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Effect of host-plant and infection with '*Candidatus* Liberibacter asiaticus' on honeydew chemical composition of the Asian citrus psyllid, *Diaphorina citri*

Faraj Hijaz[1], ZhanJun Lu[2] & Nabil Killiny[1]*

[1]*Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA, and* [2]*National Navel-Orange Engineering and Technology Research Center, GanNan Normal University, Jiangxi Province 341000, China*

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), transmits the citrus greening pathogen '*Candidatus* Liberibacter asiaticus' (*C*Las) by feeding on citrus phloem sap. Because phloem sap is rich in sugars but low in amino acids, ACP sucks large quantities and excretes most of it as honeydew. We studied the chemical composition of ACP honeydew on various host plants. Honeydew samples were analyzed with gas chromatography–mass spectrometry. Fourteen sugars, 13 amino acids, and six organic acids were detected in the honeydew of ACP. Sugars composed about 95% of the total compounds. Sucrose and trehalose were the predominant sugars, composing about 58 and 23% of the total sugars, respectively. Proline, asparagine, aspartic acid, and glutamic acid were the most abundant amino acids in ACP honeydew. The host plant and its infection with *C*Las had some effect on the honeydew composition. Glucose, chiro-inositol, myo-inositol, inositol, maltose, and turanose were lower in honeydew collected from *C*Las-infected citrus compared to that collected from non-infected trees. In *C*Las-infected citrus (pineapple sweet orange, *Citrus sinensis* L. Osbeck) and *Bergera koenigii* (L.) Spreng. [curry leaf tree (both Rutaceae)] honeydews, valine, alanine, serine, glutamine, glycine, and the organic acids were lower than in honeydew from healthy citrus. Mannose, galactose, inositol, mannitol, an unknown disaccharide, and proline were higher in the honeydew collected from *B. koenigii* than in honeydew collected from healthy citrus (pineapple sweet orange), whereas fructose, chiro-inositol, myo-inositol, trehalose, and lactic acid were lower. The findings of this study help us understand the metabolism and the nutrient needs of ACP that transmits *C*Las, the pathogen of huanglongbing in citrus.

Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a phloem-sucking insect, and it mainly infests citrus and its related species (Halbert & Manjunath, 2004). ACP is an important economic pest of citrus because it transmits '*Candidatus* Liberibacter asiaticus' (*C*Las) which causes the citrus greening disease, huanglongbing (Halbert & Manjunath, 2004). In addition to the transmission of *C*Las, direct feeding on the phloem sap and production of large amounts of honeydew may also contribute to further economic losses (Ammar et al., 2013).

Honeydew excretions of phloem-feeding insects are mainly composed of sugars and often contains small amounts of amino acids (Dhami et al., 2011). Because the phloem sap of most plants contains high amounts of sugars and low amounts of the essential amino acids (an unbalanced diet), phloem-sucking insects are able to tolerate the high sugar content while acquiring their essential amino acids (Douglas, 2006). To reduce the osmotic pressure caused by the high sugar content, some piercing-sucking insects transform excess ingested sugars to

*Correspondence: Nabil Killiny, Entomology and Nematology Department, Citrus Research & Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA. E-mail: nabilkilliny@ufl.edu

long-chain oligosaccharides and excrete them as honeydew (Douglas, 2006). Phloem sap-sucking insects are also able to get their amino acids from symbiotic microorganisms and amino acids synthetase enzymes (Wilkinson, 1998; Douglas, 2006).

To fulfill their metabolic need for essential amino acids, plant-sucking insects must consume a large volume of the phloem sap (Byrne & Miller, 1990). Materials existing in high quantity in phloem sap like sugars and water are directly passed from the posterior of the foregut to the interior part of the hindgut through the filter chamber (Roeder, 1953). The compounds discharged through the filter chamber vary from one species to another. In some insects, such as aphids, sugars and other organic and inorganic materials are generally discharged through the filter chamber and are excreted as honeydew (Roeder, 1953).

The honeydew of insects has been studied extensively. Some studies focused on the effect of honeydew composition on the attraction of ants and natural enemies (Fischer & Shingleton, 2001; Fischer et al., 2002, 2005; Woodring et al., 2004), whereas others focused on the effects of insect age (Fischer et al., 2002), host plants (Fischer & Shingleton, 2001; Fischer et al., 2005), species (Dhami et al., 2011), and diurnal changes in phloem sap composition on honeydew composition (Tarczynski et al., 1992; Taylor et al., 2012). Other studies tried to explain insect metabolism by comparing the composition of honeydew to the phloem sap of the host plants (Fischer & Shingleton, 2001; Fischer et al., 2005). In addition, some of the previous studies on honeydew composition tried to answer a specific question. For example, Byrne & Miller (1990) tried to explain why the host range of a Florida strain of tobacco whitefly, *Bemisia tabaci* (Gennadius) was wider than that of an Arizona strain, by studying the honeydew produced by both strains and the phloem sap of their host plants.

A recent study about the ultrastructure of the honeydew of ACP showed that color and texture of the honeydew produced by ACP nymphs were different from honeydew produced by adults. The nymphs produce a continous white thread of waxy honeydew excretion (Ammar et al., 2013). The length of these threads may reach many times the body length and its width ranges from 30 to 100 μm (Ammar et al., 2013). On the other hand, adult ACP males produce sticky colorless droplets (500–900 μm) (Ammar et al., 2013). In addition, the color and texture of honeydew produced by females is also different from that produced by males. In general, female's honeydew is whiter, more solid, and less sticky than honeydew from males (Ammar et al., 2013).

Surprisingly, the honeydew excretion behavior in males is also different from that in females (Ammar et al., 2013). Males excrete their honeydew directly behind their bodies after bending the end of their abdomen downward, whereas female adults push the honeydew droplets upward and sideward after twitching their wings and bending their abdomen (Ammar et al., 2013). By pushing the excreted honeydew droplets away from their body, ACP females prevent eggs and young nymphs from being contaminated or covered by their sticky honeydew (Ammar et al., 2013).

Previously, we showed that the phloem sap of pineapple sweet orange, *Citrus sinensis* L. Osbeck (Rutaceae), is rich in sugars, amino acids, and organic acids (Hijaz & Killiny, 2014). Sucrose was the most abundant sugar followed by glucose and fructose. Sucrose, glucose, and fructose made up 64, 20, and 10% of the total sugars, respectively. Sugar alcohols were also abundant in the citrus phloem sap and they composed about 5% of the total sugars. Twenty amino acids were identified and proline was the most abundant amino acid. Gamma–aminobutyric acid (GABA) was the only non-protein amino acid detected, and composed about 10% of the total amino acids. In addition, many organic acids, including maleic, fumaric, succinic, malic, benzoic, and citric acids, were detected. Malic acid was the main organic acid and it made up about 50% of the detected organic acids.

Since the discovery of citrus greening, many studies have been conducted to evaluate the effect of *C*Las infection on the level of primary and secondary metabolites of citrus. Early studies reported massive accumulation of starch in leaves from infected trees (Schneider, 1968). Fan et al. (2010) confirmed the accumulation of starch in *C*Las-infected leaves and demonstrated that the level of sucrose, fructose, and glucose were higher in mid ribs of symptomless *C*Las-infected leaves. Accumulation of sucrose and glucose was also obvious in symptomatic leaves, whereas no accumulation of fructose was observed. *C*Las also alters the nutritional status of infected citrus plants (Mann et al., 2012). It has been found that *C*Las-infected plants were lower in nitrogen, phosphorus, sulfur, zinc, and iron compared to healthy plants (Mann et al., 2012). Recently, Hijaz et al. (2013) showed that *C*Las infection significantly alters the secondary metabolite profile of sweet orange leaves. In addition to the effects on leaf metabolites, *C*Las was found to alter the nutritional status and the secondary metabolites of citrus fruits from *C*Las-infected trees (Rosales & Burns, 2011; Slisz et al., 2012).

In this study, we investigate whether the *C*Las-infection removes nutrients from the host, thereby reducing the nutrients available for the psyllid and effectively lowering the nutrients expelled in the honeydew. Our null hypothesis is that there is no difference between the honeydews of *C*Las-infected and healthy hosts. In addition, the nutrients available in the *C*Las-infected host would be similar to those of a non-preferred host species,

such as curry leaf tree, *Bergera koenigii* (L.) Spreng. (Rutaceae), as expected based on the insect's initial attraction to *C*Las-infected plants; its final settling is on healthy plants and it has been observed that the insect does not feed on *B. koenigii* in the field when citrus is available (Halbert & Manjunath, 2004). On the other hand, the fact that *B. koenigii* does not support the infection with *C*Las (Damsteegt et al., 2010) suggests that its poor nutrient profile does not meet the requirement for bacterial growth. This also suggested that the nutrients in *B. koenigii* would be different from those in healthy citrus. Investigating the chemical composition of honeydews collected from these three hosts will provide useful information about ACP nutrient needs and metabolism and may help developing an artificial diet for this insect.

Materials and methods

Diaphorina citri colonies

Healthy (*C*Las-free) colonies of *D. citri* were maintained on healthy pineapple sweet orange inside 400-mesh rearing and observation cages (Bioquip, Landam, MD, USA). Colonies were kept in temperature-controlled growth rooms set at 27 ± 3 °C, 60 ± 5% r.h., and L16:D8 photoperiod. Originally, insects were collected in 2000 from citrus groves in Polk City (FL, USA). Insect colonies were PCR tested regularly to make sure they were *C*Las-free.

Honeydew collection

Newly emerged adults of ACP were randomly collected from our colonies and starved for 6 h. After starvation, 100 adults were caged with 1-year-old healthy pineapple sweet orange, healthy curry leaf trees, or *C*Las-infected pineapple sweet orange (6 months after graft inoculation with *C*Las-positive budwood from the same tree species). Five sets for each treatment were established as described above. Five samples of the honeydew were collected from each set (25 samples per treatment). Honeydew samples were collected without gender discrimination after 1 month of exposing the plants to the insects. Honeydew droplets were collected from the plants. All samples were collected on the same day. The experiments were carried out in the growth room at 27 ± 3 °C, 60 ± 5% r.h., and L16:D8 photoperiod. Samples were stored at −80 °C until analysis. Five additional samples were collected to determine honeydew moisture content, by drying the honeydew to a constant weight at 105 °C.

Derivatization of sugars and amino acids from honeydew

For each sample, about 0.5 mg honeydew (1–5 droplets) was dried under nitrogen stream. The dried sample was mixed with 30 µl of methoxyamine hydrochloride solution (MOX) in pyridine (2%) and allowed to react for 17 h at room temperature (Gullberg et al., 2004). After methoximation, silylation reactions were induced by adding 80 µl of *N*-methyl-(*N*-trimethylsilyl) trifluoracetamide (MSTFA) for 2 h at room temperature, then 0.3 µl of derivatized sample was injected into the gas chromatograph-mass spectrometer (GC-MS) running in electron ionization mode (EI), full scan mode. Sucrose, glucose, mannose, fructose, inositol, quinic, citric, succinic, and malic acid standards were prepared in the same way and injected into the GC-MS under the same conditions to identify the components of honeydew.

The amino acid composition was determined by GC-MS after methylchloroformate (MCF) derivatization as described by Dhami et al. (2011). In summary, about 5 mg of honeydew was dissolved in 20 µl of water and then mixed with 180 µl of 1 N sodium hydroxide in 1-ml silanized conical inserts. The sample was vortexed for 1 min to dissolve the honeydew droplets and then mixed with 167 µl of methanol and 34 µl of pyridine. An aliquot of 20 µl of MCF was added and the sample mixture was vigorously mixed for 30 s. Another 20 µl of MCF was added and the sample mixture was also vigorously mixed for 30 s. The reaction was stopped by the addition of 200 µl of chloroform and vigorous mixing for 10 s. An additional 200 µl of 50 mM sodium bicarbonate was added with vigorous mixing for 10 s. The aqueous layer was discarded and 0.5 µl of the organic layer was injected into the GC-MS using the splitless mode.

Sucrose, glucose, fructose, mannose, galactose, malic acid, inositol, citric acid, quinic acid, glycine, alanine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine, histidine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, proline, serine, tyrosine, and methylchloroformate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide, sodium bicarbonate, chloroform, MOX in pyridine (2%), and MSTFA were purchased from Fisher Scientific (Pittsburg, PA, USA). These compounds were used as standards for GC-MS.

GC-MS analyses and peak identification

Derivatized samples and standards were analyzed using Clarus 500 GC-MS system (Perkin Elmer, Waltham, MA, USA) fitted with an HP-5MS column (cross-linked 5% Ph Me siloxane, 30 m × 0.25 mm × 0.025 µm film thickness). Hydrogen was used as a carrier gas and delivered at a constant flow rate of 1 ml per min. The GC temperature program was as follows: initial temperature was held at 70 °C for 5 min, then increased to 180 °C at a rate of 10 °C per min, held for 2 min, increased further to 280 °C at 10 °C per min, held for 1 min, increased to

300 °C, and finally held for 5 min. The injector and the detector temperatures were set at 220 and 260 °C, respectively.

Gas chromatograph-mass spectrometer chromatograms were analyzed using TurboMass software v. 5.4.2 (Perkin Elmer). Peaks were first identified by comparing their mass spectra with library entries (NIST, National Institute of Standards and Technology, Gaithersburg, MA, USA, 2011; and Wiley, 9th edn, John Wiley and Sons, Hoboken, NJ, USA, 2009). Compound identification was further confirmed by comparing retention times and mass spectra with authentic standards. Linear retention indices (LRI) of the detected compounds were calculated using a calibration curve generated by injecting a mixture of alkanes (C8–C20).

Statistical analysis

Statistical analysis was performed using JMP v. 9.0 (SAS Institute, Cary, NC, USA). Data were normally distributed. The percentage peak areas were calculated by dividing separate peak area of each compound by the total area. ANOVA was used to compare the relative amounts (log-transformed) of the detected compounds among honeydew samples. Post-hoc pair-wise comparisons between treatments were performed with Tukey's honestly significant difference (HSD) test.

Results

Composition of honeydew collected from ACP fed on healthy pineapple sweet orange

The moisture content of the honeydew produced by ACP adults was 20.6 ± 2.6% (n = 5). This indicates that the honeydew of ACP is highly concentrated and contains little water.

After derivatization with TMS, 20 compounds were detected in ACP honeydew (Figure 1A and 2A). These compounds could be classified into sugars, organic acids, and amino acids. Sugars were the most predominant compounds (95%) detected in honeydew after TMS derivatization (Figure 2A). Four types of sugars were detected: monosaccharides (15% of the total compounds), disaccharides (78%), trisaccharides (0.5%), and sugar alcohols (2%). Fructose and glucose composed about 99% of the monosaccharides. Galactose and mannose were found in much smaller quantities. Sucrose was the predominant disaccharide (56%), followed by trehalose (22%). Sucrose and trehalose alone composed 99.5% of the total disaccharides. Mannitol, inositol, chiro-inositol, and myo-inositol composed about 2% of the total compounds and inositol and chiro-inositol were the dominant sugar alcohols.

In addition to sugars, lactic, succinic, malic, citric, and quinic acids were detected in the honeydew of ACP. These organic acids composed about 5% of the total compounds and malic acid was the most abundant. Only one amino acid (proline) was detected in the honeydew after TMS derivatization. This result indicates that the amino acids are present in relatively low amounts in ACP honeydew compared to sugars.

To improve the amino acid and organic acid detection and analysis, additional honeydew was collected and derivatized using MCF instead of TMS. In addition, the sample weight of the derivatized sample was increased from 0.5 to 5 mg to detect those amino acids that are present at very low concentration. Thirteen amino acids and four organic acids were detected in the MCF-derivatized honeydew of ACP using GC-MS (Figure 1B). The amino acids composed about 80% of the total compounds detected and they included proline, asparagine, aspartic acid, glutamic acid, and alanine, which together comprised about 96% of the total amino acids (Figure 2B). In agreement with the TMS results, malic, citric, and succinic acids were also detected after MCF and malic acid was again the most abundant organic acid.

Effect of infection with *C*Las in pineapple sweet orange on honeydew composition

The carbohydrate composition of ACP fed on *C*Las-infected pineapple sweet orange was slightly different from that collected from ACP fed on healthy pineapple sweet orange (Figure 3). The relative amounts of fructose, mannose, galactose, mannitol, sucrose, an unknown disaccharide, trehalose, and an unknown trisaccharide were not affected, whereas a significant decrease in amounts of glucose, chiro-inositol, myo-inositol, inositol, maltose, and turanose was observed (Figure 3).

Among the 13 detectable amino acids found in ACP honeydew, valine, proline, alanine, serine, glutamine, and glycine displayed a significant difference (Figure 4A and B). Honeydew produced by ACP fed on *C*Las-infected pineapple sweet orange was significantly lower in those amino acids than that of ACP honeydew collected from healthy sweet orange. Leucine, isoleucine, threonine, aspartic acid, phenylalanine, asparagine, and glutamic acid were in similar relative amounts in ACP honeydew collected from both healthy and *C*Las-infected sweet orange (Figure 4A and B). Except lactic acid, the relative amounts of all other organic acids were significantly decreased (Figure 4C).

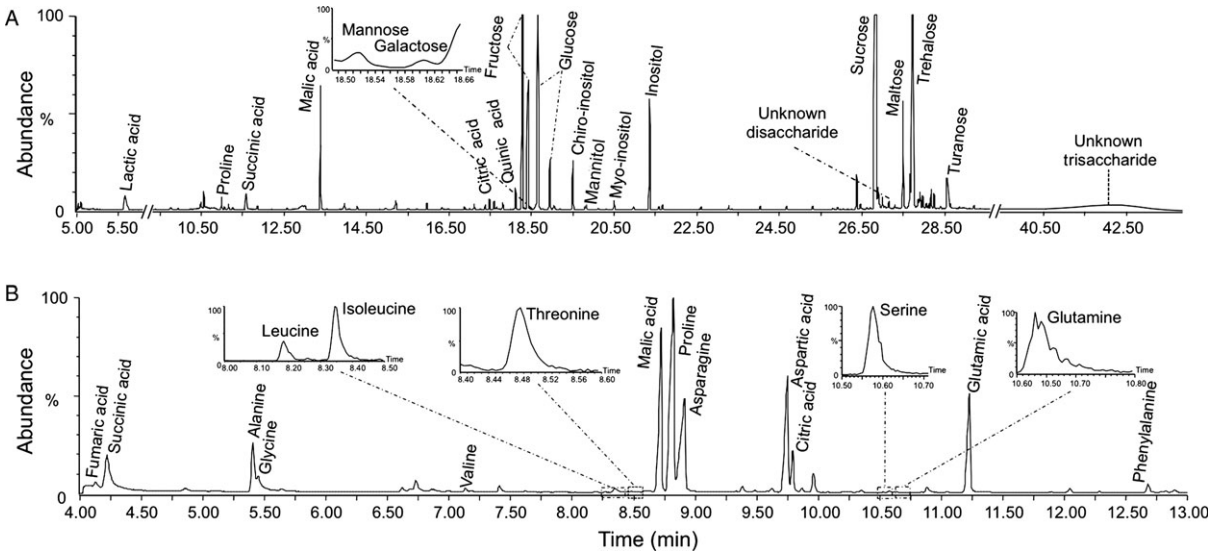

Figure 1 Gas chromatograms of derivatized honeydew samples collected from Asian citrus psyllids fed on healthy pineapple sweet orange. Samples derivatized with (A) trimethylsilyl (TMS) or (B) methyl chloroformate (MCF).

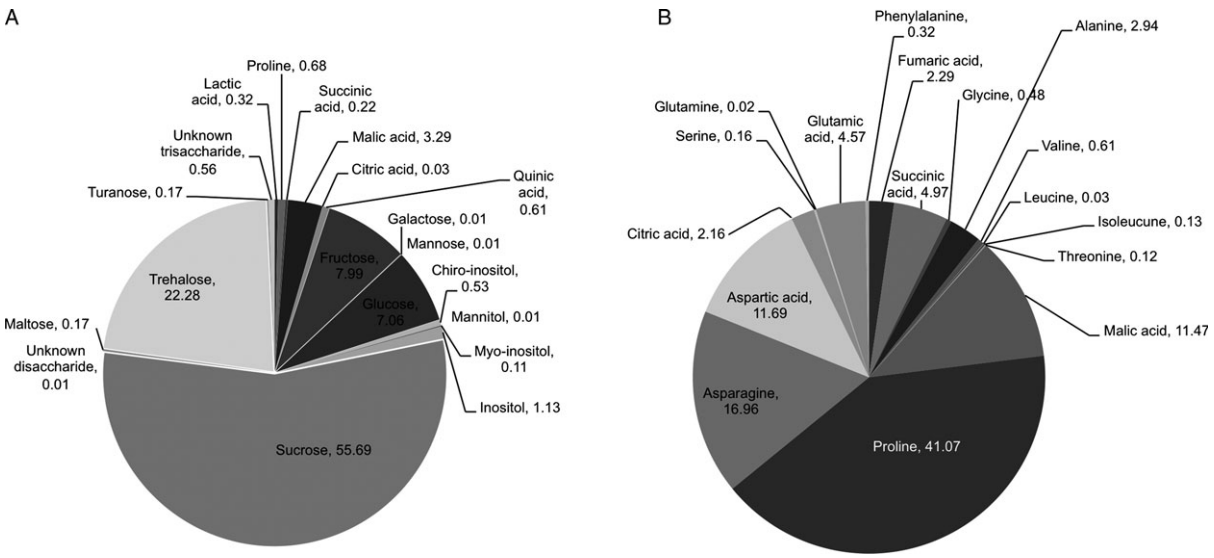

Figure 2 Composition (%) of compounds in honeydew collected from Asian citrus psyllids fed on healthy pineapple sweet orange. Samples derivatized with (A) trimethylsilyl (TMS) or (B) methyl chloroformate (MCF).

Effect of host plant (*Bergera koenigii*) on the chemical composition of ACP's honeydew

The honeydew composition of ACP fed on *B. koenigii* was different from that collected from ACP fed on healthy pineapple sweet orange. The relative amounts of inositol, mannitol, mannose, galactose, and an unknown disaccharide in honeydew collected from ACP fed *B. koenigii* were significantly higher than those in honeydew samples collected from ACP fed on healthy pineapple sweet orange. The relative amounts of fructose, chiro-inositol, myo-inostol, and trehalose were lower in honeydew samples collected form ACP fed on *B. koenigii* compared with those collected from healthy pineapple sweet orange (Figure 3). The amino acids valine, alanine, serine, glutamine, and glycine were found in lower amounts in honeydew collected form ACP fed on *B. koenigii* than in samples collected from ACP fed on healthy sweet orange (Figure 4A and B). Contrarily, proline was found in higher

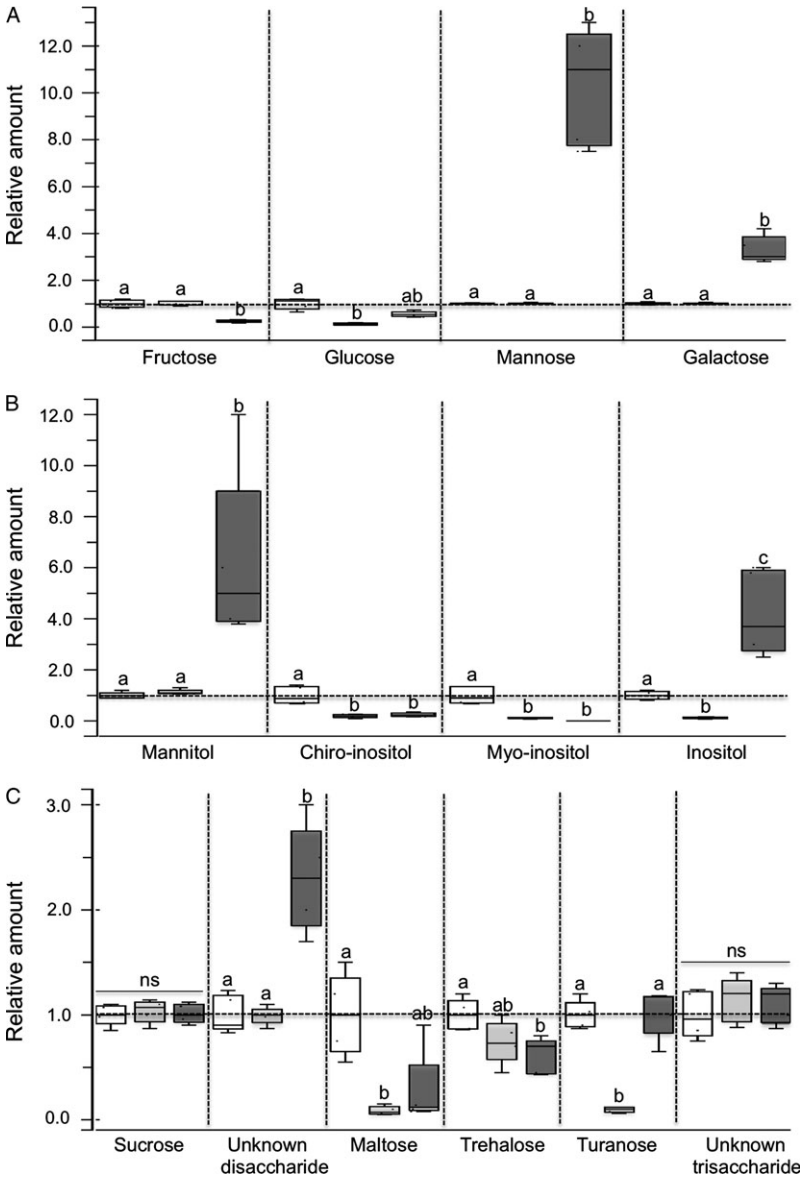

Figure 3 Relative quantification of sugars (data were normalized by dividing data points by the mean peak area for each compound found in honeydew collected from Asian citrus psyllids fed on healthy pineapple sweet orange). (A) Monosaccharides, (B) sugar alcohols, (C) di- and trisaccharides – in honeydew collected from Asian citrus psyllids fed on healthy pineaple sweet orange (white), *C*Las-infected pineapple sweet orange (light gray), and healthy *Bergera koenigii* (dark gray). Horizontal lines inside the boxes indicate the medians, the boxes indicate the interquartile ranges including 50% of the values, and the whiskers reflect the extreme values (range). Different letters capping boxes within a compound indicate significant differences between treatments (Tukey's HSD test: $P<0.05$); ns, not significant.

amount in honeydew collected from ACP fed on *B. koenigii* (Figure 4). The organic acids were found to be in lower amounts in honeydew collected from ACP fed on *B. koenigii* (Figure 4C). Malic, lactic, citric, and succinic acids were in lower amounts, whereas fumaric and quinic acids were in similar amounts in, both, sweet orange and *B. koenigii*.

Discussion

The high amounts of sugar detected in the honeydew of ACP suggest that the phloem sap of sweet orange contains much more carbohydrate than is required by ACP metabolism. Conversely, the low amount of amino acids found in ACP honeydew indicated that most were utilized by ACP and little was excreted, and that phloem sap composition overall was lower in amino acids. In fact, this is the case for most plant-feeding hemipteran insects.

Fourteen sugars were detected in ACP honeydew, many of which were also detected in the honeydew of other plant-feeding hemipterans. For example, sucrose, fructose, glucose, trehalose, maltose, and mannitol were detected in honeydew of many aphid and scale insect species (Fischer et al., 2002; Dhami et al., 2011). On the other hand, some sugars like malto-sucrose, malto-tri-sucrose, and melezitose that were detected in honeydew of some insects (Auclair, 1963) were not detected in ACP honeydew.

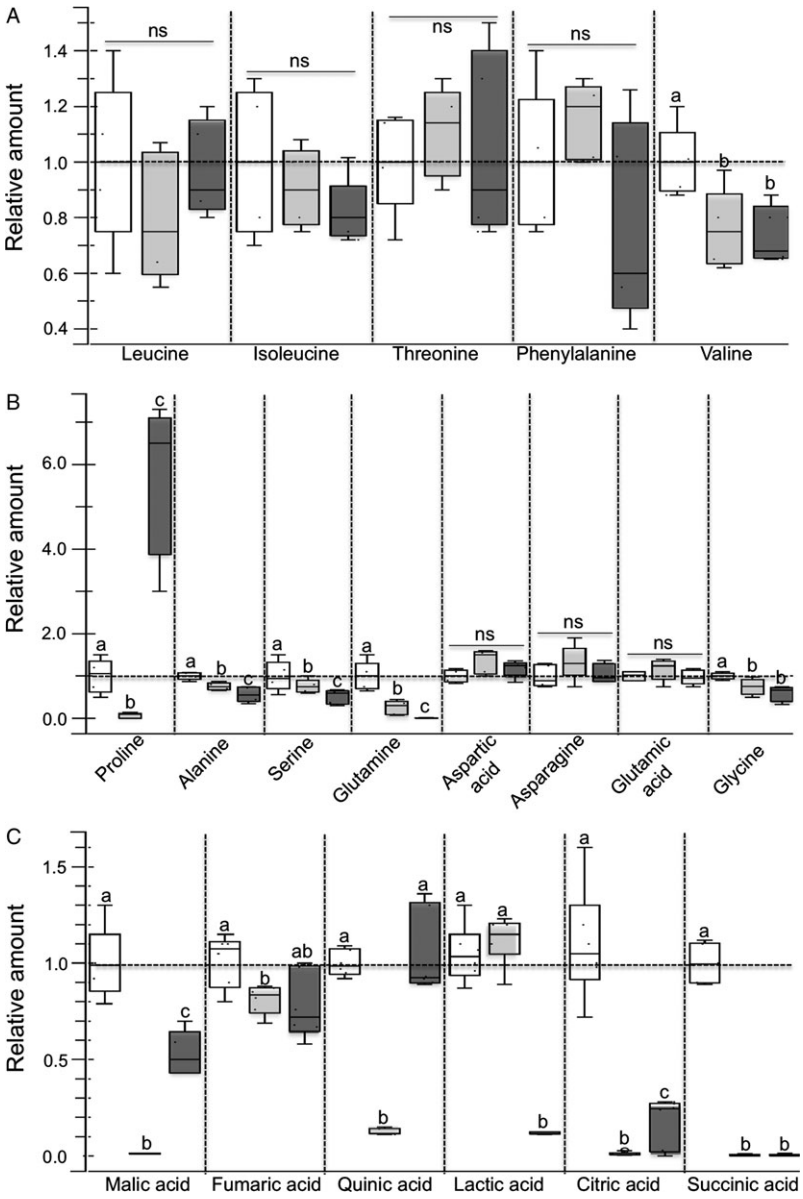

Figure 4 Relative quantification of (A) essential and (B) non-essential amino acids and (C) organic acids in honeydew collected from Asian citrus psyllids fed on healthy pineapple sweet orange (white), *C*Las-infected pineapple sweet orange (light gray), and healthy *Bergera koenigii* (dark gray) (data were normalized by dividing data points by the mean peak area for each compound found in honeydew collected from Asian citrus psyllids fed on healthy pineapple sweet orange). Horizontal lines inside the boxes indicate the medians, the boxes indicate the interquartile ranges including 50% of the values, and the whiskers reflect the extreme values (range). Different letters capping boxes within a compound indicate significant differences between treatments (Tukey's HSD test: $P<0.05$); ns, not significant.

Access to a highly sensitive technique such as GC-MS has improved detection significantly, but may have induced bias from what was observable with traditional methods used in the past. For example, 13 amino acids were identified in the honeydew of ACP after MCF derivatization. More amino acids were detected and identified with MCF than with TMS, because the MCF method is more specific for amino acids; MCF does not react with the hydroxyl group of sugars (Sobolevsky et al., 2003). All of the amino acids found in this study were reported in the honeydew of other hemipteran species (Auclair, 1963; Byrne & Miller, 1990; Fischer et al., 2002; Woodring et al., 2004; Dhami et al., 2011).

Beside sugars and amino acids, six organic acids were identified in ACP honeydew (lactic, succinic, malic, citric, fumaric, and quinic acid). Citric, malic, succinic, fumaric, and isocitric acid were also detected in honeydew of the aphid *Brevicoryne brassicae* (L.) feeding on swedes (*Brassica napobrassica* DC) (Auclair, 1963). In addition, succinate, lactate, malate, citrate, and isocitrate were detected in honeydew of scale insects such as *Ultracoelostoma* spp. and *Coelostomidia* spp., collected from black beech and Ngaio trees (Dhami et al., 2011).

Dhami et al. (2011) surmised that honeydew has a multi-trophic constitution, because it originates in the phloem sap of the host trees, passes through the gut of an insect,

and finally is modified by microbial symbionts. All of the compounds except trehalose and lactic acid detected in ACP honeydew were detected in the phloem sap of pineapple sweet orange (Hijaz & Killiny, 2014). Sucrose, glucose, fructose, galactose, mannose, and three inositol isomers were detected in the phloem sap of pineapple sweet orange (Hijaz & Killiny, 2014). Sucrose was the dominant sugar in the sweet orange phloem sap, followed by glucose and fructose. Malic, citric, threonic, quinic, succinic, and fumaric acid were also detected in the phloem sap of sweet orange (Hijaz & Killiny, 2014). In addition to sugars and organic acids, 20 amino acids were detected in pineapple sweet orange phloem sap. Proline was the most abundant amino acid in this phloem sap (Hijaz & Killiny, 2014).

In this study, proline was abundant in the honeydew of ACP. Proline is an important source of fuel for flight muscles in some insects (Candy et al., 1997). However, because the phloem sap of pineapple sweet orange contains a high amount of proline, some proline could be excreted in the honeydew along with excess sugars. On the other hand, the high levels of asparagine, aspartic acid, and glutamic acid in ACP honeydew could not originate from the phloem sap because the pineapple sweet orange phloem sap contains low levels of these amino acids (Hijaz & Killiny, 2014).

The percentage of essential amino acids to the total amino acid in the honeydew of ACP fed on pineapple sweet orange was 3%, which is lower than that reported in the phloem sap of pineapple sweet orange (7%) (Hijaz & Killiny, 2014). The amino acids methionine, cysteine, tyrosine, tryptophan, GABA, and lysine that were detected in the phloem sap of pineapple sweet orange (Hijaz & Killiny, 2014) were not detected in the honeydew of ACP. Valine, leucine, isoleucine, threonine, and phenylalanine were found at lower levels. By contrast, aspartic acid, asparagine, serine, and glutamic acid in the honeydew of ACP fed on pineapple sweet orange were higher than those reported in the phloem sap of the same host plant (Hijaz & Killiny, 2014). The amino acids level in the honeydew of phloem sap-sucking insects is affected by diet, insect metabolizing enzymes, and their endosymbionts (Wilkinson, 1998). It has been found that excess ammonia in aphid is converted to glutamine via the insect glutamine synthetase (Wilkinson, 1998). Synthesized glutamine could be directly excreted in the honeydew (Wilkinson, 1998) or converted to other amino acids such as glutamic acid, alanine, aspartic acid, isoleucine, leucine, phenylalanine, proline, and valine (Sasaki & Ishikawa, 1995). The presence of glutamine in the honeydew of aposymbiotic aphids that were fed glutamine-free diet indicated that glutamine is synthesized by insect glutamine synthetase (Wilkinson, 1998). We detected lactic acid in ACP honeydew but not in pineapple sweet orange phloem sap. Lactic acid is synthesized in insect as a product of the lactic acid metabolic cycle (Dhami et al., 2011).

Although trehalose was abundant (>23% of the total sugar) in ACP honeydew, no trehalose was detected in the phloem sap of pineapple sweet orange (Hijaz & Killiny, 2014). Trehalose is widely distributed in higher plants (Hodge et al., 2013); however, its concentration varies with species. For example, *Arabidopsis thaliana* (L.) Heynh. contains trace amounts of trehalose (0.03 mg g^{-1} dry weight) (Hodge et al., 2013). In agreement with our results, trehalose was abundant (45%) in honeydew of *B. tabaci* feeding on poinsettia but was not detected in the phloem sap of poinsettia (Byrne & Miller, 1990). Our results together with the previous results suggested that trehalose is being synthesized by these insects.

Trehalose, otherwise known as blood sugar of insects (Yu et al., 2008), is the most important disaccharide for insect flight muscles (Candy et al., 1997). Trehalose is found in the hemolymph of insects at relatively high concentration (2%, 0.06 M) (Candy et al., 1997) and is synthesized in the fat body from monosaccharides, stored glycogen, and by gluconeogenesis from many precursors like amino acids. Trehalose synthesized in the fat body is transported to the hemolymph where it is used as energy source (Candy et al., 1997; Kikuta et al., 2012). Trehalose in the hemolymph may also leak into the tubule lumens and be excreted in the honeydew (Kikuta et al., 2012).

The conversion of monosaccharides (glucose, fructose, and mannose) to trehalose in insects has many advantages. First, it converts the reactive reducing monosaccharides to less reactive (less toxic) non-reducing sugar (Candy et al., 1997). Second, it efficiently traps the sugars and enhance their uptake (Candy et al., 1997). Finally, it decreases the osmotic pressure because the osmotic pressure depends on the solute molarity and not solute weight (Douglas, 2006).

The honeydew of ACP collected from *B. koenigii* was slightly different from that collected from healthy sweet orange. This result suggests that the chemical composition of phloem sap of *B. koenigii* was relatively close to that of pineapple sweet orange. Honeydew from *B. koenigii* was higher in mannose galactose, mannitol, inositol, and proline. However, because fructose, chiro-inositol, myo-inositol, trehalose, valine, alanine, serine, glutamine, glycine, and organic acids were low in the ACP honeydew collected from *B. koenigii*, this also indicated that the phloem sap of *B. koenigii* was as rich in nutrients as that of pineapple sweet orange. In fact, field observations indicated that *B. koenigii* was not an excellent host for ACP. However, it can support a small population of ACP, including nymphal development (Halbert & Manjunath, 2004).

The honeydew of ACP collected from healthy sweet orange was slightly different from that collected from *C*Las-infected pineapple sweet orange. The amounts of glucose, chiro-inositol, myo-inostol, trehalose, and inositol decreased significantly in ACP honeydew collected from *C*Las-infected pineapple sweet orange compared to that collected from the healthy plants. A decrease in most of the amino acids and organic acid was also observed. These changes in the honeydew composition may result from the differences between the phloem sap composition of *C*Las-infected and healthy sweet orange. A recent study on the changes in carbohydrate metabolism in sweet oranges showed a significant increase in sucrose and fructose in midribs and lobes of symptomless *C*Las-infected leaves, whereas an increase in glucose level was only observed in the midribs (Fan et al., 2010).

Because the composition of ACP honeydew collected from *B. koenigii* was similar to that collected from *C*Las-infected pineapple sweet orange, this also indicated that the phloem sap of *C*Las-infected pineapple sweet orange was also poor in nutrients compared to the (healthy) control. Previous research about *C*las-infection of pineapple sweet orange on ACP feeding behaviors indicated that ACP chose healthy rather than *C*Las-infected plants as their final preferred settling point (Mann et al., 2012). This behavior has been explained by the inferior quality of infected plants compared to healthy plants (Mann et al., 2012). In fact, the chemical analysis showed that infected leaves were lower in nitrogen, phosphorus, sulfur, zinc, and iron compared to healthy leaves (Mann et al., 2012).

Previous research on the effect of host plant on the honeydew composition of phloem-sucking insects displayed contradictory results. For example, no differences were detected in the sugar composition of the honeydew from the aphid *Aphidius ervi* Haliday and the scale insect *Ultracorlostoma* spec. reared on different host plants (Hogervorst et al., 2007; Dhami et al., 2011). However, a significant increase in melezitose concentration was observed in the aphid *Chaitophorus populialbae* Boyer de Fonscolombe when reared on *Populus tremula* L. than on *Populus alba* L. A change in honeydew sugar composition of *B. tabaci* whiteflies was also observed when these strains were reared on different hosts (pumpkin or poinsettia) (Byrne & Miller, 1990). In a previous study about the effect of host plant on the honeydew sugar composition of aphid, no effects on total sugar concentration in the honeydew of *Aphis fabae* Scopoli was observed and slight variation in honeydew sugar composition were observed when feeding on various host plants (Fischer et al., 2005).

In conclusion, our results demonstrated that ACP honeydew was mainly composed of sugars, amino acids, and organic acids. All of the identified compounds in ACP honeydew except trehalose and lactic acid were found in the phloem sap of pineapple sweet orange. Host plants status affected the honeydew composition of ACP. The poor quality of *C*Las-infected pineapple sweet orange phloem sap was reflected on the honeydew composition. These data may help in understanding nutrient needs and metabolism of this psyllid. These findings along with the phloem sap composition of pineapple sweet orange (Hijaz & Killiny, 2014) could lead to develop a successful artificial diet solution for ACP. The information provided here might help in modifying gut bacteria to not be able to synthesize trehalose and result in controlling ACP.

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