

# Influences of leaf age and type, non-host volatiles, and mineral oil deposits on the incidence, distribution, and form of stylet tracks of *Diaphorina citri*

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## Abstract

Psyllids produce saliva that gels to form a protective sheath around their stylets. This saliva can be visualized as stylet tracks, and we have used the presence of tracks to study the feeding behaviour of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). Both single and branched tracks were produced by nymphs and adults, and the tracks made by males and females did not differ. Tracks dissipated from calamondin leaves within a 7-day post-feeding period after adults fed for 2 days and numbers of tracks declined with increasing maturity of calamondin leaves. In the six host plants studied, most psyllids fed abaxially from midribs and most probes traversed or terminated in the tissue (midrib, lateral vein, minor vein, or mesophyll) above which the stylets entered the leaf or leaflet. The number of tracks and landings were recorded on the six hosts in choice tests. More tracks were found in leaves or leaflets of orange jasmine, wampee, and trifoliolate orange than in sour orange, calamondin, and lemon. Orange jasmine is considered a preferred host of *D. citri*, and this is in agreement with the number of tracks found in this study, but not the number of landings. Trifoliolate orange is considered a poor host of *D. citri*, which, in this study, is reflected in the low number of landings, but not in the numbers of tracks. Our results indicate that the presence of adult psyllids on a plant may not reflect its host status, and that the presence of stylet tracks should also be determined in host preference studies. When calamondin leaves were paired with leaves of guava, billygoat weed, or greenleaf desmodium, the presence of volatiles from these plants reduced feeding by adults on calamondin and suggests that understoreys of billygoat weed or desmodium in orchards may also reduce feeding. A negative, exponential relationship between numbers of tracks per leaf and the concentrations of an agricultural mineral oil applied to leaf surfaces was found. This reduction in feeding, in conjunction with reductions in oviposition, has practical implications for suppressing psyllid populations in orchards.

## Introduction

The Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psylloidea: Liviidae) is one of two known vectors of huanglongbing (HLB), the most serious disease of citrus. *Diaphorina citri* originated in southern Asia (Hollis, 1987; Halbert & Manjunath, 2004;

Beattie & Barkley, 2009) and has spread naturally in association with human activities. It now occurs in most tropical, subtropical, and some temperate regions of Asia (extending from Arabia to Japan, the Philippines through the Indonesian Archipelago to New Guinea), in some Pacific and Indian Ocean islands, and in the Americas, including Brazil since about 1940 and the USA since 1998 (Halbert & Manjunath, 2004; Bové, 2006; Aubert, 2009; Gottwald et al., 2012).

*Diaphorina citri* was suspected (Tirtawidjaja et al., 1965) and subsequently recognized (Capoor et al., 1967)

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as a vector of huanglongbing in Asia in the 1960s, and Xu et al. (1988) observed the pathogen in haemolymph and salivary glands of the psyllid. After acquisition from phloem of an infected plant, '*Candidatus Liberibacter asiaticus*' (CLAs) [ $\alpha$ -Proteobacteria], the putative causal agent of the disease in Asia (Bové, 2006), multiplies first in the midgut epithelial cells of the psyllid, then penetrates through the basal lamina and enters into the haemocoel, where it multiplies again, before invading other host tissues, including haemocytes, nerve tissue, fat bodies, trachea, muscles, and eventually the salivary glands (Jiang, 2005). Capoor et al. (1974) and Xu et al. (1988) reported that CLAs can only be acquired and transmitted by fourth and fifth instars and adults of *D. citri*. However, Hung et al. (2004), using PCR, detected CLAs in second, third, fourth, and fifth instars.

Proportions of initially pathogen-free, symptomatic trees infected with CLAs in Asia can reach 100% within 2–3 years of establishment of orchards even under intensive insecticide applications in which psyllid populations are barely noticeable (pers. obs. of the authors; Gatineau et al., 2010). The disease can lead to negligible harvests in the first year that trees bear and can destroy orchards within 5–10 years. Depending on the region and source of planting stock, significant proportions of plants may be infected before planting by psyllid transmission in unprotected nurseries or through poor propagation practices, such as the use of marcotts and budwood from infected trees (pers. obs. of the authors; Zhao, 1981; Roistacher, 1996; Bové, 2006; Yang et al., 2006). Recent studies indicate that seasonal variation in levels of CLAs infection in *D. citri* populations are in part due to pathogen titres in plants (Hung, 2006, 2008) and influences of disease severity and host leaf age on adult feeding behaviour (Bonani et al., 2008, 2010). Levels of infection of adult psyllids in orchards with high percentages of infected trees have been reported to be as low as 1% and as high as 100% (Bové et al., 1993; Li et al., 2009). Reported acquisition rates in laboratory and screen house experiments range from low to high, and nymphs acquire the pathogen more effectively than adults (Capoor et al., 1974; Huang et al., 1984; Xu et al., 1988; Hung, 2006, 2008).

When psyllids feed, they insert their mouthparts, known as stylets, into plant tissues. They produce a proteinaceous salivary substance that gels to form a protective sheath around the stylets as they move within plant tissues (Miles, 1972). After the stylets are withdrawn, a tubular sheath or track remains (Miles, 1972; Bowling, 1980; Bing et al., 1991; Brennan et al., 2001). Some tracks, formed when stylets penetrate surfaces but no further, are called flanges (Nault & Gyrisco, 1966; Miles, 1972). These sheaths and flanges can be observed using a microscope by staining

them within unsectioned tissues from which chlorophyll has been removed (Bennett, 1934; Backus et al., 1988; Cohen et al., 1996). These procedures have been used to characterize stylet tracks (Backus et al., 1988; Bing et al., 1991; Ni & Quisenberry, 1997; Brennan & Weinbaum, 2001; Brentassi & Maldonado, 2002) and determine the host preferences and feeding behaviours of a range of sternorrhynchans (Bowling, 1980; Xu et al., 1994; Cohen et al., 1996; Brennan & Weinbaum, 2001).

A common assumption is that the psyllids feed solely on phloem, part of the vascular system of higher plants in which photosynthates, particularly sucrose, are transported. Nevertheless, according to Gullan & Martin (2003), they may ingest sap from a variety of tissue sources, including phloem, xylem, and mesophyll parenchyma and, thus, are not phloem specialists like most other sternorrhynchans. However, recent EPG studies (Bonani et al., 2010; Cen et al., 2012) have shown that *D. citri* feeds predominantly from the phloem and less frequently from the xylem. Moreover, Bonani et al. (2010) showed that *D. citri* does not feed on mesophyll parenchyma. In this study, we examined stained, cleared leaves to determine the impacts of leaf age and type on the distribution and form of stylet tracks in leaves of orange jasmine (*Murraya exotica* L.), calamondin (*Citrus × microcarpa* Bunge), sour orange (*C. × aurantium* L.), trifoliolate orange (*C. trifoliata* L.), lemon [*C. × limon* (L.) Osbeck], and wampee [huangpi: *Clausena lansium* (Lour.) Skeels] (all Rutaceae). Impacts of mineral oil deposits on oviposition by *D. citri* (Rae et al., 1997) led us to determine the effects of deposits on adult psyllid feeding behaviour based on the incidence of stylet tracks in oil-sprayed calamondin leaves. Reported repellent effects of guava [*Psidium guajava* L. (Myrtaceae)] leaf volatiles on *D. citri* adults (Cen et al., 2008; Hall et al., 2008; Zaka et al., 2010) led us to assess the possibility of leaf volatiles of billygoat weed [*Ageratum conyzoides* L. (Asteraceae)] and greenleaf desmodium [*Desmodium intortum* (Mill.) Urb. (Fabaceae)] influencing the behaviour of psyllid adults. In this instance, we determined the incidence of adult psyllid stylet tracks in calamondin leaves paired with leaves of test plants. The results of the studies are discussed in relation to acquisition and transmission of CLAs by *D. citri*.

## Materials and methods

### Insects

Adult *D. citri* were collected from orange jasmine plants growing on the campus of Zhongshan (Sun Yat-sen) University, Guangzhou, China (23°06'N, 113°17'E) and cultured on potted calamondin and orange jasmine plants in a mesh house.

### Plants and leaves

All host plants used in the study were maintained in separate pots and isolated from *D. citri* and other sternorrhynchans. They comprised orange jasmine, sour orange, trifoliolate orange, lemon, and wampee trees grown from seeds, and calamondin trees on lemon rootstocks. The orange jasmine plants were 2–3 years old, the calamondin trees 4–5 years old, and the other plants 3–4 years old. Calamondin leaves used in the study were categorized as: CI – young, newly-unfurled, soft, yellow-green leaves <30 mm long (leaf tip to leaf base, excluding the petiole); CII – mature, soft, light-green leaves >30 mm long; CIII – mature, firm, deep-green leaves >300 mm long; and CIV – mature, hard, dark green leaves longer than 30 mm and more than 3 months old. All leaves or leaflets from other host plants were mature, fully expanded, and less than 3 months old. Water and fertilizers were applied regularly to the plants and no pesticides were applied.

### Staining and clearing

Staining and clearing methods were based on Backus et al. (1988) and Cohen et al. (1996). Shoots, leaflets, and leaves on which *D. citri* nymphs and adults had fed were stained for 20–24 h at room temperature in 0.2% acid fuchsin in a 1:1 (vol/vol) mixture of 95% ethanol and glacial acetic acid, a stain developed by McBryde (1936) for examining rust hyphae and haustoria in unsectioned leaf tissue. The tissues were then cleared to remove chlorophyll by placing them in 95% ethanol for 15–30 min and then heating them in a 1:1:1 mixture by volume of 85% lactic acid, 99% glycerol, and water (LGW). Orange jasmine and calamondin shoots and CI calamondin leaves were placed in LGW liquid at room temperature then simmered in the liquid for a few minutes until they became soft and limp. CII and CIII calamondin leaves were boiled in LGW liquid for 8–10 min and 15–20 min, respectively. CIV calamondin leaves and mature leaves of other hosts were autoclaved in LGW liquid at 126 °C at 0.14 MPa for 8–10 min. Cleared leaves were preserved in fresh LGW liquid, which is suitable for storing such specimens for months. All specimens were observed at 15–125× magnification using a Leica M8 stereomicroscope.

### Experiments

*Experiment 1: incidence and distribution of nymphal stylets and stylet tracks in unfurling calamondin and orange jasmine shoots.* Points of penetration, incidence, and distribution of stylets of *D. citri* nymphs in unfurling calamondin and orange jasmine shoots were determined by randomly picking, staining, and clearing nymph-infested shoots from mesh house plants used for mass

rearing the psyllid, and examining stylets and stylet tracks of nymphs (all instars) still attached to the shoots.

*Experiment 2: adult tracks in calamondin leaves.* Over an interval of about 2 months, pairs of sternorrhynchan-free CI, CII, and CIII calamondin leaves were picked separately from 14 plants. Pairs of CIV leaves were picked from 10 plants. Leaves comprising each pair were placed separately and upright in a small, water-filled 70-ml glass bottle. Each petiole was firmly surrounded by moist cotton wool. Each leaf was then enveloped with a mesh-covered, clear glass cylinder (60 mm diameter × 140 mm high) into which 5–6 psyllids of the same sex were released. Therefore, 14 CI, CII, and CIII leaves and 10 CIV leaves were assessed for each sex. All psyllids were starved for 24 h before release into the cylinders. They were allowed to feed for 24 h at room temperature before they were removed and the leaves stained and cleared. The nature and number of stylet tracks in each leaf were then recorded.

*Experiment 3: persistence of adult stylet tracks in calamondin leaves.* The persistence of stylets tracks in young shoots with 3–4 CI leaves and in CII, CIII, and CIV leaves was compared on nine occasions (= replicates) using initially sternorrhynchan-free plants over an interval of 4 weeks. On each occasion, pairs of leaves or shoots were chosen on each plant. Each leaf or shoot was covered with a 55-mm-diameter, 100-mm-long, cylindrical mesh cage. Five adults of mixed sex were released into each cage that enclosed a leaf; eight were released into each cage that enclosed a shoot. The cages and adults were removed after 2 days, and one shoot or leaf in each set of paired shoots or leaves was chosen at random, stained, and cleared. The second shoot or leaf in each pair was picked from the plant, stained, and cleared 7 days after the cages and adults were removed. The number of stylet tracks in each shoot or leaf was counted.

*Experiment 4: adult host preferences in no-choice tests.* The nature and incidence of stylet tracks in mature orange jasmine leaflets and mature sour orange, lemon, trifoliolate orange, and wampee leaves were compared. For each host, pairs of leaflets or leaves were picked from eight plants. Tests were conducted as in Experiment 2, with the exception of the test with wampee, in which eight adult males or females were released into cylinders enclosing individual leaves. The experiment was conducted over an interval of about 2 months.

*Experiment 5: adult host preferences in choice tests.* Choice tests were conducted on five occasions (= replicates) to

determine the nature and occurrence of stylet tracks from male and female psyllids in mature orange jasmine leaflets, CII and III calamondin leaves, and mature sour orange, lemon, trifoliolate orange, and wampee leaves under light and dark conditions. On each occasion, four leaflets/leaves of similar size were picked from each of the six host plants, and maintained separately, as above, in small, water-filled glass bottles. Bottles within each of the four sets of six were then arranged equidistantly around the inner rim of a 155-mm glass Petri dish, with the adaxial surfaces of the leaflets/leaves facing inwards. Each of the four Petri dishes was then placed separately in the centre of one of four 225 × 225 × 225-mm wooden boxes, each with a clear glass top and mesh on the back. Fifty males were released into each of two boxes and 50 females into each of the other two boxes; the psyllids were starved for 24 h before they were released. One box into which males were released and one into which females were released were then covered to exclude light. Each of the other two boxes was illuminated with a 60-W fluorescent lamp and natural room light throughout the experiment. All boxes were kept at room temperature. Numbers of psyllids on each leaflet/leaf were recorded 1, 3, 5, 20, and 24 h after they were released into the boxes. The leaflets/leaves were then removed, stained, and cleared and the number of stylet tracks in each counted. The arrangement of bottles within each of the four treatments (boxes) within a replicate was identical, but differed randomly for each of the five occasions on which the experiment was conducted. The experiment was completed over an interval of 10 days.

*Experiment 6: influence of non-rutaceous plant volatiles on adult feeding.* The impact of volatiles emanating from mature guava, billy goat weed, greenleaf desmodium, and CIII calamondin leaves on feeding by adult psyllids on CII calamondin leaves was determined. In these tests, leaves of each of these plants, all in pots, were paired in situ, but separately (see below), with CII calamondin leaves in 42-mm-diameter × 150-mm-long plastic cylinders wrapped in two layers of black plastic to exclude light. For each test, the petiole of a non-host leaf or a CIII calamondin leaf was inserted into foam at one end of one of the plastic cylinders. The opposite end of the cylinder was then sealed with foam. This foam was removed after 24 h and a CII calamondin leaf was inserted, in each instance, in a similar manner to the leaf inserted in the opposite end of the cylinder. Eight adult psyllids of mixed sex were released into each plastic cylinder. The psyllids were allowed to feed on the CII leaves for 24 h. The CII leaves were then removed, stained, and cleared, and the number of stylet tracks in each counted. The experiment was repeated nine times over an interval of 2 months.

*Experiment 7: influence of mineral oil deposits on adult feeding.* Potted calamondin trees were used to determine the impact of deposits of six concentrations (= treatments) of a mineral oil on the incidence of adult stylet tracks after psyllids were allowed to feed on sprayed leaves. The oil used was SK EnSpray 99 (SK Corporation, Seoul, Korea), an *n*C24 agricultural mineral oil. Its unsulphonated residue value was  $\geq 99.8\%$ , and it contained  $\leq 0.9\%$  (vol/vol) of non-ionic emulsifiers. The glossary in Beattie et al. (2002) defines these terms. The six concentrations were water (0% oil) and 0.125, 0.25, 0.5, 1.0, and 2% aqueous emulsions of the oil. On each of five occasions (= replicates), six trees of similar size were selected, one for each treatment. On each of these five occasions, three CII leaves of similar size on each plant were tagged and then sprayed to run-off. This was achieved by spraying each adaxial and abaxial leaf surface five times with a 500-ml hand-held sprayer (Guangzhou Panyu Zhenxin Plastic, Guangzhou, China); the oil emulsions were thoroughly agitated before spraying, and spray deposits were allowed to dry naturally. Each leaf was then covered with a 42-mm-diameter, 75-mm long clear plastic cage with a white, 100-mesh netting sleeve on the proximal end and a foam plug around the petioles on the distal end. Eight adults of mixed sex were released into one cage on each plant 24 h after application of the sprays and into the other cages 48 and 72 h after application of the sprays. The psyllids were allowed to feed on the leaves for 24 h. The leaves were then removed, stained, and cleared and the number of stylet tracks in each was counted. The duration of the experiment spanned 6 weeks.

#### Statistical analysis

Data pertaining to the number of stylets within tissue types were assumed to follow a multinomial distribution. The data were then fitted with the explanatory variable, plant type, and were analysed based on an assumption of binomial errors underlying the distribution of stylets between the response factor (tissue types), and then fitted with a generalized linear model (McCullagh & Nelder, 1989). The model parameters included effects of host and tissue type and their interactions. Via a logit (log-odd) link function, a residual maximum likelihood (REML) technique was used to estimate these parameters. 95% confidence intervals were then calculated, and the significant differences between plant types were determined using their confidence intervals. Data pertaining to the percentage of probes in the different tissue types from the abaxial or adaxial surfaces of calamondin and orange jasmine were analysed based on an assumption of Poisson errors underlying the distribution of stylets between the response factor (tissue type). The model parameters for these analyses

included the effects of host, surface, instar, and tissue type and their interactions. Via logarithmic link functions, REML was used to estimate these parameters. Prior to analysis, data from leaf age categories CIII and CIV were pooled due to low frequencies within data cells. Similarly data from the fourth and fifth instars in case of the percentage of probes were also pooled.

Data relating to adult host preferences in choice tests, the influence of non-rutaceous plant volatiles on adult feeding, and the influence of mineral oil deposits on adult feeding were subjected to analyses of variance using STATISTICA, version 9.1, (StatSoft, Tulsa, OK, USA). Before analysis, the homogeneity of the variances within all data sets was checked using Levene's tests. Where the variances were not homogeneous, data were subjected to log transformations to remove or reduce heteroscedasticity. Means were separated using Fisher's LSD tests at  $P = 0.05$ . The relationship between oil concentration and numbers of stylet tracks per leaf was determined using TableCurve 2D, version 5 (Systat Software, San Jose, CA, USA).

## Results and discussion

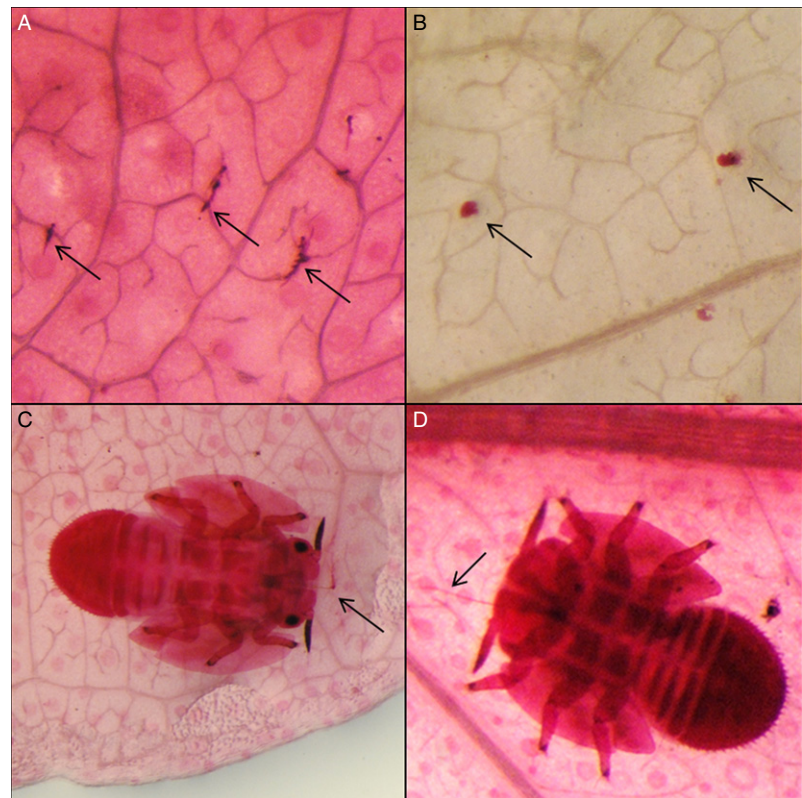
### General appearance of stylets and stylet tracks in host tissues

Stained nymphal stylets and stained tracks of nymphs and adult *D. citri* were clearly visible in cleared tissues when

viewed with transmitted light under a stereomicroscope (Figure 1). This simple staining technique permitted host preferences, preferences for probing and feeding, and impacts of leaf age, non-host volatiles, and mineral oil deposits on feeding behaviour to be easily assessed using unsectioned shoots and leaves. Flanges, the short, stubby lengths of solidified sheaths that mark the commencement of tracks, occurred on both the abaxial and adaxial surfaces of all host leaves. They were more common on mature leaves than on younger leaves, and on CIII and CIV leaves were easily dislodged during staining and clearing. Branched tracks were commonly observed and, as in other hemipterans (Bennett, 1934; Miles, 1972), may have stemmed from multiple backward and forward probes to optimize feeding on phloem.

### Nymphs

Nymphs probed buds and the adaxial and abaxial surfaces of furled and unfurled leaves of calamondin and orange jasmine. Stylet tracks of first and second instars in shoots of these plants were thinner and less evident than those of older third–fifth instars; tracks of fifth instars were comparatively thinner than those of adults (Figure 1). Stylets of all instars were observed in leaf mesophyll and all forms of vascular bundles within all shoot tissues (young stem tissues, petioles, leaf midrib, and lateral and minor veins)



**Figure 1** Stylet tracks produced by adults and nymphs of *Diaphorina citri* in host leaves. (A) tracks produced by male psyllids on a CI calamondin leaf; (B) flanges produced by adult psyllids on a CI calamondin leaf; (C) multiply branched track produced by a fifth instar feeding on calamondin; and (D) parasitised fourth instar feeding on orange jasmine. Stylet tracks or flanges are indicated by arrows.

and sometimes passed through veins, irrespective of vein shape or size. Some that passed through vascular bundles terminated in mesophyll. It was not uncommon for stylets that initially passed through mesophyll to terminate in vascular bundles of midribs and lateral veins after passing through minor veins.

#### Adults

Points where adult stylets pierced leaflet or leaf surfaces varied, and tracks terminating in either veins or mesophyll were common. Some tracks were perpendicular to leaf surfaces, and some tracks branched several times. Branching usually occurred in the mid region of a track, but some branches, usually short and fine, formed near the ends of tracks. Branching of short tracks was less common than branching of long tracks. The branches sometimes took similar directions, but most varied in length and direction. The branched tracks produced by adults often occurred only in mesophyll tissues, especially in younger leaves.

Tracks starting above midribs were generally unbranched or bifurcated, and unbranched tracks often changed direction after entering midribs. Bifurcated tracks resembled an inverted 'Y', with the two branches generally diverging within midrib vascular bundles, and with one or both branches sometimes terminating in lateral or minor veins, or in mesophyll after passing through midribs. Tracks starting above lateral veins were more variable in form than those starting above midribs, and many tracks commenced on the edge of veins. Most tracks starting above lateral veins were curved, occasionally with hook-like ends and some ran parallel to veins. Others, many branched, passed through veins and terminated in other veins or mesophyll. Tracks with bifurcated branches were more common than tracks with three or more branches. Multiple branches were often observed to cross vascular bundles in similar or different directions, with one or more terminating in nearby veins, or in mesophyll after bypassing such veins.

Tracks starting above minor veins and mesophyll were more diverse than those associated with mid and lateral veins, and most were ramose. Unbranched tracks or branches of branched tracks were generally slender and longer than tracks commencing above mid and lateral veins. They tended to traverse one or more minor, lateral or mid veins before terminating in a vein or in mesophyll. Sometimes, they ran parallel to a vein before terminating in another vein or in mesophyll. Multiple branches were common and associated with most of the above situations. Some branches ran in similar directions, others in opposite directions. Sometimes, branches crossed or terminated at the junction of two or more veins, either minor or major. In other instances, they

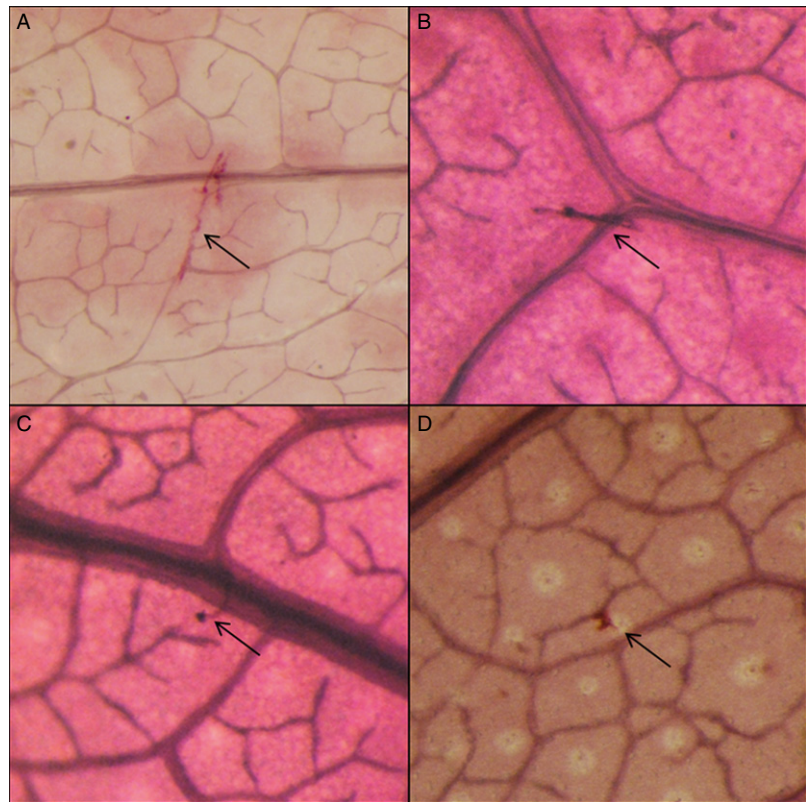
terminated in veins and mesophyll, sometimes after traversing a vein.

In general, adult tracks in CI–CIV calamondin leaves were of three types. Firstly, branched tracks that generally had more than two relatively long branches; these tracks were more common in young leaves (e.g., CI and CII leaves) than in mature leaves (e.g., CIII and CIV leaves). Secondly, short, stubby, unbranched tracks that were more common in CIII and CIV leaves than in CI and CII leaves and, finally, intermediate forms that were unbranched and not stubby. The appearance of tracks made by males and females did not differ. There were no apparent differences in track type in mature leaves of each host nor did tracks that entered adaxially and abaxially differ. However, the nature of tracks was influenced by the age of the calamondin leaves used in this study. They were readily visible, diverse, slender, relatively longer and widely distributed over the leaf surface in CI and CII leaves, but as leaves grew older the tracks became less noticeable, less diverse, relatively shorter, and narrowly distributed (Figure 2).

#### Incidence and distribution of nymphal stylets and stylet tracks in young, unfurling calamondin and orange jasmine shoots

More nymphs were found on orange jasmine (1 816) than on calamondin (436), presumably due to differing numbers of eggs being laid on the two species. No significant differences occurred between the two hosts for the proportions of stylets that pierced midribs, lateral veins, minor veins, mesophyll in leaves/leaflets, and petioles/petiolules and stems ( $P>0.05$ ) (Table 1); however, a greater proportion of tracks was found on young vascular bundle tissues in stems, rachises, and buds of orange jasmine than on calamondin ( $P<0.05$ ). The percentage of stylet tracks observed in non-leaf lamina tissues (stems, buds, petioles, petiolules, and rachises) of calamondin shoots was 53.2%, in contrast to 63.2% for orange jasmine. The high proportion of tracks in the non-leaf lamina tissues of the orange jasmine was probably due to the softer nature of these tissues compared to the other host. Within leaves/leaflets, the percentage of probes in different tissue types from both abaxial or adaxial surfaces of both hosts were generally similar with combined mean percentages of tracks being 53.3, 13.4, 13.1, and 20.2%, respectively, from midribs, lateral veins, minor veins, and mesophyll. However, residual maximum likelihood (REML) analysis indicated evidence of association between penetration sites and hosts, with probing of orange jasmine being more associated with minor veins whereas probing of calamondin was more associated with mesophyll ( $P<0.05$ ).

Proportions of stylet tracks of all instars piercing the abaxial or the adaxial surfaces of unfurled calamondin and orange jasmine shoots differed significantly between the



**Figure 2** Stylet tracks produced by adult psyllids in calamondin leaves of different ages. (A) branched track produced by a female psyllid into the lateral vein of a CI leaf; (B) multiply branched track produced by a male psyllid on a CII leaf; (C) track produced by a female psyllid into the lateral vein of a CIII leaf; and (D) stubby and branched track produced by a female psyllid into a CIV leaf. Stylet tracks are indicated by arrows.

**Table 1** Percentage of *Diaphorina citri* nymphal instars probing and/or feeding on young, unfurling calamondin leaves and young, unfurling orange jasmine leaflets from the adaxial and abaxial surfaces (n = number of stylet tracks). The P-values were calculated from residual maximum likelihood analysis

Tissue type	Calamondin	Orange jasmine	P
Midribs in leaves/ leaflets	24.3 (n = 106)	18.7 (n = 340)	>0.05
Lateral veins in leaves/leaflets	6.7 (n = 29)	4.6 (n = 83)	>0.05
Minor veins in leaves/leaflets	3.4 (n = 15)	5.2 (n = 95)	>0.05
Mesophyll in leaves/ leaflets	12.4 (n = 54)	8.3 (n = 151)	>0.05
Young vascular bundle tissues in stems, rachises, and buds	23.4 (n = 102)	30.1 (n = 547)	<0.05
Petioles/petiolules and stems	29.8 (n = 130)	33.0 (n = 600)	>0.05

two hosts (Table 2), and REML analysis showed a highly significant ( $P < 0.001$ ) association between leaf/leaflet surface and host. On calamondin, a greater number of stylet

tracks pierced the adaxial leaf surface (abaxial:adaxial ratio = 0.69:1) whereas on orange jasmine the reverse was true (abaxial:adaxial ratio = 4.26:1). In addition, on calamondin, first and second instars preferred probing and feeding on adaxial surfaces of leaves; <41% fed on abaxial surfaces. In contrast, >75% of these instars preferred to probe and feed on the abaxial surfaces of orange jasmine leaflets. Older instars showed an increasing preference for probing and feeding on the abaxial surfaces of leaves and leaflets of both hosts. These results suggest, firstly, that nymphs have marked feeding preferences in terms of the leaf surface from which they prefer to feed, secondly, that this preference appears to change as the nymphs pass through their nymphal stages and, thirdly, that their preference is different on different host plants.

#### Adult tracks in calamondin leaves of different maturity

A total of 3 756 adult tracks (single tracks and branches of branched tracks) were observed with approximately equal numbers (♀♀ 1 880, ♂♂ 1 876) being produced by both sexes. Numbers of tracks of all types observed declined markedly with increasing leaf maturity (68.3 per leaf in 28 CI leaves, 25.4 per leaf in 28 CII leaves, 30.6 per leaf in 28 CIII leaves, and 13.7 per leaf in 20 CIV leaves). Numbers of entire, branched tracks also declined from 20.2 per leaf in

**Table 2** Percentage of *Diaphorina citri* nymphal instars probing and/or feeding from the abaxial surfaces of young, unfurling calamondin leaves and young, unfurling orange jasmine leaflets (n = number of stylet tracks). The P-values were calculated from residual maximum likelihood analysis

Host	Instar				
	1	2	3	4 & 5	All instars
Calamondin	33.3 (n = 33)	40.6 (n = 13)	50.0 (n = 18)	51.4 (n = 19)	40.7 (n = 83)
Orange jasmine	76.5 (n = 189)	80.8 (n = 143)	82.7 (n = 124)	90.5 (n = 86)	81.0 (n = 542)
P	<0.001	<0.001	>0.05	<0.001	<0.001

**Table 3** Percentage of adult (male and female) *Diaphorina citri* stylet tracks<sup>1</sup> that entered calamondin leaves above leaf midribs, lateral veins, minor veins, and mesophyll, and proportions of *D. citri* stylet tracks that terminated in, or traversed (in parentheses), these tissues in relation to leaf age (CI–CIV leaves). A total of 70–84 psyllids of each sex were allowed to feed on CI–CIII leaves and 50–60 of each sex on CIV leaves; in total, 3 756 stylet tracks were observed

Tissue type	Leaf age category			
	CI	CII	CIII	CIV
Midrib	43.4 (44.4)	63.6 (61.9)	12.7 (12.7)	2.9 (2.9)
Lateral vein	8.7 (11.2)	4.5 (6.2)	17.7 (18.4)	30.4 (32.6)
Minor vein	19.1 (24.6)	10.7 (14.8)	32.8 (34.7)	43.2 (43.6)
Mesophyll	28.7 (19.7)	21.2 (17.1)	36.8 (34.2)	23.4 (20.9)

<sup>1</sup>Unbranched tracks and branches of branched tracks.

28 CI leaves to 5.9, 1.0, and 0.25 per leaf in 28 CII, 28 CIII, and 20 CIV leaves, respectively.

The sex of the insect had a significant ( $P = 0.002$ ) influence on the proportions of tracks (single or branched) traversing or terminating in midribs, lateral veins, minor veins, and mesophyll in CI–CIV leaves. A greater proportion of females preferred to feed on CI (♀♀ 55.8%; ♂♂ 46.0%) and CIV (♀♀ 8.6%; ♂♂ 5.9%) than males. Irrespective of leaf age, >70% of probing and feeding linked to midribs was tied to adult feeding activities initiated on abaxial leaf surfaces. On average, 71% of probing and feeding linked to midribs, lateral veins, minor veins, and mesophyll of CIV leaves was also tied to adult feeding activities that commenced on abaxial leaf surfaces. In contrast, <40% of probing and feeding linked to lateral veins, minor veins, and mesophyll in CI–CIII leaves was linked to abaxial surfaces. There was a clear trend for probing on midribs to decline with leaf age, and for activities linked to lateral and minor veins to increase (Table 3). These differences in points of entry and termination/traversing of stylet tracks differed significantly among leaf age categories ( $P_{\text{entry}} < 0.001$ ;  $P_{\text{termination/traversing}} < 0.001$ ). As leaves aged, tracks of stylets that entered leaves above a tissue type

increasingly terminated in, or traversed that tissue (Table 4).

Use of unsectioned leaves did not allow us to estimate proportions of stylets terminating in phloem and xylem and, therefore, preferences of *D. citri* adults for feeding on these tissues. However, as noted in the introduction, recent EPG studies have shown that *D. citri* adults feed predominantly on phloem and less frequently on xylem and do not feed from mesophyll parenchyma (Bonani et al., 2010; Cen et al., 2012). Therefore, the incidence of stylet tracks in mesophyll in our study merely reflects, in most instances, probes that did not lead to successful detection and penetration of vascular tissues. Notwithstanding these limitations of the technique, we showed that leaf age is likely to play an important role in acquisition and transmission of liberibacters by *D. citri* adults, with the probability for successful acquisition and transmission likely to be highest in infected young leaves. Despite reports of variable levels of infection of *D. citri* adults by liberibacters, predominantly CLAs, in orchards (Bové et al., 1993; Ebert et al., 2008; Hung, 2008; Li et al., 2008; Kawai et al., 2010), and likewise, variable seasonal acquisition and transmission of the pathogens (Huang et al., 1990; Bové et al., 1993; Chavan, 2004; Manjunath et al., 2008), the influence of leaf age and disease severity on acquisition and transmission by *D. citri* adults only appears to have been considered by Bonani et al. (2008). These authors reported that acquisition declined with both leaf age and disease severity and attributed the lower level of feeding on the mature leaves to a thicker layer of fibre sclerenchyma surrounding the vascular tissue. Our results suggest that vascular dimensions related to mixed CTV/HLB infections, nutrient deficiencies, and natural thickness, and distribution of fibre sclerenchyma may also influence feeding behaviour of *D. citri*.

#### Persistence of adult stylet tracks in calamondin leaves

Mean numbers of stylet tracks per psyllid observed in leaves stained and fixed immediately after adult psyllids were allowed to probe and feed on them for 2 days were highest in CI and CII leaves, noticeably lower in CIII leaves, and negligible in CIV leaves (Figure 3). Similar



**Table 4** Percentage of *Diaphorina citri* stylet tracks<sup>1</sup> that terminated in, or traversed, midribs, lateral veins, minor veins, and mesophyll of CI–CIV calamondin leaves after stylet tracks commenced in one of these tissue types. A total of 70–84 psyllids of each sex were allowed to feed on CI–CIII leaves and 50–60 of each sex on CIV leaves; in total, 3 756 stylet tracks were observed

Tissue type that tracks terminated in, or traversed	Leaf age category	Tissue type in which tracks commenced			
		Midrib	Lateral vein	Minor vein	Mesophyll
Midrib	CI	96.8	1.1	0.5	1.6
	CII	98.9	0	0.2	0.9
	CIII	100.0	0	0	0
	CIV	100.0	0	0	0
Lateral vein	CI	0.5	61.9	7.4	30.2
	CII	6.8	59.1	2.3	31.8
	CIII	0	94.3	0	5.7
	CIV	0	93.3	0	6.7
Minor vein	CI	0.4	1.9	57.1	40.6
	CII	1.9	1.9	42.9	53.3
	CIII	0	0	93.3	6.7
	CIV	0	0	95.8	4.2
Mesophyll	CI	1.1	4.8	20.4	73.7
	CII	9.8	3.3	23.8	63.1
	CIII	0	1.0	1.0	98.0
	CIV	0	0	7.0	93.0

<sup>1</sup>Unbranched tracks and branches of branched tracks.

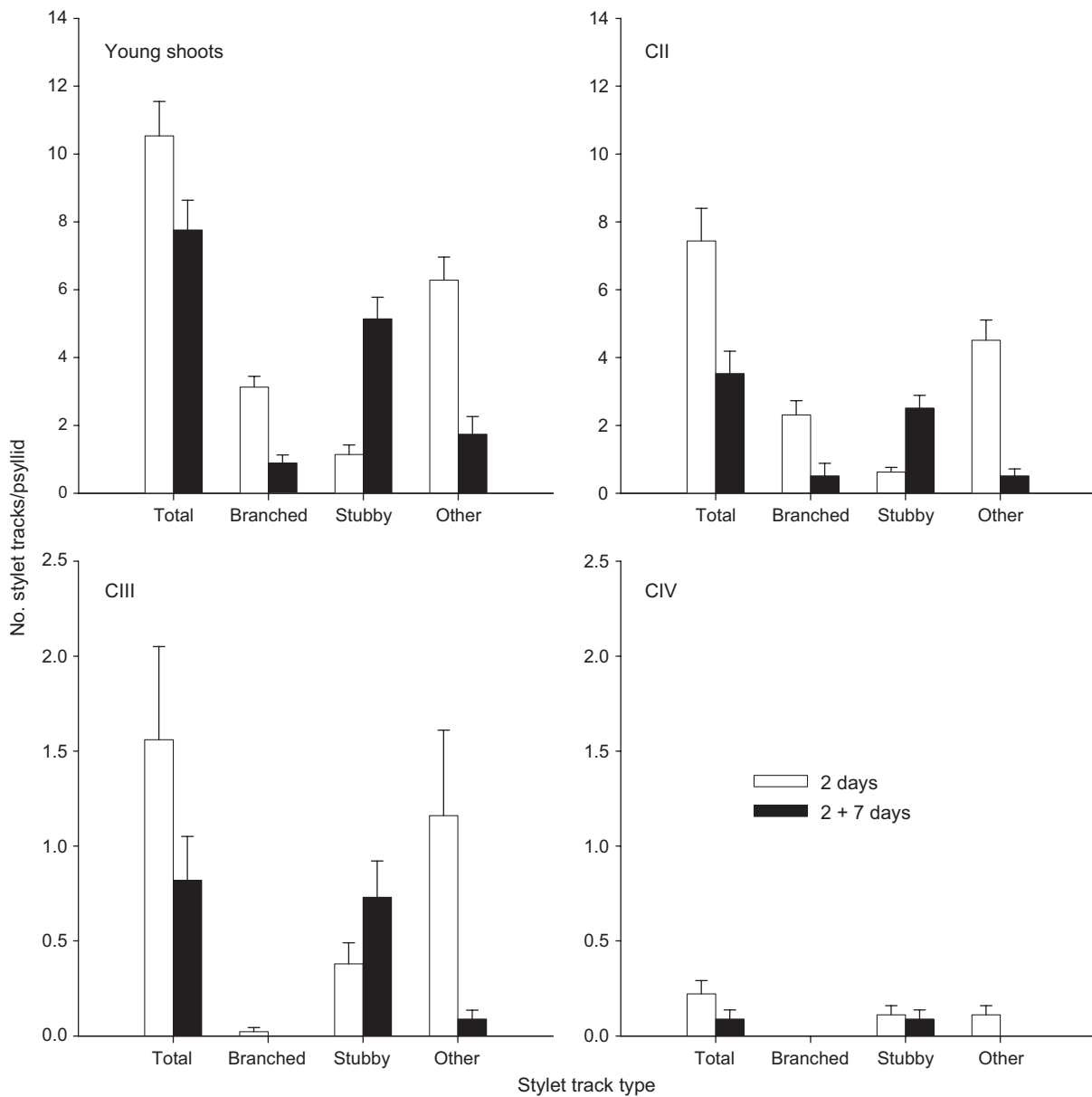
trends were evident for leaves stained and fixed 7 days after adult psyllids were allowed to probe and feed on them for 2 days. Numbers of tracks for all leaf age categories were 35.3% lower (759 compared to 1 174) 7 days after 5–8 adults fed on them, and numbers of branched tracks were 73.6% lower (87 compared to 330). In contrast, numbers of stubby, unbranched tracks for all leaf age categories were 74.6% higher in the 7 days post-feeding treatment (520 compared to 132). This indicates that persistence of tracks was inversely related to increasing leaf maturity.

Bennett (1934) observed that the stylet sheaths of beet leafhopper [*Circulifer tenellus* (Baker)] persisted for up to 20 days in sugar beet. Those of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Cicadellidae), can persist for at least 80 days in grape vine [*Vitis vinifera* L.] tissues (Backus et al., 2005, 2006). However, our results show that the dissipation of stylet tracks of *D. citri* adults in calamondin leaves was significant within 7 days after the adults being allowed to feed on the leaves. Declines in numbers of tracks per adult during this 7-day interval, and simultaneous increases in the incidence of stubby tracks in CI, CII, and CIII leaves, suggests that the sheaths were being dissolved by sap enzymes. We assume that tracks would also disappear as rapidly in tissues of other host plants of the psyllid. If so, there would be only limited scope for using stylet tracks for determining host preferences and incidence of feeding on these hosts in field situations. However, the technique could be useful for

determining tissue and host preferences within 1 week of psyllids feeding on leaves and leaflets less than 3 months old. Moreover, the technique would also allow the detection of psyllid stylet sheaths if host leaves were fixed and stained immediately after psyllid feeding.

#### Adult host preferences in no-choice tests

A total of 713 tracks were observed in host leaflets or leaves, ranging from 106 to 240 in the 16 leaflets or leaves examined for each host. Mean numbers of tracks per leaf/leaflet ranged from 6.6 in trifoliate orange to 15 in sour orange leaves (Table 5). Probing and feeding from the abaxial surfaces of orange jasmine leaflets, and trifoliate orange, lemon, and wampee leaves were more common than from adaxial surfaces, averaging, for all tissue types, 55.1, 73.0, 72.0, and 68.1% for these hosts, respectively. In contrast, >55% of probing and feeding on sour orange leaves occurred from the adaxial surface of leaves. For each host, the percentage of occasions on which feeding activity was initiated on the abaxial surfaces above particular tissues varied; however, abaxial probing and feeding on midribs was usually the highest for all tissue types (Table 5). Lowest variation in site of feeding initiation (41.2–49.0%) occurred on sour orange. On average, >91% of probing and feeding on midribs of the other hosts was initiated from abaxial leaf surfaces, and probing and feeding initiated above lateral veins, minor veins, and mesophyll of these hosts averaged 56.8, 63.1, and 56.9%, respectively.



**Figure 3** Mean (+ SEM) total numbers of stylet tracks of adult *Diaphorina citri* and the mean numbers of branched and stubby tracks in young calamondin shoots and CII, CIII, and CIV calamondin leaves immediately after adult psyllids fed on the tissues for 2 days and then 7 days after feeding ceased.

In general, for most hosts, most probes traversed or terminated in the tissue above or below which the stylet track entered the leaf or leaflet (Table 5); however, two exceptions were found. For orange jasmine, fewer probes commencing over the mesophyll ended in this tissue with a significant proportion termination in the other three tissue types. For trifoliate orange, more probes traversed/terminated in the mesophyll than were initiated above this tissue, with probes starting over the lateral and minor veins

often traversing or terminating in the mesophyll. Probing and feeding on midribs or lateral veins varied markedly among hosts (Table 5).

#### Adult host preferences in choice tests

This experiment assessed settling (landing) and feeding behavioural responses of adult male and female *D. citri* on mature orange jasmine leaflets, CII–III calamondin leaves, and mature sour orange, lemon, trifoliate orange, and

**Table 5** Percentage of stylet tracks<sup>1</sup> entering midribs, lateral and minor veins, and mesophyll from the abaxial surfaces of mature leaves/leaflets of five hosts of *Diaphorina citri*, and percentage of stylet tracks entering mature leaves/leaflets above (adaxially) or below (abaxially) each of these tissues and, in parentheses, terminating in or traversing them. A total of 713 tracks were observed

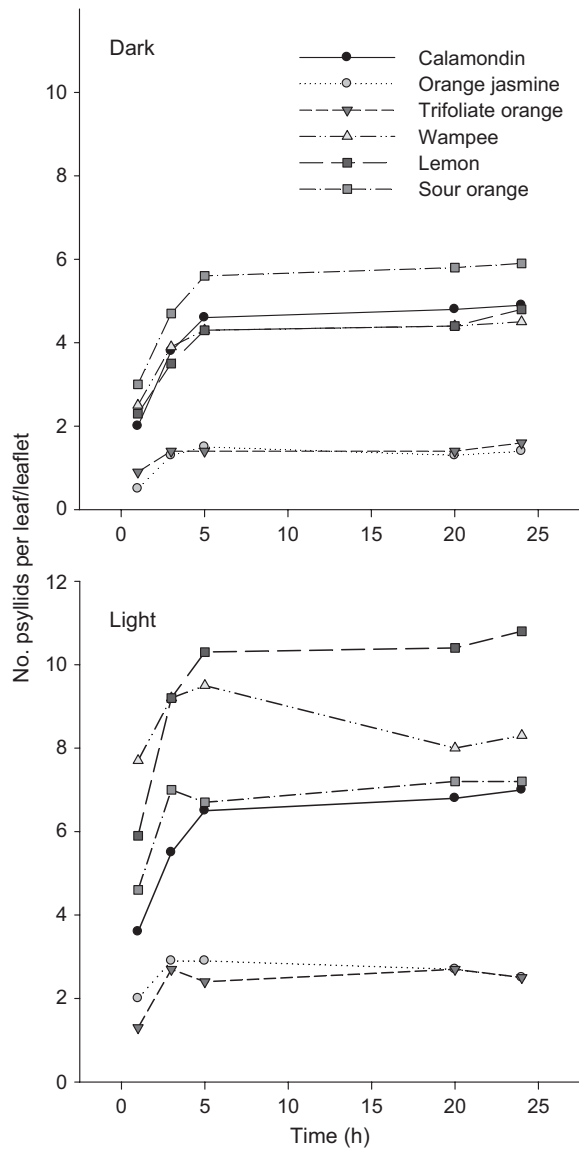
	Host				
	Orange jasmine	Trifoliolate orange	Lemon	Sour orange	Wampee
No. leaflets or leaves examined	16	16	16	16	16
No. tracks observed	109	106	140	240	118
% stylets entering tissues abaxially					
Midrib	75.0	94.4	96.2	41.7	100.0
Lateral vein	33.3	53.8	75.5	41.5	64.5
Minor vein	50.0	84.6	47.8	49.0	70.0
Mesophyll	62.2	59.2	68.4	41.2	37.7
% tissue types above which stylets entered leaves/leaflets and terminated in or traversed (in parentheses)					
Midrib	33.0 (35.8)	17.0 (17.0)	18.6 (18.6)	5.0 (5.0)	3.4 (3.4)
Lateral vein	5.5 (11.9)	17.9 (12.3)	34.3 (35.0)	27.5 (27.1)	30.5 (26.3)
Minor vein	20.2 (25.7)	36.8 (24.5)	34.3 (32.8)	41.3 (40.8)	28.0 (25.4)
Mesophyll	41.3 (26.6)	28.3 (46.2)	12.9 (13.6)	26.3 (27.1)	38.1 (44.9)

<sup>1</sup>Unbranched tracks and branches of branched tracks.

wampee leaves. Numbers of psyllids on leaflets or leaves were recorded 1, 3, 5, 20, and 24 h after 50 adults of each sex were released into separate cages in either darkness or light. Data for numbers of psyllids observed at each interval were log transformed and differences recorded at each interval analysed separately. Differences in sex ( $F_{1,96} = 0.8-23.16$ ,  $P = 0.08-0.37$ ), and for host\*light ( $F_{5,96} = 0.42-1.34$ ,  $P = 0.25-0.84$ ), host\*sex ( $F_{5,96} = 0.22-0.57$ ,  $P = 0.72-0.95$ ), light\*sex ( $F_{1,96} = 0.001-0.24$ ,  $P = 0.62-0.98$ ) and host\*light\*sex ( $F_{5,96} = 0.34-0.89$ ,  $P = 0.49-0.88$ ) interactions were not significant at each assessment time. However, differences in host ( $F_{5,96} = 9.72-15.82$ ,  $P < 0.001$ ) and light ( $F_{1,96} = 17.83-28.17$ ,  $P < 0.001$ ) were significant at each interval. Combined data for sex were, therefore, subjected to a factorial analysis of variance to determine the effects of time, host, and light. Each of these factors had significant impacts: time ( $F_{4,540} = 14.68$ ,  $P < 0.001$ ), host ( $F_{5,540} = 65.87$ ,  $P < 0.001$ ), and light ( $F_{1,540} = 120.62$ ,  $P < 0.001$ ). Differences in the time\*host ( $F_{20,540} = 0.35$ ,  $P > 0.9$ ), time\*light ( $F_{4,540} = 0.31$ ,  $P > 0.8$ ), and time\*host\*light ( $F_{20,540} = 0.12$ ,  $P > 0.9$ ) interactions were not significant, but the host\*light interaction was significant ( $F_{5,540} = 3.44$ ,  $P < 0.005$ ). The host\*light interaction is evident in Figure 4: average numbers of adults landing per leaflet or leaf in darkness did not exceed six in contrast to light in which mean numbers of adults landings per leaf on calamondin, lemon, sour orange, and wampee) in the 5, 20, and 24-h observations ranged from  $>6$  to  $>10$ . There was no significant effect of sex or light on the numbers of stylet tracks nor were any of the two-way or three-way interactions statistically significant. Therefore, the

data for sex and light were combined. In contrast to the numbers of psyllid adults landing on host leaves and leaflets, significantly higher numbers of stylet tracks were observed on orange jasmine leaflets and wampee and trifoliolate orange leaves than on sour orange, calamondin, and lemon leaves ( $F_{5,96} = 6.10$ ,  $P < 0.001$ ) (Figure 5).

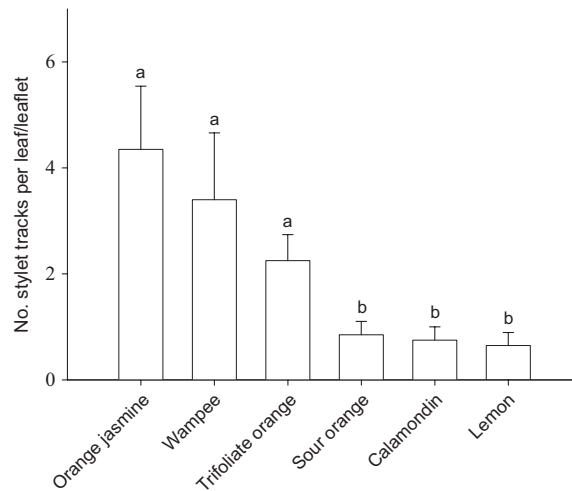
Host preference studies have been performed by Aubert (2008) and Westbrook et al. (2011). Aubert (2008) compared 19 hosts under controlled conditions based on adult feeding, oviposition, and nymphal development, and Westbrook et al. (2011) evaluated 87 rutaceous genotypes as hosts of *D. citri* under field conditions based on numbers of eggs, nymphs, and adults. Aubert (2008) ranked orange jasmine as a preferred host and Westbrook et al. (2011) ranked it as a favoured host; this is in agreement with the number of stylet tracks found in this study, but not the number of landings. Aubert (2008) suggested that trifoliolate orange was an occasional host, whereas Westbrook et al. (2011) found this species to be a poor host as trifoliolate orange selections ranked 85th and 86th and concluded it is partially resistant to *D. citri*. The number of landings on trifoliolate orange leaves in this study indicated that it is not a good host whereas, in contrast, the numbers of stylet tracks indicated that it is a favoured host. The differences between our study and those of Aubert (2008) and Westbrook et al. (2011) may be due to experimental conditions, as this study was limited to responses of adult *D. citri* to leaflets and leaves in small cages in a laboratory and have limited relevance to host preferences under field conditions. However, despite differences, our results indicate that the mere presence of adult psyllids on a host plant



**Figure 4** Average number of *Diaphorina citri* adults observed landing on leaves or leaflets of orange jasmine, wampee, trifoliolate orange, sour orange, calamondin, and lemon in choice tests in either the light or the dark.

may not accurately reflect its status as a host. Our data also suggest that the presence of stylet tracks in host plant tissues should be determined in host preference studies on *D. citri*. Landings may reflect attractiveness of a host based on the host's visual and chemical cues, whereas the presence of stylet tracks may reflect acceptance for successful feeding.

The landing behaviour of adult *D. citri* may be influenced by physical factors related to host leaves/leaflets, for example in this test, leaf size. Orange jasmine leaflets and



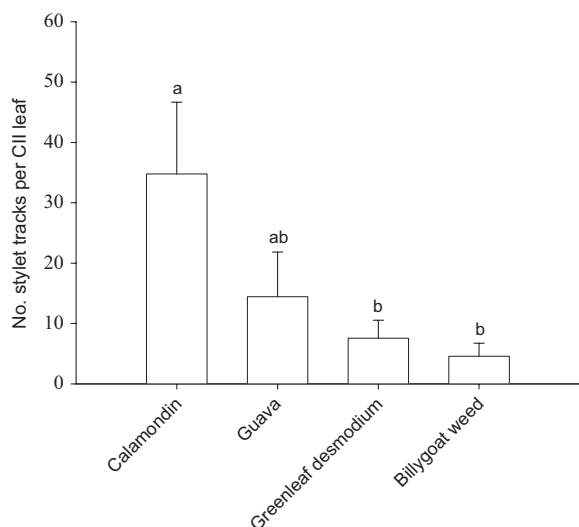
**Figure 5** Average (+ SEM) number of stylet tracks of *Diaphorina citri* in leaves or leaflets of six hosts (orange jasmine, wampee, trifoliolate orange, sour orange, calamondin, and lemon) in a choice test. Bars labelled with the same letter are not statistically different (Fisher LSD test:  $P > 0.05$ ).

trifoliolate orange leaves are small compared to the relatively large leaves of sour orange, lemon, and wampee and the medium-sized leaves of calamondin. The numbers of adults landing on orange jasmine leaflets and trifoliolate orange leaves were low throughout the 24-h observation period in contrast to the higher numbers of the other three species (Figure 4). This may suggest a relationship between leaf size and adult *D. citri* landing behaviour.

Impacts of light and dark on the settling behaviour of adult *D. citri* were recently reported by Sétamou et al. (2010). They found that colonization of potted plants, and subsequent oviposition on these plants, was significantly higher in light than in dark. They also reported that illumination significantly increased responses of adults to host plant volatiles in a Y-tube olfactometer. Our results also suggest that settling may be greater in the light than in the dark, at least on certain species.

#### Influence of non-rutaceous plant volatiles on adult feeding

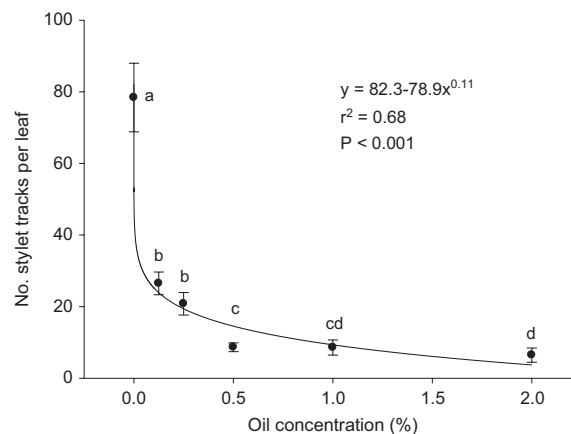
Numbers of stylet tracks per CII calamondin leaf differed significantly ( $F_{3,32} = 3.54$ ,  $P < 0.05$ ) among treatments (Figure 6). Numbers of tracks in the presence of guava leaf volatiles ( $14.4 \pm 7.04$  tracks) were 58.5% lower than in the presence of CIII calamondin leaves ( $34.7 \pm 11.9$ ), but the difference was not significant. Numbers of tracks per leaf in the presence of either of the other non-hosts of the psyllid were >75% lower than in the control but not significantly lower than in the guava leaf treatment (Figure 6).



**Figure 6** Influence of CIII calamondin (control), guava, greenleaf desmodium, and billygoat weed leaf volatiles on the mean ( $\pm$  SEM) number of stylet tracks of *Diaphorina citri* adults in CII calamondin leaves paired separately with each of the former. Bars labelled with the same letter are not statistically different (Fisher LSD test  $P > 0.05$ ).

Reports from Vietnam indicate that ingress of *D. citri* and HLB into densely planted citrus orchards is slowed, and subsequent incidence of the psyllid and disease reduced, when guava is interplanted with citrus (Beattie et al., 2006; Hall et al., 2008; Nguyen et al., 2008; Gottwald et al., 2010). These effects appear to be related to repellent effects of guava leaf volatiles on adult psyllids (Zaka et al., 2010). In our study, feeding by adult *D. citri* on calamondin leaves was reduced compared to the control, when adults were enclosed with paired CII calamondin and guava leaves in small cages. This outcome was probably influenced by the design of the experiment, as some adults would have also fed on CIII leaves in the paired calamondin leaves treatment. This level of feeding was not assessed. However, feeding was significantly reduced when CII calamondin leaves were paired with leaves of either billygoat weed or greenleaf desmodium. If such impacts can be demonstrated in orchards, cultivation of these plants may be more suitable for restraining *D. citri* populations than interplanting with guava. If so, these plants would be particularly useful in situations where cultivation of guava may not be profitable, where the fruit are subject to attack by fruit flies, and other circumstances where interplanting citrus with guava may not be a suitable option.

Billygoat weed and greenleaf desmodium are grown as understorey, green manure crops in orchards in southern China to enhance incidence, biodiversity, and the



**Figure 7** Relationship between mean ( $\pm$  SEM;  $n = 15$ ) number of stylet tracks of *Diaphorina citri* and deposits of an nC24 agricultural mineral oil applied to CII calamondin leaves in 0 (water), 0.125, 0.25, 0.5, 1, and 2% (vol/vol) aqueous emulsions.

effectiveness of natural enemies of arthropod pests (Liang & Huang, 1994; Liu et al., 1999a,b, 2001). Greenleaf desmodium is also grown as a forage crop (Liang & Huang, 1994; Liu et al., 2001). Billygoat weed has been used since the late 1970s as a source of pollen to sustain populations of phytoseiid predators of citrus red mite [*Panonychus citri* (McGregor)] in citrus orchards (Huang et al., 1978; Liang & Huang, 1994). It produces allelochemicals, including ageratochromene, demethoxy-ageratochromene,  $\beta$ -caryophyllene,  $\alpha$ -bisabolene, and E- $\beta$ -farnesene, which in laboratory olfactory tests have been shown to attract the predators of *P. citri* and/or repel the mite itself (Kong et al., 2005). The concentration of volatiles in *A. conyzoides*-intercropped citrus orchards can be so high that an unpleasant odour can be detected (Kong et al., 2005). Olfactory studies, preferably based on electroantennograms, are required to determine if these and/or other volatiles repel *D. citri*. Field tests are required to determine if populations of *D. citri* are lower in orchards with dense understoreys of billygoat weed than in orchards in which the weed is not present. Similar research is required for greenleaf desmodium, volatiles of which, including (E)- $\beta$ -ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene and other sesquiterpenes, are also known to repel noctuid and pyralid stem borers (Khan et al., 2000, 2010).

#### Influence of mineral oil deposits on adult feeding

In this part of the study, psyllids were released onto leaves treated with six concentrations of oil 24, 48, and 72 h after spraying. There was no effect related to the time psyllids

were released after leaves were sprayed and no oil\*time interaction; therefore, data for the three times were combined and the results presented in Figure 7. Highly significant differences occurred among treatments ( $F_{5,84} = 29.54$ ,  $P < 0.001$ ) and numbers of stylet tracks per leaf fell dramatically by >65% and then to >90% as oil concentrations in sprays used to form oil deposit on leaves increased from 0.125 to 2%. Impacts of the 0.5–2% treatments were greater than impacts of the 0.125 and 0.25% treatments.

The steep, negative exponential relationship derived for numbers of stylet tracks per leaf vs. oil concentration in the emulsions resembles the relationship reported by Beattie et al. (1995) for oviposition by citrus leafminer (*Phyllocnistis citrella* Stainton) on citrus leaves sprayed with 0.125–1% aqueous emulsions of an nC21 horticultural mineral oil (HMO), and steep declines for feeding and oviposition by greenhouse thrips [*Heliothrips haemorrhoidalis* (Bouché)] on sweet orange fruit and mature mango leaves (*Mangifera indica* L.) sprayed with 0.5–1.5% aqueous emulsions of the same oil (Liu et al., 2002).

Our study is the first to report impacts of deposits of 0.125–2% aqueous emulsions of mineral oil on feeding by adult *D. citri*. Previously, Rae et al. (1997) reported significant impacts of deposits of 0.25–1% (vol/vol) aqueous emulsions of an nC22 HMO on oviposition by *D. citri*. Leong et al. (2002) reported that 0.3–0.5% sprays of an nC24 AMO were as effective as two synthetic pesticide treatments for suppression of *D. citri* populations in Sarawak, Malaysia. In addition, Leong et al. (2012) reported that weekly 0.35% applications of an nC24 AMO was as, or more, effective for suppressing *D. citri* populations as synthetic pesticide treatments applied at the same frequency and significantly reduced oviposition and feeding. At the end of the experiment, levels of HLB in the treatments, as determined by PCR, were 42% in the control, 11% in the oil, and 9 and 23% for the two pesticide treatments (Leong, 2006). Thus, the impacts of mineral oil deposits on feeding and oviposition of *D. citri* have practical implications for suppressing populations of the psyllid in orchards, nurseries, and in other circumstances.

Our studies demonstrated that the visualization of stylet tracks is a simple technique that can be used in conjunction with other methods to assess the feeding behaviour and preferences of *D. citri* adults. However, before using this technique, it is important to determine the longevity of the tracks after insertion of stylets, as this study has shown that their longevity is different in leaves of different ages and also suggests that longevity can vary significantly in different host-insect combinations. In addition, this technique provides information about the tissues in which the stylet tracks terminate or

traverse thereby providing further information about the activities of the insect on the host.

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