CORRELATION OF AN ELECTRICAL PENETRATION GRAPH WAVEFORM WITH WALKING BY ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE)

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Electrical penetration graph (EPG) monitoring of insect feeding is a rigorous means of observing and quantifying the otherwise invisible feeding of piercing-sucking arthropods (Walker 2000). EPG is being used to study how the feeding behavior of the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama) changes in response to insecticide residues (Serikawa et al. 2010; Serikawa et al. in press), in an effort to improve the efficacy of widespread insecticide treatments against this insect in Florida citrus. ACP is the vector of the putative causal agent of Huanglongbing (HLB) disease, *Candidatus* Liberibacter asiaticus, the cause of millions of dollars in damage to the citrus industry in Florida (Muraro & Morris 2009). The primary means of disease management at this time is insecticide treatment of vectors; however, it is presently unknown whether insecticides prevent performance of the feeding behaviors of ACP required for pathogen transmission.

Coarse-scale EPG waveforms for ACP have been defined via standard EPG correlation methods (Bonani et al. 2010). Additional, finer-scale waveform correlations have been conducted with over 300 EPG recordings of ACP (Youn et al. unpublished data). When the stylets of an EPG-recorded insect were inserted into a plant, a sudden increase in voltage occurred, denoting the beginning of feeding. Immediately prior to this voltage surge, the waveform was usually flat near 0 V (true baseline). But, when longer non-probing durations occurred, it was noticed that ACP produced 2 types of baseline waveforms that always were visually correlated with psyllid movements. These visual observations were consistent for 100% of the insects recorded in the above studies. The current study describes correlation of these 2 new baseline EPG waveforms for ACP that allow the user to determine when the insect is moving versus standing still.

Psyllids used for this study were originally collected in citrus orchards near the University of Florida Citrus Research and Education Center, Lake Alfred, Florida, and maintained in a greenhouse on 6-8 mo-old seedlings of sweet orange, *Citrus sinensis* (L.) Osbeck (cv 'Hamlin'). Greenhouse conditions were 27 ± 1 °C, 63 ± 2% RH, and photoperiod of 14:10 h L:D. For EPG recordings, a 3-4 cm length of 25 µm-diam gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) was attached to the scutellum of each 2-5 d-old female ACP, using a droplet of silver conducting paint (Ladd Research Industries, Burlington Vermont; N-butyl acetate solvent). The free end of the gold wire was connected to the headstage amplifier of a Giga8 DC EPG monitor (EPG Systems, Wageningen University, The Netherlands). The monitor's plant electrode was inserted into the plant soil, introducing a DC applied signal. Waveforms were digitized using a DI-710-UL USB analog-to-digital board (Dataq Instruments, Akron, Ohio) and displayed using Windaq Lite ver. 2.40 software (Dataq Instruments) on a Dell desktop computer. Five psyllids were recorded on young leaves of Hamlin sweet orange plants, for each of 2 treatments: control (unaltered) psyllids, or psyllids whose tarsi were painted with clear nail polish (470A Clear Nail Fortifier, Markwins Beauty Products, Inc., City of Industry, California) then allowed to dry for 1 h before EPG recording. Scanning electron microscopy (SEM) was performed to observe the tarsal morphology of ACP and the effect of nail polish. Legs of 5 adult psyllids were excised under 40 × (Leica, Wild MC3 stereomicroscope, Heerbrugg, Switzerland) and the tarsi were painted in the same manner as for EPG using the same nail polish. The painted legs were fixed overnight in 3% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.2. Legs were washed 4 times in phosphate buffer 10 min each,

then post-fixed in 2% osmium tetroxide for 4 h at room temperature, then rinsed twice in phosphate buffer 10 min each. Thereafter, painted legs were mounted on aluminum stubs via double-sided copper sticky tape (3M, St. Paul, Minnesota), kept in a desiccating chamber (25 ± 1 °C, 10 ± 1% RH) overnight, and then critical point dried (LADD, Burlington, Vermont) for 1 h. Legs were sputter-coated with gold/palladium (40:60) in a LADD SC-502 (Burlington, Vermont) high-resolution sputter coater, and subsequently examined with a Kevex® S-530 (Hitachi, Tokyo, Japan) SEM operated at 20 kV.

The 2 baseline waveforms are herein designated NP (for non-probing) (Fig. 1A, B) and Z (opposite end of the alphabet from feeding waveform designations) (Fig. 1A, C). Waveform NP is an irregular waveform with highly variable-amplitude peaks and valleys (Fig. 1B), but rarely higher in amplitude than the spike that is recorded upon initial stylet insertion (Fig. 1A). The relative amplitude was 0.5-80% of the highest psyllid waveform peaks at the start of stylet penetration. In contrast, waveform Z is nearly flat, showing almost no voltage variability (Fig. 1C). Visual observation while waveforms were being recorded indicated that NP occurred when the insect was walking on the leaf, sometimes while simultaneously tapping the plant surface with its labium; Z was recorded when the insect was standing still. We hypothesized that waveform NP could be caused by structural features of the psyllid tarsi, perhaps scratching, grasping or otherwise making close contact with the plant surface, such that minor electrical conductivity occurred between the insect and electrified plant. SEM of the ACP tarsus showed prominent tarsal claws and a large, pad-like arolium between the claws (Fig. 2A). The surface of the arolium was covered with a dense carpet of papillae with finger-like extensions (Fig. 2B). When all tarsi of live psyllids were painted with clear nail polish prior to EPG recording (Fig. 2D; compare with Fig. 2C), the distinction between the 2 baseline waveforms was abolished. Psyllids with painted tarsi were able to stand and walk, but in either case waveform Z was recorded (similar to Fig. 1C), not NP. We concluded that the minor peaks and valleys of waveform NP were caused by tarsal structures making electrical contact with the plant, and that labial tapping did not contribute to the waveform.

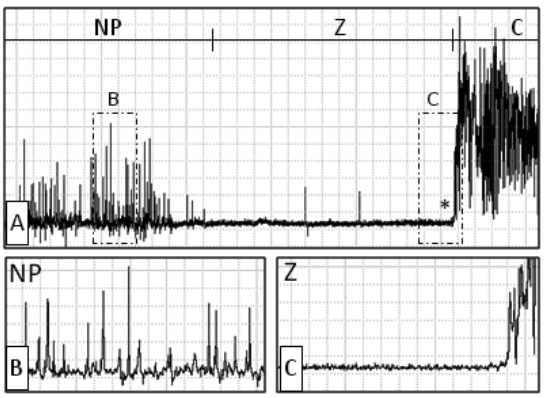

Fig. 1. A. Overview of representative NP (walking) and Z (standing still) waveforms just before stylets are inserted (denoted by *; beginning of waveform C). Sections in dashed boxes B and C are expanded (X axis) in parts B and C. Y axis: ~10% relative amplitude/division; X axis: 2.0 s/division. B. Waveform NP, expanded from box B in part A. C. Waveform Z, expanded from box C in part A. For both A and B, Y axis: ~10% relative amplitude/division; X axis: 2.0 s/division.

Distinct walking and standing waveforms are often seen in recordings of large auchenorrhynchan (e.g. sharpshooter leafhoppers) (Backus, unpublished data) and heteropteran hemipterans (Backus et al. 2007) using AC EPG monitors. In contrast, a review of all DC EPG papers published in the last 20 yr showed that the baseline was usually completely flat, with no variation in voltage that could be due to insect movement (Jin & Baoyu 2007; Cid & Fereres 2010). Therefore, this is one of the first times (cited in the literature) that 2 distinct baseline waveforms have been recorded and correlated for a hemipteran using the Giga8 monitor and DC applied signal. The walking waveform (NP) is particularly useful for EPG insecticide studies, because it documents movement vs. paralysis of the insect, an early indication of intoxication prior to death (Serikawa et al. in press). Thus, in many circumstances, EPG monitors can be used as a general actigraph to complement its functions in detecting feeding.

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Summary

The present study characterized and correlated 2 EPG waveforms, NP and Z, recorded when the Asian citrus psyllid (ACP) is walking/labial dabbing or standing still, respectively, prior to insertion of the stylets into the plant to begin feeding. The variable-voltage NP waveform was correlated with electrical contact caused by structures on the psyllid tarsus, such as tarsal claws or texture of the arolium, imaged using SEM. This conclusion is supported because covering the tarsi with smooth, clear nail polish abolished the electrical conductance. Despite walking by nail polish-treated insects, only the flat Z waveform (true baseline) was displayed. This is the first time that

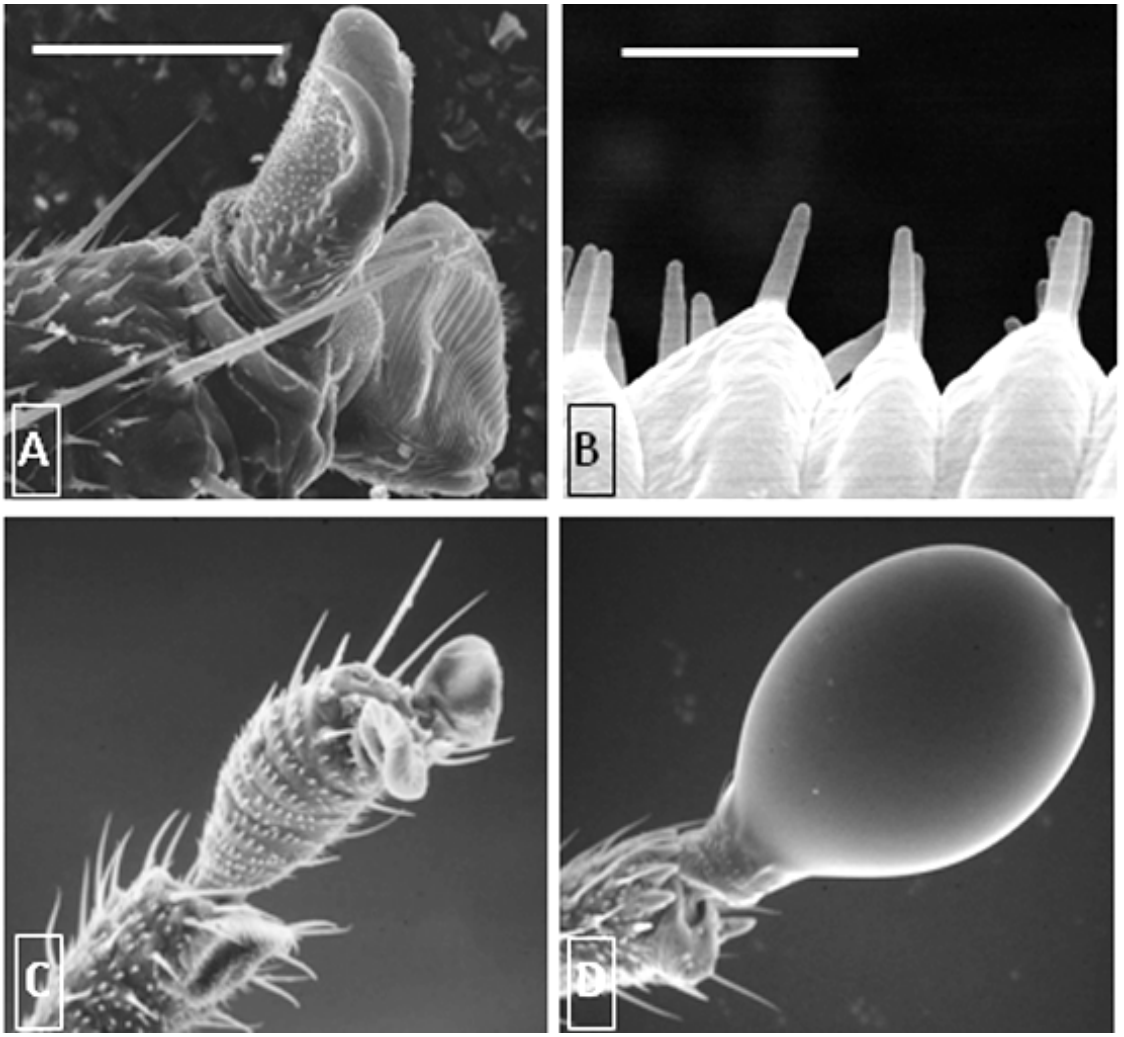

Fig. 2. A - D. Scanning electron micrographs of the tarsi of Asian citrus psyllids. A. Untreated, control insect's tarsus showing one claw and both arolia. Scale bar 30 µm. B. Close up of texturing papillae on the dorsal surface of an untreated arolium, showing the finger-like extensions. Scale bar 2 µm. C. Distal portion of a leg, including tarsus, of an untreated, control insect. D. Distal portion of a leg of a treated insect whose tarsus was encased in clear nail polish.

a walking waveform is detected for a sternorrhynchan hemipteran using the Giga8 EPG monitor. The walking waveform will be useful for numerous EPG studies of ACP.

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