane (Matsumoto et al. 1969, Nakashima et al. 1993, Wongkaew et al. 1997). The SCWL phytoplasma is an obligate parasite in the phloem of the host cells, which cannot be cultured in vitro in cell-free media (Lee and Davis 1986). The phytoplasma is transmitted predominantly by *M. hiroglyphicus* or infected seed cane (Hanboonsong et al. 2002, 2006), probably in a persistent–propagative manner (Weintraub and Beanland 2006). Our previous study showed that the phytoplasma is widely distributed throughout the body of the insect vector and transmitted transovarially from mother to offspring (Hanboonsong et al. 2002). Except for the eradication of infected plants, no efficient method of disease control has been developed, and no SCWL-resistant sugarcane varieties have been released. Consequently, the management of insect vectors offers a promising approach to reduce and control SCWL. A better understanding of the vector feeding behavior and pathogen interactions can assist in developing strategies to prevent or control the spread of the disease.

The most rigorous means of studying hemipteran feeding behavior involve the electrical penetration graph or electropenetrography (EPG) technique. This method uses an electronic monitor that enables the real-time tracking of the otherwise undetectable probing behavior of insects with piercing–sucking mouthparts. The technique was first introduced by McLean and Kinsey (1964), who developed the alternating current (AC) EPG system and then, was improved by Tjallingii (1978, 1988) using a direct current (DC) EPG system. Electropenetrography has been used in numerous studies on piercing or sap-sucking insects, such as for the localization of plant resistance factors (Annan et al. 2000, Alvarez et al. 2006, Diaz-montano et al. 2007, Marchetti et al. 2009, Crompton and Ode 2010), insect resistance to insecticides (Butler et al. 2012, Kang et al. 2012, Jacobson and Kennedy 2014, Civolani et al. 2014), and insect vector feeding behavior and transmission mechanisms (Tjallingii 1988, Backus and Bennett 1992, Backus 2000, Fereres and Moreno 2009, Bonani et al. 2010). Originally used in aphid studies, EPG has been applied to other sap-sucking insect groups, including planthoppers (Seo et al. 2009, AB Ghaffar et al. 2011), leafhoppers (Miranda et al. 2009, Stafford and Walker 2009, Jin et al. 2012, Trebicki et al. 2012), whiteflies (Johnson and Walker 1999, Walker and Janssen 2000, Fereres and Moreno 2009), psyllids (Civolani et al. 2011), and thrips (Joost and Riley 2005, Kindt et al. 2006). Here, we used the DC-EPG system to characterize the probing and ingestion behavior of *M. hiroglyphicus*, correlating waveforms with plant tissue histological data, including location and detection of the salivary sheath secreted during insect feeding.

The objectives of this study were to: 1) monitor and characterize the DC-EPG waveforms produced by *M. hiroglyphicus* when probing and feeding on sugarcane plants, and 2) investigate the correlation between the produced waveforms and probing behavior using histological data. The results provide basic information on the feeding characteristics of *M. hiroglyphicus*, which can be used in further studies on vector behavior, transmission mechanisms, and sugarcane resistance to SWCL phytoplasma.

Materials and Methods

Insects

A leafhopper (*M. hiroglyphicus*) colony was collected from a sugarcane field at Udon Thani Province, northeast Thailand, in July 2015 (rainy season), using light traps (black light) between 6:00 and 8:00 p.m. The insects were bred on 8-wk-old disease-free sugarcane plants in caged pots under controlled glasshouse conditions at 28 ± 2 °C, 70–80% relative humidity, and natural light. Only adult *M. hiroglyphicus* insects in the age range of 5–7 d were used in the study.

Plant Material

Disease-free sugarcane plants (*Saccharum* spp. cv. Khon Kaen 3) derived from tissue culture were obtained from the Khon Kaen Field Crops Research Center; plants were grown and maintained in nethouses at 30 ± 2 °C (room temperature). For EPG recording, 6–8-wk-old plants (two- to four-leaf stage) were used. The activity of individual *M. hiroglyphicus* females and males while feeding on the middle section of the second sugarcane leaf (one insect per leaf) was recorded using the EPG system.

Electrical Penetration Graph

A Giga-4 DC-EPG system (manufactured by W.F. Tjallingii, Wageningen University, The Netherlands) with 1 Giga-ohm (10^9 Ω or 1 GΩ) input resistance was used for recording EPGs. The substrate voltage electrode (2 mm in diameter, 10 cm in length) was inserted into the soil of the potted plants, and the substrate voltage was adjusted so that the EPG signals ranged from –5V to +5V. The adult leafhoppers were starved for 1 h , transferred to glass tubes (females and males separated), and chilled in a freezer at –20 °C for 2–3 min to reduce their metabolic rate, minimizing any effects on their subsequent behavior, and also to improve insect handling. The insects were immobilized by tweezers under a stereomicroscope, and a gold wire electrode (3 cm in length, 20 µm in diameter) was attached to the dorsal mesothorax, between the wings, with commercial silver conducting paint. The other end of the gold wire electrode was connected to a copper extension wire, whereas the opposite end of this extension wire was attached to a brass pin that was inserted into the input connector of the first-stage amplifier. Each insect was connected to one of the primary amplifier inputs (1 GΩ input resistance) before being placed on the sugarcane leaf. The plant, insect, and amplifiers were placed inside a Faraday cage, to cancel any external noise sources, in a temperature-controlled laboratory at 28 ± 2 °C. The electrical signals produced by the probing activities of the leafhoppers were recorded on a computer using STYLET 3.8 software (Tjallingii and Hogen Esch 1993). The EPG output was set at 50–100× gain, and the data were analyzed using PROBE 3.0 (Wageningen Agricultural University, The Netherlands). Each leafhopper was recorded for 10 hours per day (10:30 a.m.–8:30 p.m.), and a total of 25 females and 20 males were analyzed.

Characterization of EPG Waveforms

Electropenetrography waveforms were described based on their standard characteristics, including maximum peak voltage per waveform (mV), frequency (Hz), voltage level (extracellular, positive; or intracellular, negative), and electrical origin (resistance [R] or electromotive force [emf]). In order to determine the electrical origin, voltage adjustments to positive and negative levels were done over different periods for each waveform (Johnson and Walker 1999, Jin et al. 2012). The amplitude, frequency, and voltage level were identified based on the appearance of output signals (Stafford and Walker 2009, Jin et al. 2012, Trebicki et al. 2012). The mean and standard errors of amplitude and frequency were calculated based on 45 waveform events (reflecting the probing of 25 females and 20 males). The Fast Fourier Transform (FFT) tool of STYLET 3.8 was used to identify the fundamental frequency of each waveform, providing an auto-power spectrum graph (i.e., frequency analysis of the first 10 s of the waveform).

After measuring the typical waveforms related to insect probing, the following nonsequential response variables were calculated to describe stylet penetration in the sugarcane leaf by *M. hiroglyphicus* individuals during the 10-h access period: 1) mean number of waveform events per insect (NWEI), 2) mean waveform duration per insect (WDI in min), 3) mean waveform duration per event per insect (WDEI in min), and 4) mean proportion of recording time (%; Backus et al. 2007).

Plant Tissue Histology

We also investigated the correlation between EPG probing waveforms and the position of the insect stylets inside the plant tissue (as indicated by salivary sheath termini in the plant tissue). In total, 60 adult *M. hiroglyphicus* females were EPG-monitored while feeding on the middle section of the second leaf of the sugarcane plant (one insect per plant) under the same conditions used for collecting EPG data. Once an EPG waveform of interest (A, B, C, D, or E) was

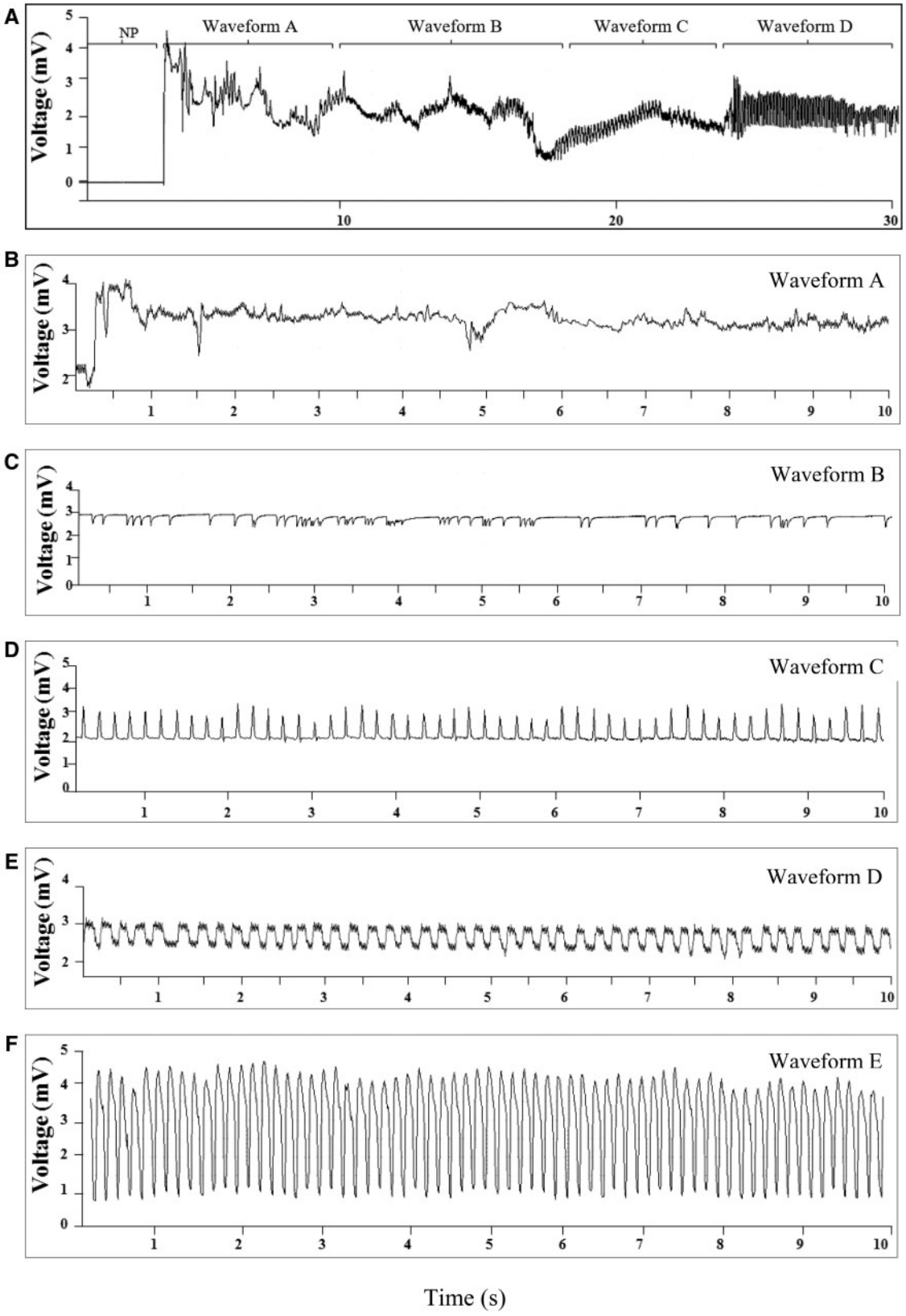

Fig. 1. Electrical penetration graph waveforms recorded during the feeding of the leafhopper *M. hiroglyphicus* on sugarcane leaves. (**A**) Overview of nonprobing and waveforms A, B, C, and D; (**B**) Waveform A; (**C**) Waveform B; (**D**) Waveform C; (**E**) Waveform D; and (**F**) Waveform E.

recorded, the insect was rapidly removed from the plant. This process was repeated using 12 insects for each waveform. For visualizing the salivary sheath termini, ~0.5 cm^2 of leaf tissue that contained the salivary sheaths was cut and treated with acid fucsin (1% aqueous) for 20 min, washed in distilled water, dried on filter paper, fixed in 6% paraformaldehyde overnight, and then, dehydrated in an ethanol series (70–100%) at 30 ± 2 °C (room temperature). A Historesin embedding Kit (Leica Heidelberg, Germany) was used for plastic infiltration and embedding. Serial transverse sections (9 μm in thickness) were cut using a rotary microtome (Shando Manual Microtome), colored with 1% toluidine blue solution (Sakai 1973), and then mounted on slides for photomicrography.

Data Analysis

Variable values were calculated and compared among individuals using the Kruskal–Wallis test combined with the Steel–Dwass multiple comparison test at $\alpha = 0.05$. Waveforms were compared between females and males using the Mann–Whitney U test, whereas pairwise comparisons were carried out using the Fisher's least significant difference (LSD) at $P < 0.05$. Descriptive and analytical statistics were computed using Statistical Analysis System 9.1 (SAS Institute, Inc.).

Results

Waveform Characteristics of *M. hiroglyphicus* Based on EPG Recording

Matsumuratettix hiroglyphicus preferred to probe near the midrib or vein on the sugarcane leaf surface during EPG recording. Both female and male leafhoppers produced all six different types of EPG waveforms during probing on sugarcane plants. These waveforms included the following: no stylet penetration (NP); stylet pathway through epidermis, mesophyll, and parenchymal cells (waveform A); contact at the bundle sheath (waveform B); salivation into phloem sieve elements (waveform C); phloem sap ingestion (waveform D); and short ingestion time of xylem sap (waveform E). A detailed description of each waveform characteristics is as follows:

The NP, or baseline, waveform was the first that recorded (Fig. 1A) and revealed the nonprobing activities of the insect. The NP had no frequency and zero voltage level (Table 1). However, small irregularities were detected when the leafhoppers walked on the plant surface or touched it with the tip of their labium.

Waveform A was the first waveform generated in all feeding probes (Fig. 1A). It was characterized by sharp positive peaks, an almost vertical increase in the voltage from the baseline, and a jagged, irregular pattern (Fig. 1B). Waveform A had a higher amplitude than the other waveforms, was exclusively extracellular, and the electrical origin of the signal was R (Table 1). The FFT spectrum of waveform A showed variable frequencies between 0 Hz and 5 Hz, with those of 0.01–0.1 Hz being the most predominant (Fig. 2A).

Waveform B appeared to alternate with waveform A (Fig. 1A). It was characterized by sequences of sharp peaks with negative deflections of large amplitude (Fig. 1C). Waveform B exhibited a regular pattern with a comparatively higher frequency at the beginning, was exclusively extracellular, and the electrical origin of the signal was emf (Table 1). The FFT spectrum of waveform B generally showed frequencies between 0.4 Hz and 1 Hz (Fig. 2B).

Waveform C showed a distinctive pattern that was typically interrupted and followed by waveform D events. It was characterized by low voltage fluctuation amplitude with sharp upward spikes (Fig. 1D). The median amplitude of waveform C was the lowest among the probing waveforms. Its voltage level was always positive and low in amplitude, indicating extracellular activity, and the electrical origin of the signal was emf (Table 1). The FFT spectrum of waveform C showed frequencies between 4 Hz and 5 Hz (Fig. 2C).

Waveform D showed a highly regular repetitive pattern of high-amplitude square plateaus (5 peaks/s). Its amplitude was higher than that of waveform B and C, but lower than that of waveform A and E (Fig. 1E). Waveform D generally occurred after waveform C, but with a higher frequency. The mean voltage level of D was extracellular, and the electrical origin of the signal was emf (Table 1). The FFT spectrum of waveform D showed frequencies between 4 Hz and 6 Hz (Fig. 2D).

Waveform E always occurred after waveform B. It had a regular pattern, similar to that of waveform D, but with rapid sequences of sharp peaks, with less variation in the depth of downward spikes (Fig. 1F). It had higher amplitude than waveforms B, C, and D, but lower amplitude than waveform A. The mean voltage level was always extracellular, and the electrical origin was emf (Table 1). The FFT spectrum revealed a primary frequency of 7–8 Hz (Fig. 2E).

Correlation Between EPG Waveforms and Salivary Sheath Termini in Plant Tissue

An overview of the leaf tissue in relation to the salivary sheathing is shown in Fig. 3A.

In the case of waveform A, one of the 12 sheath tips ended in the epidermis (short-duration probing of waveform A), three in the mesophyll, and six in vascular parenchymal cells (long-duration

Table 1. Characteristics of electrical penetration graph waveforms and corresponding probing behavior of the leafhopper *M. hiroglyphicus* during feeding on sugarcane

Waveform	Amplitude (mv)[a]	Frequency (Hz)	Voltage level	Electrical origin	Biological meaning	
					Location	Activity
NP[b]	–	–	–	–	(Not applicable)	Nonprobing surface activity
A	4912 ± 401.0	Variable	e	R	Epidermis/mesophyll/parenchyma	Pathway phase, start of penetration
B	3038 ± 248.0	0.4–1	e	emf	Bundle sheath	Bundle sheath penetration
C	2475 ± 202.8	4–5	e	emf	Phloem	Phloem salivation
D	3059 ± 249.8	4–6	e	emf	Phloem	Phloem ingestion
E	3129 ± 255.5	7–8	e	emf	Xylem	Xylem ingestion (nonphloem)

[a] mean ± SE

[b] NP, nonprobing; e, extracellular; R, resistance; emf, electromotive force

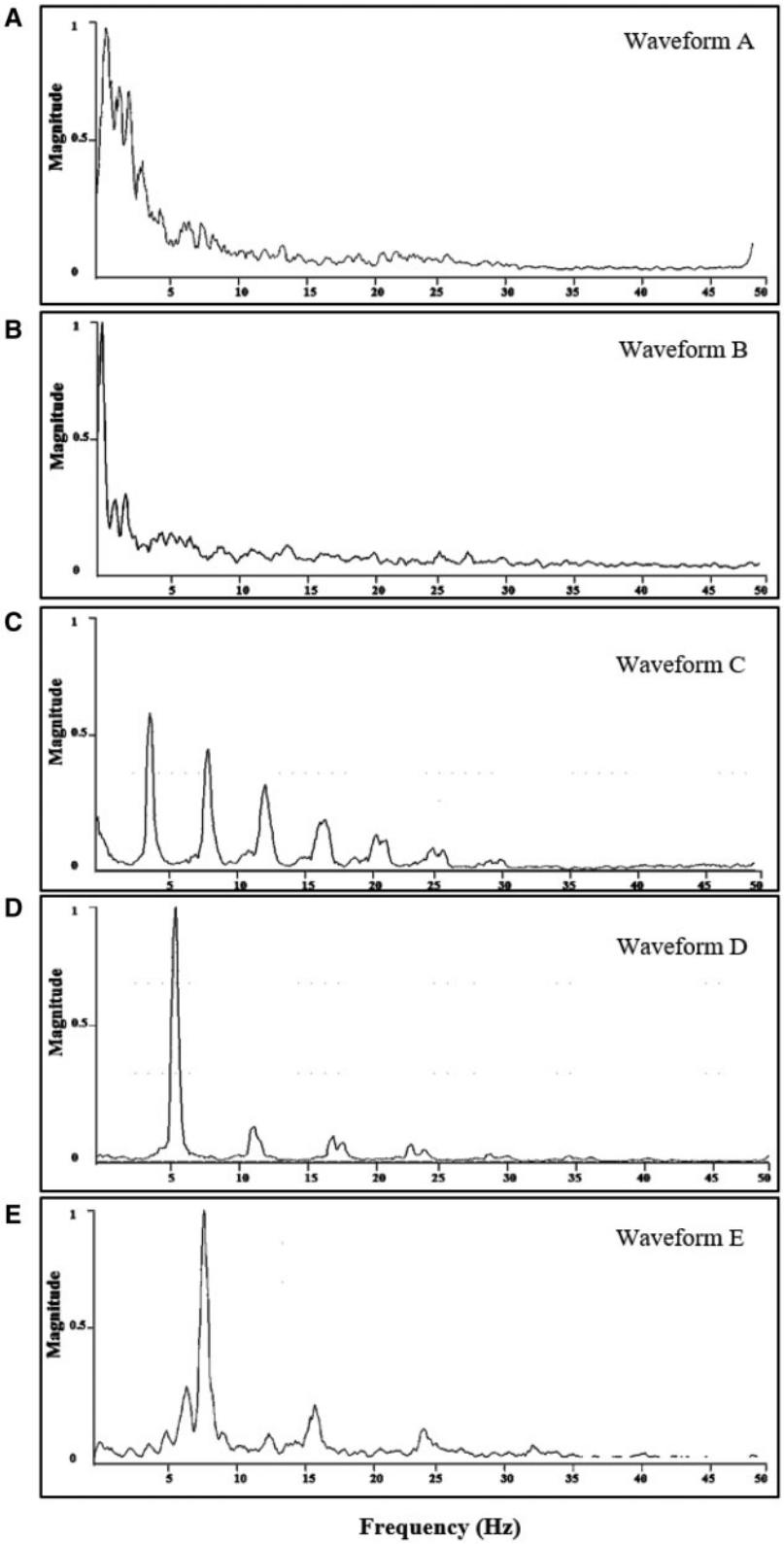

Fig. 2. Spectral analysis of waveforms recorded during the feeding of the leafhopper *M. hiroglyphicus* on sugarcane leaves. (**A**) Waveform A; (**B**) Waveform B; (**C**) Waveform C; (**D**) Waveform D; and **E**) Waveform E.

probing of waveform A; Fig. 3B). In the case of waveform B, 10 of the 12 sheath tips ended in bundle sheath cells (Fig. 3C). For waveform C, four of the 12 salivary sheath termini reached phloem cells, and eight terminated in the phloem (Fig. 3D). In the case of waveform D, all the 12 salivary sheaths ended in the phloem (Fig. 3E). Thus, waveform D was related to probing activities in the plant phloem. In the case of waveform E, all of the 12 salivary sheaths ended in the xylem (short-duration probing; Fig. 3F).

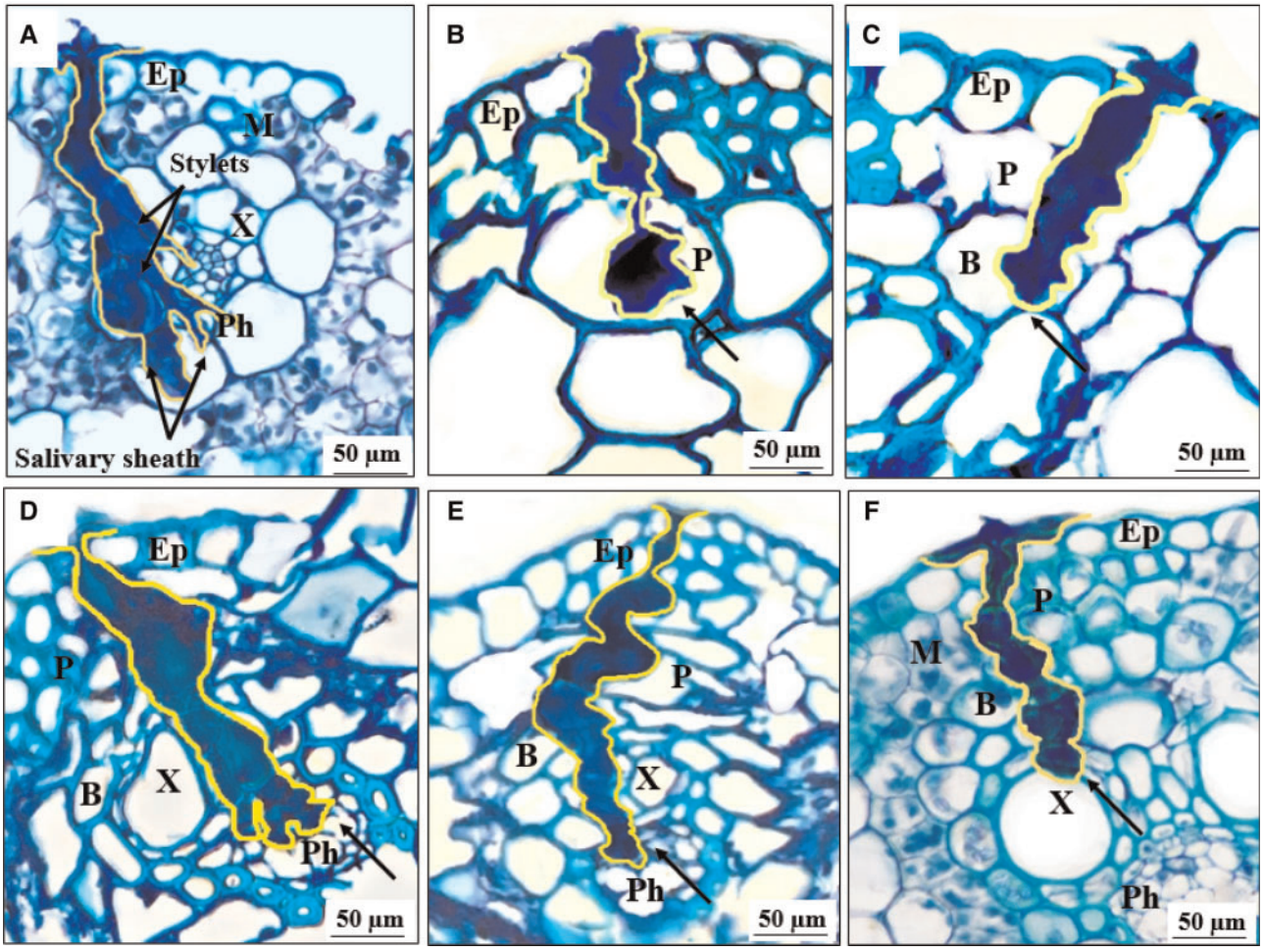

Fig. 3. Light microscope images of cross-sections of sugarcane leaves containing salivary sheaths of *M. hiroglyphicus*. (**A**) Overview of complete salivary sheath starting in the epidermis and ending in the phloem after a terminated probe on sugarcane leaf (40×). (**B**) Salivary sheath ending in the epidermis and parenchyma during waveform A (40×). (**C**) Salivary sheath ending in bundle sheath cells during waveform B (40×). (**D**) Salivary sheath ending in the phloem during waveform C (40×). (**E**) Salivary sheath ending in the phloem during waveform D (40×). (**F**) Salivary sheath ending in the xylem during waveform E (40×). Ep, epidermis; B, bundle sheath; M, mesophyll; P, parenchyma; Ph, phloem; X, xylem. Unlabeled arrows indicate the end-point of salivary sheath (40×).

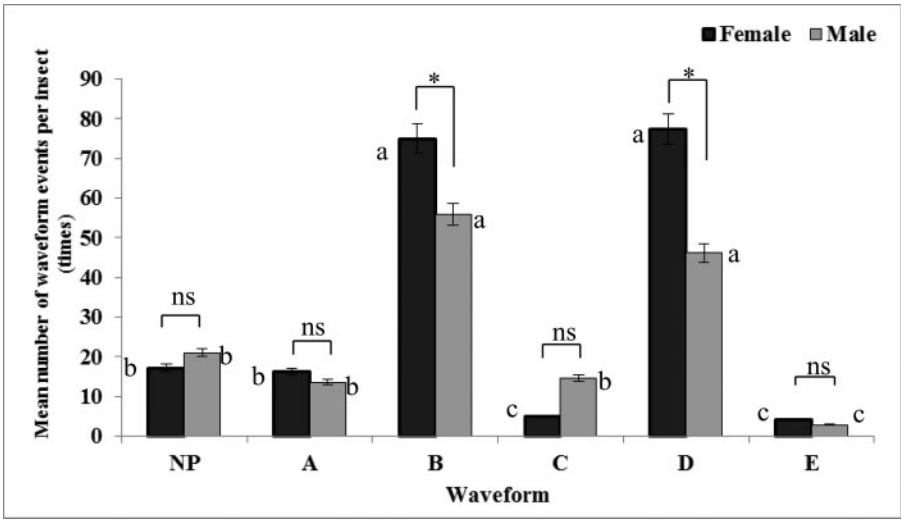

Fig. 4. Comparison of the mean number of waveform events per insect (NWEI) in 10 h, between females and males using the Kruskal–Wallis test, with the Steel–Dwass multiple pairwise comparison test at $\alpha = 0.05$. Significant differences within each gender are marked with different letters (female, left bar; male, right bar). Gender-based values were compared using the Mann–Whitney U test ($P = 0.05$). Analysis results are shown above each bar (*, $P < 0.05$; ns, not significant).

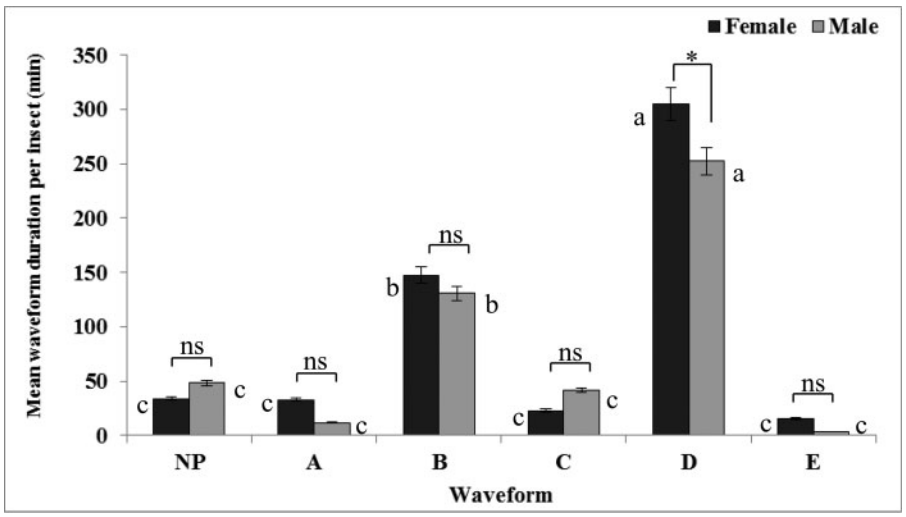

Fig. 5. Comparison of the mean waveform duration per insect (WDI) between females and males using the Kruskal–Wallis test, with the Steel–Dwass multiple pairwise comparison test at $\alpha = 0.05$. Significant differences within each gender are marked with different letters (female, left bar; male, right bar). Gender-based values were compared using the Mann–Whitney U test ($P < 0.05$). Analysis results are shown above each bar (*, $P < 0.05$; ns, not significant).

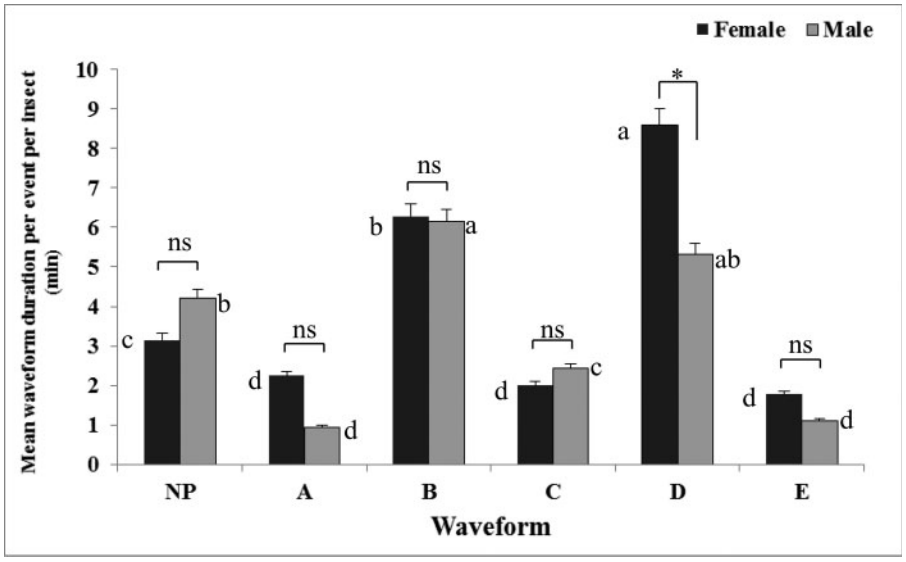

Fig. 6. Comparison of the mean waveform duration per event per insect (WDEI) between females and males using the Kruskal–Wallis test, with the Steel–Dwass multiple pairwise comparison test at $\alpha = 0.05$. Significant differences within each gender are marked with different letters (female, left bar; male, right bar). Gender-based values were compared using the Mann–Whitney U test ($P < 0.05$). Analysis results are shown above each bar (*, $P < 0.05$; ns, not significant).

Probing Behavior of *M. hiroglyphicus*

All six probing waveforms were observed in both female and male insects. The NWEI, WDI, and WDEI did not differ significantly between waveforms B and D (Figs. 4–6, respectively), and these waveform values were dominant in both females and males. However, the mean waveform duration per event differed significantly between the two sexes for waveforms (Fig. 6; $P < 0.05$). Overall, waveform D was the most dominant in both females and males (56.4% in females; 51.7% in males), whereas waveform B was the second most dominant (27.0% in females; 24.1% in males). The mean number of waveforms B and D was significantly higher in females than in males (Fig. 4; $P = 0.021$ for waveform B; $P = 0.016$ for waveform D). The NP was of short duration in both females and males (6.2% in females; 8.9% in males), and the males typically spent more time in nonprobing activities than females (Fig. 5). The mean proportion of recording time was very high for waveform D and very low for waveform E, in both females and males (Fig. 7). In addition, >90% of the recording time was spent in probing ingestion (waveform D; Fig. 7).

Discussion

Biological Meaning of *M. hiroglyphicus* Waveforms

This is the first published EPG study describing the probing and ingestion behavior of the leafhopper *M. hiroglyphicus*. Some of *M. hiroglyphicus* probing waveforms showed similarities to those observed in other sap-sucking insect groups such as other leafhoppers (Lett et al. 2001, Stafford and Walker 2009), aphids (Tjallingii 1978, 1988; van Helden and Tjallingii 2000), planthoppers (Seo

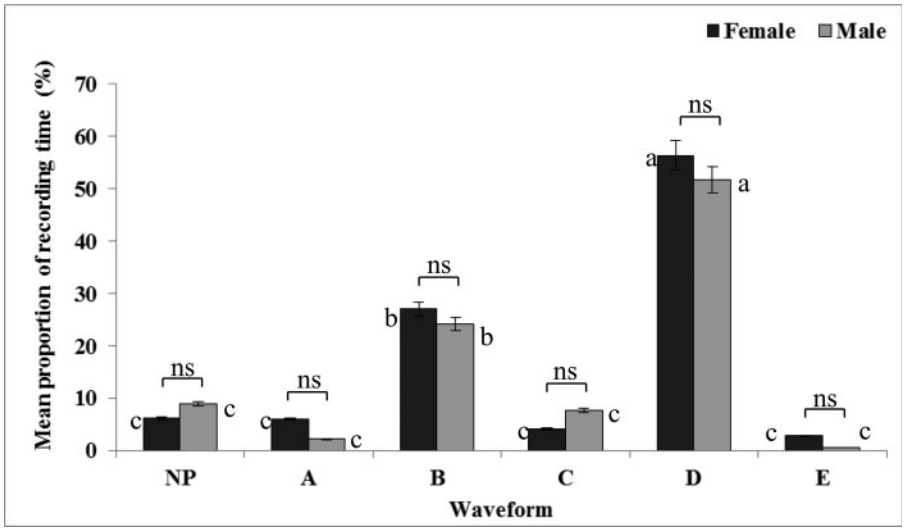

Fig. 7. Comparison of the proportion of waveform recording time between females and males using the Kruskal–Wallis test, with the Steel–Dwass multiple pairwise comparison test at $\alpha = 0.05$. Significant differences within each gender are marked with different letters (female, left bar; male, right bar). Gender-based values were compared using the Mann–Whitney U test ($P < 0.05$). Analysis results are shown above each bar (ns, not significant).

et al. 2009, Trebicki et al. 2012), and whiteflies (Prado and Tjallingii 1994, Lei et al. 1999).

Waveform A showed a sustained high voltage from the baseline, and its amplitude was higher than that of other waveforms. Waveform A was observed in every probe and was similar to that observed in the pathway phase of other leafhoppers, but especially to that pathway of the beet leafhopper *Circulifer tenellus* (Baker), which is the vector of beet curly top virus (Stafford and Walker 2009). The amplitude of waveform A was higher than that of other waveforms, probably representing probing and the secretion of the sheath trunk and short branches, similarly as observed in other leafhoppers (Backus et al. 2005). Waveform A always occurred after the NP phase and was recorded in all instances of probing. During this phase, the insect produces gelling saliva, which creates the salivary sheath that lubricates the stylet as it penetrates the tissue. Based on the tissue cross-section results, the salivary sheath was left in the sugarcane leaf during waveform A recording, supporting the hypothesis that waveform A corresponds to the pathway phase or the penetration of the epidermis. However, the location of the stylet tip depended on the duration of insect probing and the depth of the plant cell structure. In the case of waveform A, short-duration probing corresponded to the presence of salivary sheath in the epidermis, whereas long-duration probing corresponded to the presence of salivary sheath in the mesophyll or parenchymal cells. Similar results were reported in the Asian citrus psyllid *Diaphorina citri* Kuwayama that waveform C corresponded to the presence of salivary sheath termini in parenchymal cells (Bonani et al. 2010); in the leafhopper *Empoasca vitis* (Göthe), waveform E1 corresponded to channel cutting or salivary sheath phase (Jin et al. 2012); and in the *Orosius orientalis* (Matsumura), waveform O1 corresponded to the presence of salivary sheath in the epidermis, mesophyll, and all tissues (Trebicki et al. 2012).

Waveform B showed sequences of sharp peaks with negative deflections of high amplitude, similar to those of waveform C of the leafhopper *Cicadulina mbila* Naudé (Lett et al. 2001), the Asian citrus psyllid *D. citri* (Bonani et al. 2010), and the leafhopper *C. tenellus* (Stafford and Walker 2009). In the present study, waveform B appeared after waveform A and was relatively longer in duration. During waveform B, salivary sheaths were clearly observed in the leaf cross-sections, wherein the salivary sheath branch crossed into bundle sheath cells (Fig. 3C). Similar results were reported in the *Homalodisca coagulata* (Say) using AC-EPG, and showed that waveform B was correlated with probing activity in parenchymal cells (Backus et al. 2005).

Waveform C had a distinctive shape, with low amplitude of voltage fluctuation and sharp upward spikes. Waveform C was similar in shape to waveform E1 of the psyllid *D. citri* (Bonani et al. 2010) and waveform PC2 of the *Cacopsylla pyri* L. (Civolani et al. 2011), which corresponded to the location of stylet tips in the phloem and were correlated with salivation. Therefore, we hypothesized that waveform C represents the stylet tip position in a phloem sieve element and the stylet activity of sheath or watery salivation.

Waveform D, a highly rhythmic and regular waveform, occurred after waveform C, and showed a low voltage level similar to waveform C. In addition, histological analysis showed that the salivary sheath was clearly present in phloem cells during waveform D. Waveform D resembles other reported waveforms corresponding to ingestion by salivary sheath-feeding leafhopper species, such as waveform 5 of the psyllid *C. mbila* (Lett et al. 2001) and the common brown leafhopper *O. orientalis* (Trebicki et al. 2012), or waveform N4 of the brown planthopper (Seo et al. 2009). These findings support the hypothesis that waveform D corresponds to phloem ingestion and penetration into the sieve element.

Waveform E showed rapid sequences of sharp peaks with little variation in the depth of the downward spikes, and high frequency. Dugravot et al (2008) reported that this waveform pattern was likely related to the activity of the cibarial pump. Waveform E had a similar shape to waveform N5 of *Nilaparvata lugens* (Stål) (Seo et al. 2009), waveform B of the leafhopper *C. mbila*, as well as waveforms G and Xc of the leafhoppers *Bucephalogonia xanthophis* (Berg) and *C. tenellus*, respectively (Lett et al. 2001, Miranda et al. 2009, Stafford and Walker 2009). In all cases, these waveforms were correlated with active ingestion in xylem cells and involved short-duration probing. The combined waveform and salivary-sheath data in the present study suggested that waveform E might be related to the active ingestion of fluids and correspond to xylem-related insect activity, similar to waveform O1 of the common brown leafhopper *O. orientalis* (Trebicki et al. 2012).

Quantitative Comparison of Probing Data Between *M. hiroglyphicus* Females and Males

The probing behavior results showed that male leafhoppers spent more time in NP behavior than their female counterparts, with females probing more frequently and for longer duration in the phloem than males. Thus, females have a higher probability of acquiring phytoplasma, and once infected of inoculating the phytoplasma into plant tissue. These gender differences probably make females more effective vectors than males and may partially explain previously reported gender differences in transmission efficiency (Hanboonsong et al 2006). However, the gender-related ability to transmit pathogens is also species specific. For example, Chuche and Thiéry (2014) reported that the *Scaphoideus titanus* Ball males, the leafhopper vector of grapevine flavescence dorée phytoplasma, probe more frequently and for longer duration in the phloem than females, and thus, they have a greater probability of acquiring and inoculating the phytoplasma. Our study showed that *M. hiroglyphicus* females spent more time in ingestion, searching for phloem cells, and salivation, resulting in a greater efficiency in the acquisition and inoculation of SCWL phytoplasma and thus, exacerbating disease spreading.

In conclusion, our study provided a better understanding of probing, disease transmission, and epidemiology. Our data could be used for the future screening of sugarcane resistance to the insect vector and disease as well as to develop a sustainable control strategy for SCWL.

Acknowledgments

This study was supported by the Royal Golden Jubilee Ph.D. program of the Thailand Research Fund and Khon Kaen University (PHD54K0162). We would like to thank Dr. Elaine Backus, USDA-ARS, Parlier, CA , for her critical review and suggestions that helped us to improve the quality of this manuscript.

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