6.4 Characterization of electrical penetration graphs of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus

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Abstract

For decades the Asian citrus psyllid, *Diaphorina citri* Kuwayama has been known as a vector of phloem-limited bacteria associated to citrus Huanglongbing (HLB), *Candidatus* Liberibacter spp. However, some aspects of transmission remain poorly understood, such as the feeding activities inside the plant tissue that result in bacterial acquisition and inoculation. This research was carried out to investigate stylet penetration and feeding activities of *D. citri* on seedlings of *Citrus sinensis* (L.) Osbeck cv. ‘Pêra’ by using the electrical penetration graph (DC-EPG system) technique. EPG waveforms were described based on amplitude, frequency, voltage level and electrical origin of the observed traces during stylet penetration on plant tissues. The main waveforms were correlated with histological observations of salivary sheath termini in plant tissues, in order to determine the putative location of stylet tips. The behavioral activity was also inferred based on waveform similarities in relation to other Sternorrhyncha, particularly aphids and whiteflies. By analyzing 8-h EPGs of 20 adult females, five waveforms were described: (C) salivary sheath secretion and extracellular stylet pathway through epidermis and parenchyma; (D) first contact with phloem tissue (distinct from other waveforms reported for Sternorrhyncha); (E1) putative salivation in the phloem; (E2) probably phloem ingestion; and (G) putative xylem ingestion. *D. citri* always initiate a probe with stylet pathway through epidermis and parenchyma (C), with mean duration of 10 min. After waveform C, stylet withdrawal from the plant and return to non-probing status (Np) is the most frequent event (86.2%), especially in the first 2 h of recording. The probability of phloem contact after C is 12.5%. Waveform D is always observed upon phloem contact, followed by E1 (salivation; mean duration of 1.55 min). After E1, there is a 45% probability of phloem ingestion (E2), but return to pathway activity (C) is a more frequent outcome (55%). This insect only reached the phloem vessels after an average of 155 min following the onset of the first probe. Despite the long time to reach the sieve elements, phloem ingestion is the main activity of *D. citri* within the plant, with average total duration of 206.1 min (42.9% of the recording time). Waveform G was observed after C with a very low frequency (1.3%) and mean duration of 23.7 min. These data on probing behavior of *D. citri* are basic to determine stylet activities and time periods required for acquisition and inoculation of *Candidatus* Liberibacter spp. in citrus, as well as to establish effective control tactics for preventing HLB spread.