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ORIGINAL PAPER

What are the nutritional needs of the pear psylla *Cacopsylla pyri*?

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Abstract
Phloem sap is the diet of numerous sap-sucking insects, such as aphids or psyllids, which use it as a source of carbon and nitrogen. *Cacopsylla pyri* is a phloem sap-sucking insect specialised in pear trees that can cause great damage to most pear tree-growing regions. The main goal of our study is to determine the food requirements of *C. pyri* and to quantify the nutrients uptaken from the plant (sugars and amino acids), with a comparison between the composition of the phloem sap and the composition of the egested honeydew. We highlighted that phloem sap is composed of two sugars, sorbitol and sucrose and both are ingested by *C. pyri*. Seventeen free amino acids were also found in the phloem sap, including eight essential amino acids, serine, histidine, threonine, arginine alanine, valine, isoleucine, phenylalanine, leucine and lysine. Two essential amino acids were not found in the pear tree phloem sap (methionine and tryptophan), and two other amino acids (asparagine and glutamine) were egested in high amounts by both the adult females and the larvae. This probably indicates that these four amino acids are synthesised by the pear psylla endosymbiont(s). Finally, three amino acids (valine, glutamic acid and aspartic acid) were consumed only by the adults and not by the larvae and probably participate in actions only performed by adults, such as flying, jumping or reproduction.

Keywords *Cacopsylla pyri* · Phloem sap · Honeydew · Amino acid · Sugar

Introduction

Phloem sap contains essential components, such as sugars, amino acids, inorganic ions, proteins, mRNAs, small RNA proteins and peptides, that are needed for plant nutrition and development (Dinant et al. 2010). Although it represents a rich mixture generally devoid of toxins and feeding deterrents, it is exclusively consumed by a very restricted range of specialised feeders belonging to the Hemiptera order, such as aphids or psyllids (Karley et al. 2002; Douglas 2006). Moreover, some experimental evidence has shown that the composition of phloem sap can impact insect performances by affecting their location and acceptance (Sandström and Pettersson 1994; Hunt et al. 2010). A detailed analysis of phloem sap composition and what is potentially assimilated by the insects that feed on it is therefore essential to understand the adaptations the insects have developed and the physiology of their nutritional needs.

In most plant species, carbohydrates assimilated from CO_2 are exported into the phloem exclusively in the form of sucrose, with concentrations ranging from approximately 0.8 to 1.5 M (Riens et al. 1991; Lohaus et al. 1998; Lohaus and Moellers 2000; Woodring et al. 2004). Other plant species, such as members of the Scrophulariaceae, Plantaginaceae or Oleaceae families, also translocate oligosaccharides of the raffinose family (Knop et al. 2001; Öner-Sieben and Lohaus 2014) or sugar alcohols, such as mannitol or sorbitol, in addition to sucrose (Nadwodnik and Lohaus 2008).

The concentration of total amino acids in phloem sap is generally in the range of 50–200 mM (Lohaus et al. 1998; Lohaus and Moellers 2000; Sandström and Moran 2001; Woodring et al. 2004). In insects, ten amino acids are

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considered essential (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), whereas the others are considered non-essential and can be synthesised or derived from the essential ten (Dadd 1985; Douglas 2006; Hunt et al. 2010). Although up to 20 different amino acids have been found in phloem sap (Lohaus et al. 1998; Lohaus and Moellers 2000; Sandström and Moran 2001), phloem sap is not an ideal diet for insects because of its high osmotic pressure, its lack of some essential amino acids and the low ratio of amino acids compared to sugars (Douglas 2006, 2009). Furthermore, the compositions of plant amino acid profiles rarely match insect food needs (Taylor et al. 2012). Therefore, to fulfil their metabolic need for essential amino acids, phloem feeders ingest phloem sugars at rates in excess of their carbon requirement, and high concentrations of unassimilated sugars are egested as honeydew (Wilkinson et al. 1997). Honeydew is indeed mainly composed of water and sugars (98% of the dry weight), but it also contains amino acids, a high diversity of proteins and secondary plant compounds (Völkl et al. 1999; Leroy et al. 2011a; Sabri et al. 2013). The honeydew composition may vary among insects (species, age classes) (Sandström and Moran 2001; Fischer et al. 2002; Woodring et al. 2004) based on the host plant species (Hendrix et al. 1992), the host plant quality and the sugar concentration in the diet (Fischer et al. 2002; Hale et al. 2003). For aphids, most of the studied honeydew compositions comprised a mix of monosaccharides (mainly fructose, glucose), disaccharides (sucrose, trehalose, maltose) and trisaccharides (melezitose, raffinose, fructose maltose) (Völkl et al. 1999).

The European pear psylla *Cacopsylla pyri* L. (Hemiptera:Psyllidae) is one of the most important pests of European pear trees (*Pyrus communis* L.) and can cause heavy economic losses in most pear tree-growing regions (Civolani 2012). *C. pyri* is a sap-sucking insect that causes direct damages to trees, weakening the trees and reducing their production by nutrient substraction. *C. pyri* also produce indirect damages caused by their high honeydew excretion, which enables the development of sooty moulds. Moreover, *C. pyri* transmit the phytoplasma *Candidatus phytoplasma pyri* (Seemuler and Schneider 2004), which causes the pear decline disease that reduces tree vigour and can be fatal to trees (Civolani 2012). Little is known about the nutritional physiology of this psyllid pest and its amino acid requirements. Many sap feeders live in strong association with obligate endosymbionts that produce essential amino acids, such as *Buchnera aphidicola* in aphids (Van Ham et al. 1997; McCutcheon et al. 2009; Douglas 2009). Psyllid insects host the obligate endosymbiont *Carsonella ruddii* (Thao et al. 2000). However, this bacteria species shows a strong genome reduction, and about half of the biosynthesis pathways for essential amino acids are completely or partially missing, which causes uncertainties related to the true role of *C. ruddii* as essential amino acid providers. Due to the sap feeder's unbalanced diet, it is difficult to understand how these species can thrive on phloem sap and how they obtain essential amino acids (Feldhaar and Gross 2009), even if secondary endosymbionts were identified (Thao et al. 2000). Although some cosymbionts still contain arginine and tryptophan pathways, this is not always the case (Sloan and Moran 2012; Hansen and Moran 2014).

Moreover, pear tree phloem sap has never been characterised before this study, probably because of the difficulties involved in the rearing of pear trees and the extraction of pure phloem sap. The main goal of our study was to determine the food needs of *C. pyri* and to quantify the nutrients taken from the plant through a comparison of the composition of the phloem sap and the egested honeydew. Sugars and amino acids were the two metabolites analysed in this paper, as they are the principal carbon and nitrogen sources utilised by phloem sap feeders (Dadd 1985).

Materials and methods

Plants

We used pear trees (*Pyrus communis*) of the Williams cultivar (between 1 and 2 years old and 0.75–1 m high). Plants were obtained from Battistini Vivai (http://www.battistirebschule.it/) and stored in a climatic room at a controlled temperature of 22 °C.

Psyllids

The pear psyllids of the species *C. pyri* that were used for the experiments were initially collected from a population sampled in the experimental pear orchard of Proefcentrum voor Fruitteelt, Sint-Truiden, Belgium, in the pear orchards of Pcfruit (http://www.pcfruit.be) (Sint-Truiden, Belgium) and maintained in the laboratory on pear trees with the following climatic conditions: 24 °C, 60% RH and L16D8.

Phloem exudate collection

The phloem exudate was collected from uninfested plants in January 2015 through the method described in Hijaz and Killiny (2014). For this technique, pieces of bark of approximately 2 cm long were rinsed with demineralised water (pH 6.60) and dried with Tork paper© to avoid xylem contamination. They were placed in a 0.5-ml Eppendorf tube with a small hole at its bottom. This tube was placed inside a 2-ml Eppendorf tube. The samples were then centrifuged at 12,000 rpm for 15 min at 4 °C, to allow the phloem sap to descend into the 2-ml Eppendorf tube under the influence of centripetal force (Hijaz and Killiny 2014). In this way, the

exudates were mainly composed of phloem sap; however, as the bark may be wounded during the experiments, the exudates may contain a lesser amount of xylem and cell sap. The collected phloem exudates were then stored at − 20 °C until analysis.

Honeydew collection

Infested plants were placed in a room with the following climatic conditions: 24 °C, 60% RH and L16D8. Adult female and larvae honeydew secretions were collected directly on the plants. All larvae honeydew samples were collected on the same day from the direct emissions of stage three larvae with a Pasteur pipette. The female honeydew is composed of semi-solid droplets. Therefore, the samples were collected manually with a fine brush. To obtain a sufficient amount of female honeydew for chemical analysis, the samples were taken every day for 5 days. Sampled plants were placed in a room with the following climatic conditions: 24 °C, 60% RH and L16D8. Samples were stored at − 20 °C until analysis.

Phloem exudate and honeydew analysis

The pH of the Williams pear tree phloem exudate was measured using a Van London-Phoenix 5473901 Glass Micro pH Electrode. Thirteen replicates were done for the pH analysis of phloem exudate.

Sugars

Phloem exudates, female honeydew and larvae honeydew samples were analysed using a Dionex ICS-5000 system (Dionex corp., Sunnyvale, CA). This system consisted of a Dionex ICs-5000 pump configured for a standard gradient analytical pump, an Eluent Generator (EG), a detector/chromatography module thermally regulated with a 25-ml injection loop, an AS-AP autosampler and an ED40 electrochemical detector equipped with an amperometric cell. The cell comprised an approximately 1-mm-diameter gold working electrode, a glass and Ag/AgCl combination reference electrode (Dionex) and a titanium counter electrode consisting of the cell body. The chromatographic separation of the carbohydrates was performed as described by Goffin et al. 2009, on a CarboPac PA100 column (250 × 4 mm) in combination with a CarboPac PA10 guard column (40 × 4 mm) at a flow rate of 1 ml/min. Both columns were packed with an identical microporous, polymeric anion-exchange material and were installed in the thermal compartment at 30 °C. The sample injection volume was 25 ml. The mobile phase consisted of 100 mM sodium hydroxide (Eluent 1), 600 mM sodium acetate in 100 mM sodium hydroxide (Eluent 2), 500 mM sodium hydroxide (Eluent 3) and Milli-Q water (Eluent 4) from the Direct-Pure UP Ultrapure and RO Lab Water system (Rephile). The following elution programme was used: 100% of Eluent 1 for 10 min, followed by a 38-min acetate gradient to reach a concentration of 132 mM, a 7-min cleaning step (50/50 of Eluent 1 and Eluent 3) and finally a 10-min conditioning time. The detection of the targeted carbohydrates and the processing of chromatographic data were performed as described by Goffin et al. (2009). The peak area was used as the analytical measurement. Five standard solution mixes of myoinositol, sorbitol, glucose, fructose, sucrose, raffinose and stachyose with concentrations ranging from 1 to 40 mg/l were used. Subsequently, for the chromatographic determination of the carbohydrates in the phloem exudate and the female and larvae honeydew, the standard solution mixes containing the seven carbohydrates were analysed. The data collected were analysed with Dionex Chromeleon 6.80 SP3 Build 2345 software. Twelve and five replicates were done for the phloem exudate analysis and honeydew analysis, respectively. These analyses were conducted in the lab of Professor Aurore Richel (Université de Liège Gembloux Agro-bio Tech, Gembloux, Belgium).

Amino acids

The assays were performed by HPLC (Pharmacia/LKB) according to Riens et al. 1991. After precolumn derivatization with *o*-phthaldialdehyde, the amino acid derivates were separated on a 4-mm particle size reversed-phase column (Merck, Darmstadt, Germany) with an acetonitrile gradient in 18 mM potassium phosphate, pH 7.1. The derivates were detected by fluorescence. Proline (an amino acid with a secondary amino group) could not be detected with this method. Amino acid standards (Sigma-Aldrich, Germany) were measured in parallel (0–20 µM), and a calibration curve was created for each amino acid. The evaluation of the chromatograms was performed with the integration program PeakNet 5.1 (Dionex). Twelve and eighteen replicates were done for the phloem exudate analysis and the honeydew analysis, respectively. These analyses were conducted in the lab of Professor Gertrud Lohaus (Bergische Universität, Wuppertal, Germany).

Statistical analysis

The comparison between the concentration of each sugar or amino acid and the analysed substances (phloem exudate, female honeydew and larvae honeydew) were done using the Kruskal–Wallis rank sum test followed by Dunn tests. All tests were performed using GraphPad Prism version 5.01 for Mac OS (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com) and applied under two-tailed hypotheses; the significance level p was set at 0.05.

Results

The average pH of the phloem exudate samples was 5.83 ± 0.18.

Sugars

The total sugar concentrations were 2223.24 mM, 151.59 mM and 661.53 mM for the pear tree phloem exudate, the female honeydew and the larvae honeydew, respectively. Four sugars were identified in the phloem exudates; the main sugar was sorbitol (61% of the total sugar concentration), followed by sucrose (17%), glucose (12%) and fructose (10%). All these sugars were also found in the honeydew of both females (sorbitol: 87%, glucose: 5%, fructose: 3%, sucrose: 5%) and larvae (sorbitol: 84%, glucose: 2%, fructose: 2%, sucrose: 12%) (Fig. 1). By comparing the sugar concentrations in the different analysed substances, we observed that sorbitol, fructose and glucose had significantly lower quantities in the larvae honeydew than in the pear tree phloem sap, with an intermediate value for the female honeydew (Table 1). Furthermore, glucose had a lower quantity in the honeydew of both larvae and adults than in the phloem sap (Table 1).

Amino acids

The total amino acid concentration was 5.60 mM in the pear tree phloem exudate and 18.66 mM and 13.40 mM in the female and larvae honeydew, respectively. Seventeen amino acids were found in the pear tree phloem exudate, with eight essential amino acids (arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine and valine) and one non-protein amino acid (GABA) among them (Fig. 2). The amino acid composition of the phloem exudates was mainly dominated by glutamic acid (26% of the total concentration) and aspartic acid (13%), followed by arginine, alanine, asparagine, threonine, serine, GABA, valine, glycine, glutamine and lysine, all situated between 1 and 10%. Isoleucine, leucine, phenylalanine, tyrosine and histidine had the lowest amino acid proportions, with values < 1%. The essential amino acids represented approximately 25% of the total amino acids. Finally, cysteine, methionine and tryptophan were not found in the phloem sap.

When we compared the concentration of each amino acid in the phloem sap with those in the two honeydew types, we observed that seven amino acids were present in significantly lower quantities in both the female and larvae honeydew than in the phloem exudate: serine, histidine, arginine, alanine, phenylalanine, leucine and lysine (Table 1) (Fig. 2).

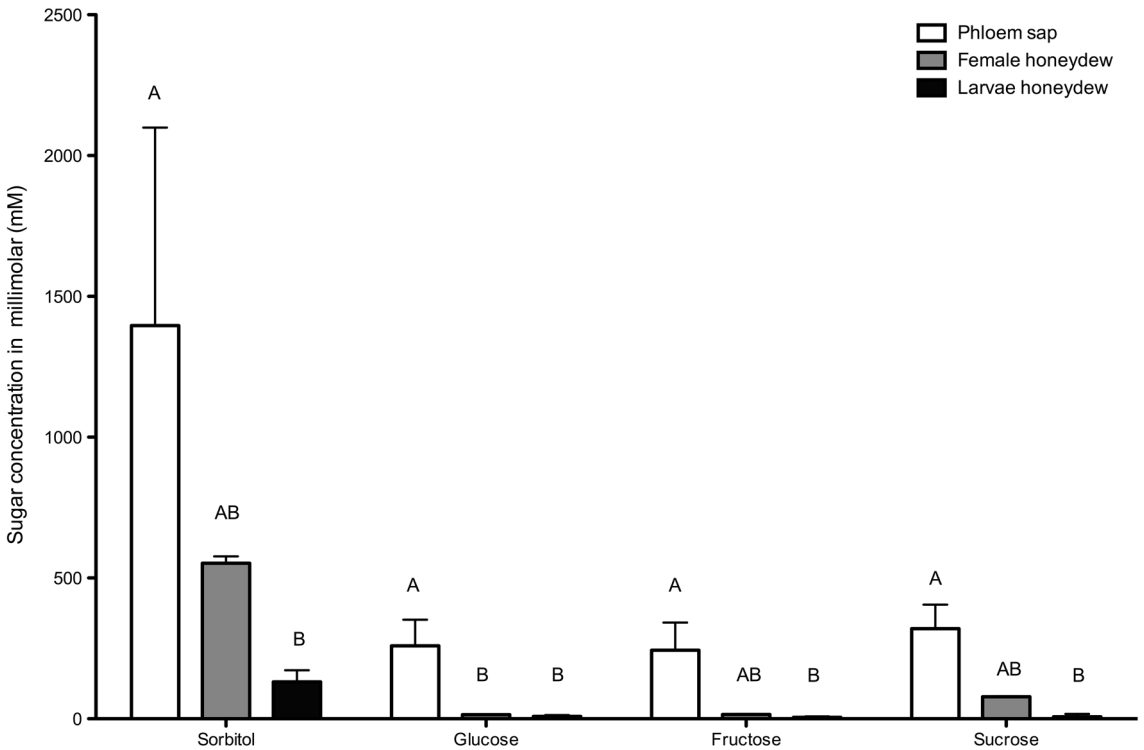

Fig. 1 Mean (± standard deviation) concentration of individual sugars in the pear tree phloem sap in white ($n = 12$), in the *C. pyri* female honeydew in grey ($n = 4$) and in the *C. pyri* larvae honeydew in black ($n = 4$). For each considered sugar, different letters represent significant differences according to the post hoc tests ($P < 0.05$)

Table 1 Results of the Kruskal–Wallis analysis of variances and multiple comparison Dunn's tests between the phloem sap, female honeydew and larvae honeydew for all sugars and amino acids

	Kruskal–Wallis results	Dunn post hoc tests		
		Phloem sap vs. female honeydew	Phloem sap vs. larvae honeydew	Female honeydew vs. larvae honeydew
Sorbitol	KW = 14.63, P < 0.001	P > 0.05	P < 0.01	P > 0.05
Glucose	KW = 13.94, P < 0.001	P < 0.05	P < 0.01	P > 0.05
Fructose	KW = 14.63, P < 0.001	P > 0.05	P < 0.01	P > 0.05
Sucrose	KW = 14.64, P < 0.001	P > 0.05	P < 0.01	P > 0.05
Aspartic acid	KW = 31.74, P < 0.001	P < 0.001	P > 0.05	P < 0.001
Glutamic acid	KW = 14.07, P < 0.001	P > 0.05	P < 0.01	P < 0.001
Asparagine	KW = 27.32, P < 0.001	P < 0.001	P < 0.01	P > 0.05
Serine	KW = 9.72, P < 0.01	P < 0.05	P < 0.05	P > 0.05
Histidine	KW = 24.48, P < 0.001	P < 0.001	P < 0.001	P > 0.05
Glutamine	KW = 28.02, P < 0.001	P < 0.001	P < 0.001	P > 0.05
Glycine	KW = 21.32, P < 0.001	P > 0.05	P < 0.05	P < 0.001
Threonine	KW = 0.84, P > 0.50	–	–	–
Arginine	KW = 8.54, P < 0.001	P < 0.05	P < 0.05	P > 0.05
GABA	KW = 14.51, P < 0.001	P > 0.05	P > 0.05	P < 0.001
Alanine	KW = 46.77, P < 0.001	P < 0.001	P < 0.001	P > 0.05
Tyrosine	KW = 7.54, P < 0.05	P > 0.05	P > 0.05	P < 0.05
Valine	KW = 13.10, P < 0.01	P < 0.01	P > 0.05	P < 0.05
Isoleucine	KW = 5.32, P > 0.05	–	–	–
Phenylalanine	KW = 46.78, P < 0.001	P < 0.001	P < 0.001	P > 0.05
Leucine	KW = 17.59, P < 0.001	P < 0.01	P < 0.001	P > 0.05
Lysine	KW = 40.07, P < 0.001	P < 0.001	P < 0.001	P > 0.05

Furthermore, we noted that histidine, phenylalanine and lysine were not found or were under the detection limit in the female and larvae honeydew. In contrast, the quantities of seven amino acids in both the larvae and the female honeydew were higher than or equal to those in the phloem exudate: asparagine, glutamine, glycine, threonine, arginine, GABA, tyrosine and isoleucine (Table 1) (Fig. 2). Finally, aspartic acid, glutamic acid and valine were present in lower quantities in the female honeydew than in the larvae honeydew and phloem exudate (Table 1) (Fig. 2).

Discussion

Our paper is the first to determine the pear tree phloem sap composition and to compare it with the composition of pear psylla honeydew. Our methodology allowed us to determine the part of the phloem sap used by the psyllid and the change in its composition from ingestion to egestion, therefore elucidating the nutritional requirements of the psyllid. Moreover, the knowledge about the honeydew composition may provide interesting insights into the role of the honeydew, particularly regarding its interactivity with natural enemies (Sabri et al. 2013).

According to the literature, phloem sap is generally neutral or alkaline. The low pH level we observed in our phloem exudate sample could be due to contamination by xylem (Kollar and Seemüller 1990; Dinant et al. 2010; Hijaz and Killiny 2014) and/or to the demineralised water used to rinse our samples, as atmospheric CO_2 easily dissolves in water to produce H_2CO_3. However, risks of xylem contamination were minimised by rinsing the bark samples with demineralised water, and the pH of this water, 6.60, could not have decreased the pH of the phloem exudate sample to a level of 5.80. Two other non-exclusive hypotheses may explain this pH level: (1) according to Hijaz and Killiny (2014), a high amount of organic acids could be responsible for a decrease in phloem sap pH. (2) Another explanation may be the presence of both sorbitol and boron in the pear tree phloem sap. Indeed, sorbitol, which is the main carbohydrate in Rosaceae (Zhang et al. 2014), and boron, which is an essential micronutrient required for all plant nutrition (Hu et al. 1997), are known to form a complex. This complex causes a decrease in pH (Böeseken 1949) and an increase in boron mobility in the plant (Brown and Hu 1996).

Concerning the sugar composition of our phloem exudate samples, the near-equal amounts of fructose and glucose suggest a release of plant sucrose invertase from the wounded surface of the bark, which transforms sucrose

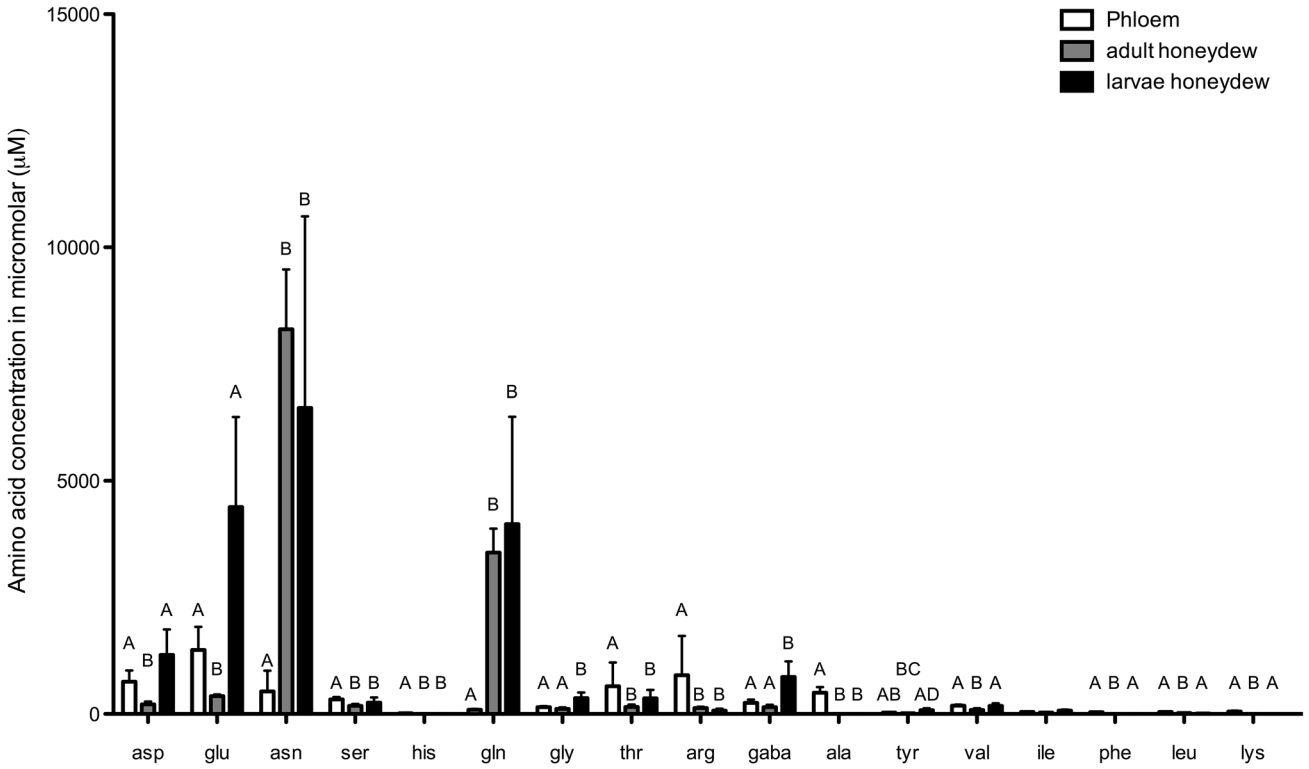

Fig. 2 Mean (±standard deviation) concentration of individual amino acids in the pear tree phloem sap in white ($n=12$), in the *C. pyri* female honeydew in grey ($n=18$) and in the *C. pyri* larvae honeydew in black ($n=18$). For each considered amino acid, different letters represent significant differences according to the post hoc tests ($P<0.05$)

into glucose and fructose (van Helden et al. 1994). We can, therefore, conclude that sorbitol and sucrose constitute the totality of the phloem exudate sugars, which corresponds to the results for phloem sap obtained from peach tree (Moing et al. 1992; Nadwodnik and Lohaus 2008) and pear (Zhang et al. 2014). However, the exceptionally high concentration of sugars, compared to the values in the literature, indicate that evaporation occurred in our phloem exudate samples. Collecting pure phloem sap samples through the stylectomy technique would help us better determine the composition of pear tree phloem sap.

From a qualitative point of view, the sugar composition of the honeydew was the same as that observed in our phloem sap samples, as it was composed of sorbitol sucrose, glucose and fructose. Sucrose ingested by phloem sap-feeding insects is hydrolysed to its monosaccharide constituents (glucose and fructose), and the fact that we observed the same amount of glucose and fructose in the honeydew of both the larvae and adults suggests that these two sugars are used in the same way. However, we do not know if the glucose and fructose in the honeydew samples were only there due to the psyllid alimentary tract or were also present because of sucrose invertase activity continuing after excretion due to the microbiota or a microbial contamination (van Helden et al. 1994; Leroy et al. 2011b). It would be interesting to analyse the bacterial and sugar compositions of honeydew over time to test this hypothesis and to better understand the pear psyllid food needs.

From a quantitative point of view, the total amounts of sucrose, fructose and glucose in the honeydew were low compared to those in the phloem sap samples. This clearly indicates that sucrose is consumed and assimilated by the psyllid as an energy source (Wilkinson et al. 1997). It can be used as a respiratory substrate and for the synthesis of other sugars, such as trehalose or mannitol (Vashishtha et al. 2013). The sorbitol, which was the main sugar found in the phloem exudates, was consumed by both the larvae and the females. However, it seems that this sugar was not assimilated in high quantities by the psyllids, as it represented more than 80% of the total sugar concentrations for both the larvae and the females. *C. pyri* presents two seasonal forms, a summer and a winter form; the former form was used in this study. *C. pyri* overwinters as an adult and is still very active at cold temperatures, as it terminates ovarian diapause mid-to-late winter and lays first-generation eggs at the end of winter (Schaub et al. 2005). Sorbitol is a compatible organic osmolyte that protects organisms from the cold (Hendrix and Salvucci 1998). The capacity for polyol accumulation may

change seasonally, and many insect species initiate polyol synthesis at low temperatures (Hance and Boivin 1993; Dhami et al. 2011; Sadeghi et al. 2012). It is, therefore, possible that *C. pyri* changes its feeding in winter to ingest and accumulate more sorbitol and increase its resistance to low temperatures, as has been shown in the common Pistachio psylla species, *Agonoscena pistaciae* (Sadeghi et al. 2012). Measuring the ingestion of sorbitol in the winter form of *C. pyri* could be an interesting next step for this study.

The lower amino acid than sugar concentration in the pear phloem exudates corresponds to the results for other plant species (Lohaus et al. 1998; Lohaus and Möllers 2000; Woodring et al. 2004; Lohaus and Schwerdtfeger 2014). However, we obtained a relatively low total concentration of amino acids compared to the values reported in the literature (Lohaus et al. 1998; Lohaus and Moellers 2000; Sandström and Moran 2001; Woodring et al. 2004). Psyllids, especially the larvae, produce a large quantity of honeydew (Civolani 2012), probably to fulfil their nitrogen needs (Wilkinson et al. 1997), and compensate for this low level of amino acids in the pear tree phloem sap. Concerning the amino acid composition, the patterns observed for the three analysed substances (Larvae honeydew, female honeydew and phloem sap) were completely different. Similar to the sugars, the difference in the amino acids found in the honeydew may be derived from the biotransformation by the psyllid or from the psyllid alimentary tract (van Helden et al. 1994). Moreover, if the psyllid digestion system is similar in psyllids and aphids, the amino acids found in the honeydew mainly come from alimentation (Febvay et al. 1999) and also from the biosynthesis activity of endosymbionts (Sandström and Moran 2001; Sloan and Moran 2012). Our results suggest that a suite of amino acids, serine, histidine, arginine, alanine, phenylalanine, leucine and lysine, was consumed by both the larvae and the adult females of *C. pyri*, as the amount of these amino acids was lower in their honeydews than in the phloem exudates. These seven free amino acids, including five essential amino acids, are probably used for protein synthesis, and they can also be used to maintain the free amino acid pool in tissues, as a respiratory substrate, or to synthesise other molecules (Sandström and Moran 1999, 2001). Moreover, it has been shown for *Phenacoccus herren* (Hemiptera: Pseudococcidae), another phloem sap-feeding insect, that the two main amino acids found in the phloem exudates, aspartic acid and the glutamic acid, have a primarily phagostimulatory function rather than a nutritive function (Calatayud et al. 2002). It could be interesting to test if this is also the case for *C. pyri*. Our study also revealed that, different from the two aphid species *Acyrthosiphon pisum* and *Megoura viciae*, which consume asparagine and glutamine (Leroy et al. 2011a), *C. pyri* seems to egest these two amino acids. Indeed, asparagine and glutamine were found in higher proportions in the honeydew than in the phloem sap for both the larvae and the females. This suggests that the psyllids received an oversupply of asparagine and glutamine from another source, which could be endosymbionts (Sandström and Moran 2001) or the insect itself. Indeed, it is possible that endosymbionts and/or the insects produce glutamate dehydrogenase and/or glutamine synthetase, the two main enzymes used to recycle ammonia waste into amino acids (Hansen and Moran 2014). Other essential amino acids can be then synthesised from glutamine (Leroy et al. 2011a).

Furthermore, we note that three amino acids were not present in the plant sap or were under the detection limit; of these, two are considered essential, methionine and tryptophan, and one is not, cysteine. This probably means that methionine and tryptophan, which are considered essential for insects, are synthesised by the pear psylla endosymbiont(s) at concentrations corresponding to the needs of their host. Indeed, endosymbionts can improve and complete insect diets by using their ability to produce amino acids (Sandström and Moran 1999). In aphids, the amino acid composition of the ingested phloem sap is completely different from that found in their tissues, and they need the biosynthetic capabilities of their endosymbiont to fulfil their dietary requirements (Sandström and Moran 2001). Discovering which endosymbiont(s) and which metabolic pathways are involved in the production of these two amino acids could be an interesting next step of this study.

The non-protein amino acid GABA is known to have a direct action on invertebrate neuromuscular junctions after assimilation and to reduce herbivory by pest phytophagous insects (Bown et al. 2006). A study by Bown et al. (2006) showed that the activity of glutamate decarboxylase, the cytosolic enzyme responsible for GABA synthesis, is optimal in an acidic environment, which seems to be the case of the pear tree phloem sap. It is therefore probable that pear trees develop a GABA defence against herbivory and that the larvae of *C. pyri* overcome this defence by not assimilating this amino acid and instead excreting it.

According to our results, three amino acids, valine, glutamic acid and aspartic acid, were consumed only by the adults and not by the larvae. These three amino acids are known to be present in the insect cuticle (Rockstein and Agosin 1978). Psyllid larvae, which differ according to developmental stage, present different colours than psyllid adults. Indeed, first to third instar larvae are creamy yellow, while, from the fourth instar larvae to the adult form, the individuals transition from greenish-brown to dark brown (Chang 1977). Moreover, larvae and adults present two different behaviours, as larvae spend most of their time eating on the tree covered by a drop of their own honeydew (Chang 1977), probably not only to avoid predation or parasitism but also to reduce the loss of water through the tegument. It is, therefore, possible that the sclerotinisation level of the cuticle differs between the adult and immature stages, and,

in *C. pyri*, the need for valine, glutamic acid and aspartic acid for pigmentation and sclerotinisation starts later in its development.

Concerning the consumption of glutamic acid only by the adult and not by the larvae, it has been shown in the locust species *Schistocerca gregaria* that this amino acid is a neurotransmitter for muscle 135 cd of the musculus extensor tibiae (jumping muscle), and it can be accumulated by the insect (van Marle et al. 1985). As adult psyllids, similar to adult locusts, are able to jump, it seems logical to think that adult psyllids are also able to accumulate glutamic acid and use it as a neurotransmitter. However, this hypothesis remains to be tested. A final explanation concerning the assimilation of these three amino acids by the adults and not by the larvae is that they participate in the synthesis of proteins and in abilities that only concern adults, such as flying or reproduction.

In conclusion, this paper shows the pear tree phloem sap composition at the sugar and amino acid level and links it to the nutrition physiology of *C. pyri*. In particular, we highlighted the nutrients included in the diet of *C. pyri*, with sucrose as the main sugar ingested by the psyllid and with seven amino acids, including five essential amino acids, serine, histidine, arginine, alanine, phenylalanine, leucine and lysine. Furthermore, we suggest that at least four amino acids are synthesised by psyllid endosymbionts; these are methionine and tryptophan, essential amino acids and that were not found in the phloem sap, and asparagine and glutamine, which were massively egested in the honeydew. We also underline different roles for three amino acids (valine, glutamic acid and aspartic acid) in the adult females and the larvae, as they were only consumed by the adult females; this indicates they participate in abilities that only concern the adults, not the larvae (flying, jumping or reproduction).

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