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Feeding behaviour of the Asiatic citrus psyllid, *Diaphorina citri*, on healthy and huanglongbing-infected citrus

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Abstract

Diaphorina citri Kuwayama (Hemiptera: Sternorrhyncha: Psyllidae) is a vector of huanglongbing, a disease of citrus that in Asia is caused by '*Candidatus* Liberibacter asiaticus' (α-Proteobacteria) (Las). Acquisition of Las by *D. citri* appears to be variable, and this variability may be due to the suitability of the host plants and their tissues for acquisition. Therefore, this study aimed to determine the effect of symptom severity of the disease on the feeding behaviour of *D. citri*. Use of an electrical penetration graph showed that the pathway phase of *D. citri* consisted of four waveforms, A, B, C, and D; waveforms A and B have not been reported for *D. citri* before. The remaining waveforms, E1, E2, and G, conform to those described before for *D. citri*. The duration of the non-penetration period did not differ between healthy or infected plants. However, in moderately and severely symptomatic plants, the duration of the pathway phase increased, whereas the phloem phase was shorter. In all diseased plants, the times to first and sustained salivation in the phloem were longer than those in control plants, with the times being related to symptom severity. As symptom expression increased, the percentage of time spent by psyllids salivating during the phloem phase increased; however, the percentage of time spent in phloem activities reduced gradually from ca. 74% in the control plants to ca. 8% in the severely symptomatic plants. In contrast, the percentage of time spent on xylem activities increased, as did the proportion of psyllids feeding from xylem. The differences in the durations of the E waveforms on plants showing different levels of symptom expression may account for differences in acquisition found amongst studies; therefore, future work on the acquisition and transmission of Las needs to carefully document symptom expression.

Introduction

In Asia, the Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psyllidae), is the only known indigenous vector of huanglongbing (HLB), a devastating disease of citrus that is widely known as citrus greening (Aubert, 1990; da Graça, 1991; Halbert & Manjunath, 2004). In Asia, the disease is caused by a gram-negative, phloem-limited bacterium, '*Candidatus* Liberibacter asiaticus' (α-Proteobacteria) (Las) (Bové, 2006). Las can be acquired by second, third, fourth, and fifth instars and adults (Hung et al., 2004) but is transmitted only by fourth and fifth instars and adults (Capoor et al., 1974; Xu et al., 1988a,b). The bacterium has been detected in haemolymph and salivary glands of *D. citri* (Chen et al., 1973; Xu et al., 1985, 1988a; Jiang, 2005), and infected adults retain infectivity throughout their lives (Capoor et al., 1974; Xu et al., 1988a; Hung et al., 2004). Hung et al. (2004) and Inoue et al. (2009) showed that the bacterium multiplies within its insect host.

Acquisition of Las by *D. citri* appears to be variable. Capoor et al. (1974) studied the minimum acquisition

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period by taking adult psyllids that had fed for known periods on infected plants and placing them on to healthy sweet orange. They found that 45–50% of trees became infected if the psyllids had fed for 1 min, whereas 100% of trees became infected if the adults had fed for 30 min or longer. Inoue et al. (2009), who assessed acquisition using single step PCR assays, also found that a large proportion (88%) of adult psyllids contained the bacterium after an access period of 24 h. In contrast, using real-time PCR assays, Rogers et al. (2008) found that only 20–30% of psyllids that had fed on plants as adults acquired the bacterium, and Su (2008) reported that less than 5% of adults acquired Las after feeding on diseased citrus plants over a period of 1 day. Some of these reported differences in acquisition access period may be due to differences in experimental protocols. However, they may also be due to differences in regional populations of the psyllid or in the suitability of the host plant for acquisition, as Bonani et al. (2008a) found that psyllids fed on mature Las-infected citrus plants did not acquire the bacterium, whereas 50% of psyllids fed on young, infected but asymptomatic plants became infected.

The feeding behaviour of sap sucking insects can be monitored electronically using the electrical penetration graph (EPG) technique of Tjallingii (1988, 2000) and, using this technique, three feeding phases have been identified: pathway, phloem, and xylem (Prado & Tjallingii, 1994; Lei et al., 1999). Each of these phases has characteristic waveforms due to changes in the conductivity of the food and saliva canal of the insect, differences in the conductivity of the plant tissues from which it is feeding, as well as electromotive forces generated within the aphid or plant (Tjallingii, 1985a; Walker, 2000). These waveforms have been associated with particular feeding behaviours such as stylet movements, secretion of different types of saliva, and sap ingestion (Tjallingii, 1978, 1985b, 2006; Tjallingii & Hogen Esch, 1993), and at least seven waveforms have been found for aphids (Prado & Tjallingii, 1994), the most commonly studied group of insects. Waveforms A, B, and C form part of the pathway phase during which the stylets penetrate the plant tissue and the salivary flange and sheath are formed (Tjallingii, 2006). Waveforms E1 and E2 occur during the phloem phase and constitute salivation into plant tissue (E1) and ingestion of phloem sap (E2) (Mentink et al., 1984; Tjallingii, 1985b; Prado & Tjallingii, 1994). Activities during waveform F are thought to be extracellular and due to mechanical stylet activities (Tjallingii, 1988), whereas waveform G occurs during xylem sap ingestion (Tjallingii, 1988).

Recently, Bonani et al. (2008b, 2010) used the EPG technique to investigate the feeding behaviour of *D. citri* and transmission of Las and described five waveforms for adult female psyllids. Waveform C was associated with stylet penetration, salivary sheath secretion, and other stylet pathway activities in epidermis and parenchyma. Waveform D was associated with initial contact with the phloem, E1 with salivation into phloem sieve tubes, E2 with phloem sap ingestion, and G with xylem feeding. Bonani et al. (2008a) found differences in phloem feeding by *D. citri* on citrus leaves of various ages, with 50% of psyllids starting phloem feeding on young leaves within 5 h, whereas on mature leaves only 15% of psyllids fed from phloem within this time period. The authors proposed that these differences in the acquisition of Las may be due to a thicker layer of fibre cells in mature leaves, reducing phloem feeding. Therefore, in this study, we used plants of the same age, but exhibiting different levels of symptom expression to determine the effect of symptomatology on the feeding behaviour of *D. citri*, to see whether this may be a factor affecting the acquisition of Las.

Materials and methods

Psyllids

Adult psyllids were collected from disease-free, ornamental plants of *Murraya exotica* L. (Rutaceae) from the campus of South China Agricultural University (23°09′N, 113° 20′E) and reared for several generations on young plants of *M. exotica* in a greenhouse at South China Agricultural University, Guangzhou. Female adults were used for EPG studies, and all psyllids were put in glass tubes and deprived of food for about 4 h prior to experimentation.

Production of infected plants

Twenty disease-free, 20-month-old plants of *Citrus reticulata* Blanco 'Sunki' [syn. *Citrus sunki* (Hayata) Hort. ex Tanaka] (Rutaceae) were grafted with 3–4 buds from 'Shatangju' mandarin (*C. reticulata*) trees severely infected with Las in August, 2008; this was repeated in each of the two subsequent months. The buds were randomly selected to optimize the chance of infection in each set of seedlings being as similar as possible. An additional 20 plants were left ungrafted and set aside for use as pathogen- and symptom-free controls. Thus, four sets of plants were produced. Before grafting, all plants, including the control plants, were pruned so that each plant was ca. 300 mm tall, and all branches were removed. The plants were kept free of the Asiatic citrus psyllid and other pests. Eight months after the first plants were graft inoculated, the plants of each group were used to assess psyllid feeding using an EPG. At the time of assessment, the plants either showed no symptoms of HLB (control plants) or were deemed to have severe, moderate, or mild symptoms (see Results and discussion).

Real-time PCR

DNA was extracted and purified from ca. 200 mg of plant tissue using an HP Plant DNA kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Each sample was assayed for the presence of DNA from Las by real-time PCR. The real-time PCR reaction components and conditions were as described by Li et al. (2006) using the primers HLBp, HLBas, and HLBr, designed to amplify the 16S rDNA gene of the bacterium.

EPG analysis

A direct current Giga-4 EPG was used to monitor psyllid feeding behaviour using the method of Tjallingii (1978). A 30-mm gold wire (20 μm diameter) was attached to the dorsum of test insects using a water-based silver glue. The wire was connected to the EPG amplifier, which had an input resistance of 10^9 ohm and a gain of 50× (Tjallingii, 1985a, 1988). The other electrode was put in the soil of the potted plant. The test material was located in a Faraday cage to shield it from external, electric noise at an ambient temperature of 25 ± 2 °C. All signals recorded were analysed by using Probe 3.4 software (supplied by W.F. Tjallingii, Wageningen, The Netherlands).

Before assessment, the youngest, fully-expanded leaf at the top of the test plant was fixed with its abaxial surface uppermost onto a non-conducting support. A psyllid with an attached gold wire was hung above the abaxial leaf surface and then lowered onto the midvein, at which point the output from the EPG was recorded. Each leaf was only used for one insect and one leaf was used per plant. Stylet activity was recorded continuously for an 8-h period. An EPG was generated for a psyllid feeding on each of the 20 plants of each of the four sets of plants.

Statistical analysis

Data concerning numbers of events or durations were analysed by one-way analyses of variance (ANOVA) using Statistical Analysis System (SAS Institute, version 8.1, Cary, NC, USA) and Statistica (StatSoft, version 9.1, Tulsa, OK, USA). For these data sets, the homogeneity of the variances was checked using Levene's test before analysis. Where the variances were not homogeneous, they were subjected to log transformations to remove or reduce heteroscedasticity. Percentage data were subjected to arcsine transformations before analysis. Means were separated using Duncan's Multiple Range tests at $\alpha = 0.05$. Differences between the proportions of psyllids feeding on the phloem or xylem from healthy and HLB-infected mandarins were determined using contingency χ^2 tests.

Results and discussion

Real-time PCR and symptoms of plants infected with Las

Real-time PCR revealed that all plants in the three sets that were graft-inoculated contained the bacterium. The first set of graft-inoculated plants were deemed to have severe symptoms (Figure 1). These symptoms included a reduction in leaf size and leaves with a leathery surface, severe interveinal chlorosis, vein corking, and prominent raised veins. The second set graft-inoculated plants were deemed to have moderate symptoms that included a reduction in leaf size, patches of interveinal chlorosis, and raised veins. The third set of graft-inoculated plants had mild symptoms that included a reduction in leaf size and a general reduction in chlorophyll. The mean concentration of Las DNA in the control plants was below that considered to be positive, whereas the mean concentrations in the infected plants increased with increasing symptom severity. However, increasing concentrations of DNA may not relate to increasing numbers of live bacteria and, for future studies on acquisition period, pre-treatment of plant tissues with ethidium monoazide (Trivedi et al., 2009) should be used as this technique allows discrimination between cells that are alive or dead (Rudi et al., 2005; Nocker & Camper, 2006; Wang & Levin, 2006).

Adult feeding patterns on healthy plants

The waveforms produced by the psyllids in this study (Figures 2–4) were similar to those for *D. citri* reported by Bonani et al. (2010) and those of aphids (Tjallingii, 1988). In this study, the pathway phase consisted of four

Figure 1 Leaf symptoms on three sets of 'Sunki' mandarin plants graft inoculated with 3–4 buds from 'Shatangju' mandarin trees severely infected with '*Candidatus* Liberibacter asiaticus' compared to a leaf from a healthy, non-inoculated plant. Numbers are mean (± SE) bacterial DNA concentrations (fg μl^{-1}) from real-time PCR from 15 plants of each symptom group. Means followed by the same letter are not significantly different (Duncan's Multiple Range test: $P>0.05$). Concentrations <7 fg μl^{-1} are not considered as positive.

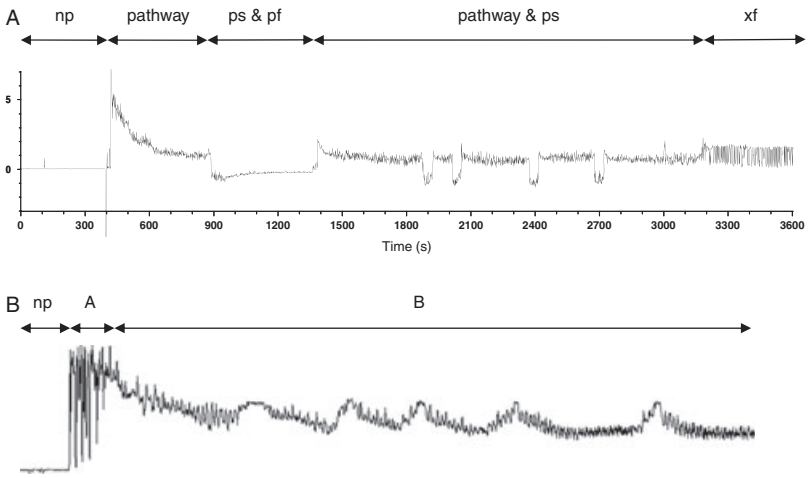

Figure 2 Typical electrical penetration graph (EPG) of an adult Asiatic citrus psyllid feeding on a healthy 'Sunki' mandarin. (A) EPG trace for the first 1 h after the start of assessment (np = non-penetration; ps = salivation within the phloem; pf = phloem ingestion; xf = xylem ingestion). (B) Waveforms expressed for the 30 s after the start of penetration (A = initial stylet penetration and salivation; B = epidermis/mesophyll sheath salivation).

waveforms, A–D. Waveforms A–C are common in aphids but only waveforms C and D were previously reported for *D. citri* by Bonani et al. (2010). In addition to occurring in aphids, waveform A has been found in a diverse range of insects including the cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) (Calatayud et al., 1994), the African corn leafhopper (*Cicadulina mbila* Naudé) (Lett et al., 2001), and the whiteflies *Trialeurodes vaporariorum* (Westwood) (Janssen et al., 1989) and *Bemisia tabaci* (Gennadius) (Jiang et al., 1999). The irregular nature of this waveform occurs as a result of alternating good and bad connections during the establishment of electrical contact between the stylet and plant tissues and, in aphids, occurs shortly after the secretion of a salivary flange (Tjallingii, 1988).

Waveform B consists of slow waves that typically had a frequency of 0.2–0.3 Hz interspersed with waves of smaller amplitude and higher frequency with the interval between the slow waves increasing as the probing progressed (Figure 3A). The frequency of the slow waves is similar to that found by Tjallingii (1988) in aphids, in which the slow waves are associated with the production of gelling saliva and the high frequency waves with stylet movements (Tjallingii, 1988). Waveform B is absent from *P. manihoti* (Calatayud et al., 1994), *T. vaporariorum* (Janssen et al., 1989), and *B. tabaci* (Jiang et al., 1999). The third waveform to occur, C (Figure 3B), has a frequency of 14–19 Hz and is interspersed with a B-like rhythmical component. This was the first waveform observed in *D. citri* by Bonani et al. (2010) and was associated with the production of the salivary sheath and other stylet pathway activities through parenchyma.

Waveform D, as defined and described by Bonani et al. (2010), lasts on average ca. 15 s, which is shorter than that reported by Bonani et al. (2010). The first 6 s consist of

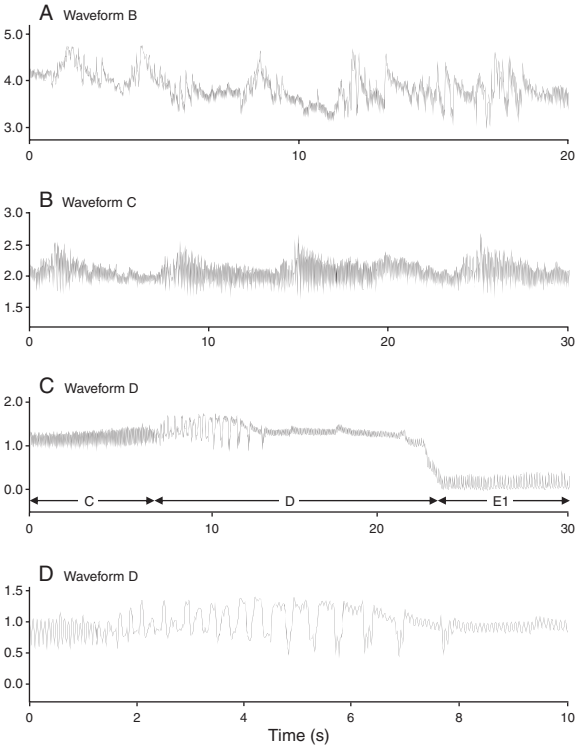

Figure 3 Typical electrical penetration graphs of an adult Asiatic citrus psyllid feeding on a healthy 'Sunki' mandarin. (A) typical waveform B; (B) typical waveform C containing B-like rhythmical components; (C) typical waveform D; (D) enlargement of waveform D.

voltage fluctuations that increase and then decrease in amplitude, with the duration of these fluctuations gradually increasing (Figure 3C and D). After this time, the voltage remains constant before dropping down when E1

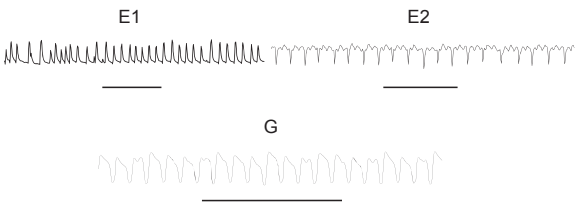

Figure 4 Typical electrical penetration graph of an adult Asiatic citrus psyllid feeding on a healthy 'Sunki' mandarin. Waveforms typical of salivation into the phloem (E1), sustained phloem ingestion (E2), and xylem ingestion (G) (scale bars = 1 s).

occurs. Superimposed upon these fluctuations is a series of smaller voltage drops with a frequency of ca. 13 Hz. All parts of waveform D occur at an extracellular voltage. This waveform has not been found in other insects including the pear psylla, *Cacopsylla pyricola* (Foerster) (Ullman & McLean, 1988), but the inability to detect this waveform in the pear psylla may be due to the use of an AC EPG system. The activities that occur during this waveform are unknown, but Bonani et al. (2010) suggest that they may be associated with initial contact with the phloem, as they occur prior to a potential drop (pd), and in their study, salivary sheath termini were found in the phloem during this waveform.

During the pathway phase in aphids, many pds occur due to intracellular punctures by the stylet tips (Tjallingii & Hogen Esch, 1993). Similar pds occur in the cassava, striped, and mango mealybugs (*P. manihoti*, *Ferrisia virgata* Cockerell, and *Rastrococcus invadens* Williams, respectively) (Calatayud et al., 1994), in the whiteflies *Bemisia argentifolii* Bellows & Perring (synonym of *B. tabaci* biotype B), *Parabemisia myricae* (Kuwana) (Walker & Perring, 1994), and *T. vaporariorum* (Janssen et al., 1989), and in the leafhopper *Cicadulina storeyi* China (Kimmins & Bosque-Perez, 1996). However, the duration of the pds varies amongst these insect types as does their frequency and fine structure, with mealybugs and whiteflies producing fewer punctures. In contrast, pds were not found in our study nor in that of Bonani et al. (2010). This suggests that the psyllid does not make intracellular punctures during the pathway phase, which may have consequences for host plant selection and the acquisition and inoculation of non-persistently transmitted viruses. Johnson & Walker (1999) proposed that aphids use intracellular punctures made early in a probe for host selection, whereas later probes were used for sieve element location. If this is correct, *D. citri* can locate sieve elements without the need to puncture cells on the way to this tissue.

The remaining waveforms, E1, E2, and G (Figure 4), conform to those described by Bonani et al. (2010). In aphids, salivary secretion into the phloem occurs during E1, whereas passive ingestion of sieve element sap that is mixed with saliva occurs during E2 (Tjallingii, 1988; Prado & Tjallingii, 1994). The waveforms produced by *D. citri* during E1 and E2 are similar to those of aphids. Bonani et al. (2010) found salivary sheath termini in the phloem during both phases and bacterial acquisition occurred only when psyllids were able to sustain E2 for at least 1 h. Therefore, the activities that are occurring during E1 and E2 in psyllids are likely to be the same as those in aphids. Waveform G is associated with ingestion from xylem vessels in aphids (Spiller et al., 1990). The similarity of the G waveform of *D. citri* with that of aphids suggests that the same activity is occurring; there is, however, no direct evidence for this. The frequency of the xylem waveform is due to the contraction and relaxation of pump muscles (Dixon, 1998; Harrewijn et al., 1998; Douglas, 2003), and xylem ingestion may help insects to resist dehydration (Spiller et al., 1990; Powell & Hardie, 2002; Pompon et al., 2010); however, it will not sustain them. In this study, waveform G was observed from only three individuals of the 20 tested female psyllids on the uninfected control plants, whereas most psyllids on infected plants exhibited this behaviour.

Feeding patterns of psyllids on huanglongbing-infected plants

The average (n = 20) durations of periods of non-penetration, the pathway phase, and phloem and xylem ingestion by adult, female *D. citri* on healthy or infected mandarins are shown in Figure 5. The duration of non-penetration did not differ significantly between healthy or infected plants, irrespective of symptom severity. However, in moderately and severely symptomatic plants, the duration of the pathway phase increased, especially in the severely symptomatic plants where the duration of this phase was ca. 4× that in the healthy control plants. In contrast, the duration of the phloem phase was significantly shorter in infected plants. This was mainly due to a significant reduction in sustained phloem ingestion. In contrast to phloem feeding, the duration of xylem feeding increased markedly and significantly in the HLB-infected plants, with the duration of feeding increasing in relation to symptom severity.

The time to first salivation and sustained salivation in the phloem (E1 >2 min) are shown in Figure 6A. In all infected plants, these times were significantly longer than in the controls, with the times being related to the level of symptom severity. Infection also increased the number of single salivation events in the phloem (Figure 6B). The number of salivation plus phloem-feeding events and the number of sustained periods of phloem ingestion were only reduced significantly in the severely symptomatic

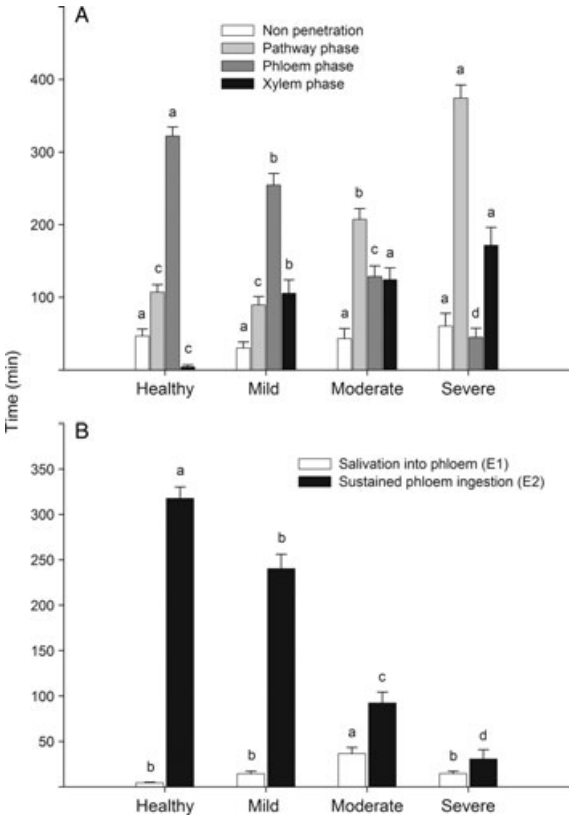

Figure 5 Mean (+ SE; n = 20) durations of (A) periods of non-penetration, the pathway and phloem phases, and xylem feeding, and (B) salivation and sustained (>2 min) phloem ingestion of adult, female *Diaphorina citri* on healthy 'Sunki' mandarins or mandarins infected with huanglongbing showing a range of symptom severity. Within each behaviour type, means capped by the same letter are not significantly different (Duncan's Multiple Range test: P>0.05).

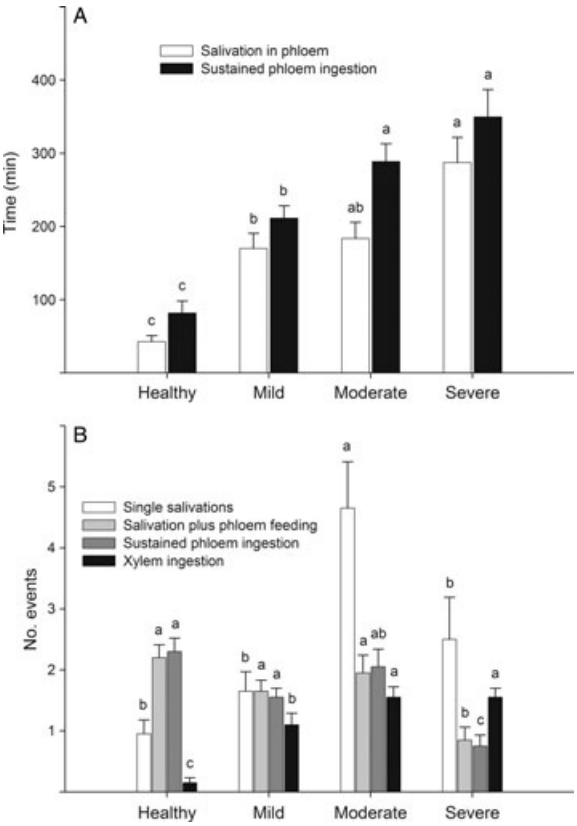

Figure 6 (A) Mean (+ SE; n = 20) time from the commencement of EPG recording to first salivation in the phloem and to first sustained phloem ingestion and (B) the mean (+ SE) number of single salivations (i.e., E1 not followed by E2), salivation followed by phloem feeding, sustained (>2 min) phloem ingestion, and periods of xylem feeding by adult, female *Diaphorina citri* on healthy 'Sunki' mandarins or mandarins infected with huanglongbing and showing a range of symptoms. Within each behaviour type, means capped by the same letter are not significantly different (Duncan's Multiple Range test: P>0.05).

plants (Figure 6B). Infection increased the number of times that the psyllids fed from the xylem of infected plants compared to healthy control plants.

As the plants became more diseased, the percentage of time the psyllids spent salivating in the phloem phase (E1) increased significantly. However, the percentage of time spent in all phloem activities (salivation plus ingestion) (Figure 7) reduced gradually from ca. 74% in the control plants to ca. 8% in the severely symptomatic plants. In contrast, the percentage of time spent in xylem activities increased significantly from 0.8% on healthy plants to 22–41% on symptomatic plants. In addition to infection affecting the durations and percentage of time spent feeding from the xylem and phloem, infection also increased the proportion of psyllids feeding from the xylem (χ^2 = 52.8, d.f. = 3, P<0.0001) (Table 1), whereas the proportion of psyllids feeding from the phloem was reduced (χ^2 = 26.7, d.f. = 3, P<0.0001) on the severely symptomatic plants only.

The probing process can be divided into two phases, with the events prior to first phloem contact (pathway phase) being associated with host plant recognition, whereas those events that occur after phloem contact being associated with phloem acceptance and ingestion (Lei et al., 1999). Several studies have shown that the pathway phase is prolonged on plants that are poor hosts. Calatayud et al. (1994), working with the cassava mealybug, found less pathway activity on host plants compared with non-hosts. Givovich & Niemeyer (1995), working with two aphids, *Rhopalosiphum maidis* Fitch and *Rhopalosiphum padi* (L.), on wheat plants, and Paul et al. (1996),

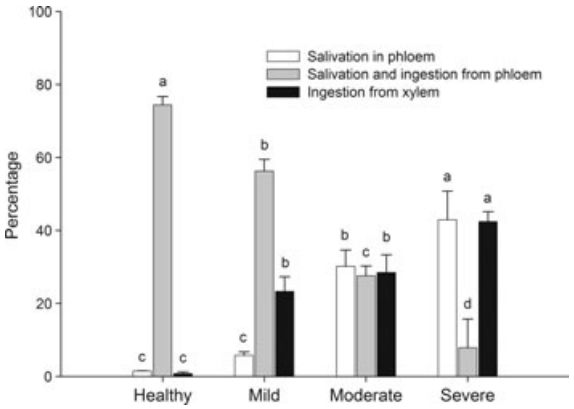

Figure 7 Mean (+ SE; n = 20) percentage of the total probe time in the phloem spent salivating in phloem, salivating and ingesting from phloem, and feeding from the xylem by adult female *Diaphorina citri* on healthy 'Sunki' mandarins and mandarins infected with huanglongbing and showing a range of symptoms. Within each behaviour type, means capped by the same letter are not significantly different (Duncan's Multiple Range Test: P>0.05).

Table 1 Number (out of 20) of female *Diaphorina citri* feeding from phloem or xylem of healthy 'Sunki' mandarins or huanglongbing-infected mandarins showing a range of symptoms

	Healthy	Mild	Moderate	Severe
Phloem ingestion	20	20	20	12
Xylem ingestion	3	17	20	20

working with the hop aphid, *Phorodon humuli* (Schrank), on hops, found that the pathway phase was longer on resistant plants. Ponder et al. (2000), working with the aphid *R. padi*, found that insects on nitrogen-deficient plants took longer to reach the phloem and the authors suggested that these insects may be experiencing problems locating the phloem. In this study, both the duration of the pathway phase and the time to E1 were longer on diseased plants, and this too may not only be due to *D. citri* having problems locating phloem vessels but may also be due to difficulties in physically penetrating the tissues of infected plants. Plants infected with Las may have a sclerenchyma layer with denser lignin deposits (Aubert, 1988) and Folimonova & Achor (2010) have also shown changes to the intercellular substances between sieve elements that cause a swelling of the middle lamella.

In this study, the duration of E1 was extended on Las-infected plants, whereas the duration of E2 was reduced. A number of studies have shown increased durations of E1 by aphids on resistant plants (van Helden & Tjallingii, 1993; Caillaud et al., 1995; Ramírez & Niemeyer, 1999; Garzo et al., 2002; Tjallingii, 2006), and Ponder et al. (2000) found that E1 of *R. padi* was longer on nitrogen-deficient plants. The prolongation of E1 suggests that some factor is delaying acceptance of the phloem or is preventing sap ingestion (Ponder et al., 2000; Tjallingii, 2006). In addition, Tjallingii & Mayoral (1992) have suggested that saliva may induce changes in plant metabolism that makes the phloem sap more acceptable to the insect, and Garzo et al. (2002) suggested that prolonged E1 and reduced E2 may indicate a reduced ability to suppress phloem wound responses. In Las-infected plants, the extended E1 and reduced E2 may be due to the phloem being a less suitable food source or the psyllid being less able to overcome phloem wound responses. As infection with Las progresses, the cambium produces more phloem cells that eventually become distorted, crushed, and necrotic (Tirtawidjaja, 1972, 1980; Su & Huang, 1990; Etxeberria et al., 2009; Kim et al., 2009); thus, affected tissue may be an unsuitable feeding source. In addition, *pp2* is up-regulated in infected plants and callose deposition is increased (Kim et al., 2009), which may further deter feeding. Although psyllids prefer feeding from the phloem, because of the barrier to sap uptake from this tissue, they exhibit a higher incidence and greater duration of xylem ingestion as a strategy for maintaining water balance (Spiller, 1990; Pompon et al., 2010).

In conclusion, this study has confirmed the production of the waveforms shown by Bonani et al. (2010) and, in addition, has also shown the presence of waveforms A and B during the feeding of *D. citri*. The differences in the duration of the E waveforms on plants showing different levels of symptom expression may explain the differences in acquisition of Las by *D. citri* found amongst studies, and it will be important for future studies on acquisition and transmission of this pathogen to carefully document the level of symptom expression.

Acknowledgements

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