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How Do Free-Living, Lerp-Forming, and Gall-Inducing Aphalaridae (Hemiptera: Psylloidea) Affect the Nutritional Quality of *Eucalyptus* Leaves?

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Abstract

About 380 described species of Psylloidea occur on *Eucalyptus* in Australia. These show differences in diversification, feeding behavior, and apparent patterns of development. We analyzed the quality of nutrients used by three species of Aphalaridae belonging to different feeding guilds on three species of *Eucalyptus*. We evaluated the quantity and quality of total nonstructural carbohydrates (TNC), $\delta^{13}C$ and $\delta^{15}N$ isotope ratios, amino acids, and fatty acids. In general, TNC levels were greater in infested leaves than in uninfested leaves of the three species of *Eucalyptus*. TNC levels in the leaves of *E. macrorhyncha* F. Mueller ex Bentham were the highest and in *E. globulus* Labillardière the lowest. Total masses of amino acids and fatty acids were the greatest in the leaves of *E. globulus* infested by *C. eucalypti* (Maskell), followed by leaves of E. sideroxylon A. Cunningham ex. Woolls infested by the species of *Glycaspis* Taylor, and the lowest values were in the leaves of *E. macrorhyncha* infested by species of *Synglycaspis* Moore. In general, $\delta^{13}C$ increased in *C. eucalypti*-infested leaves of *E. globulus*, *Glycaspis* sp. infested leaves of *E. sideroxylon,* and *Synglycaspis* sp. infested leaves of *E. macrorhyncha*. Nitrogen-isotope ratios ($\delta^{15}N$ values) were not significantly different in infested and uninfested leaves. The free-living *C. eucalypti* stress *E. globulus* leaves more intensely by its group-feeding behavior, whereas the gall-inducing species of *Synglycaspis* stresses *E. macrorhyncha* leaves in such a way to elicit a response with a novel phenotypic expression, *viz.*, the gall. The lerp-forming species of *Glycaspis* utilize nutrients, especially sugars, the excess of which is secreted to build their characteristic lerp.

Keywords: amino acid, carbon, fatty acid, infested leaf, nitrogen

Dietary requirements of herbivorous insects vary in quality and quantity depending on their developmental stage. Immature stages of Hemiptera have been shown to require greater quantities of amino acids to build structural proteins necessary for metamorphosis compared with the adults (Offor 2010). Insect infestation modifies nutrient mobilization in plants. Either enhancement or decline in the photosynthetic efficiency after insect infestation can arise due to pressure placed on plants by feeding insects: one of them being able to produce defense compounds (Schwachtje and Baldwin 2008). Insect feeding on leaves increases carbon allocation to roots, whereas insect feeding on roots increases nitrogen allocation to shoots (Schmidt et al. 2009). Plant response varies when fed upon by different insects. For example, sap-sucking insects, such as *Bemisia tabaci* (Hemiptera: Aleyrodidae) feeding by on *Gossypium hirsutum* (Malvaceae), results in an increase in sucrose concentration (Schmidt et al. 2009), whereas no change in glucose, fructose, and sucrose occurs in *G. hirsutum* infested by *Aphis gossypii* (Hemiptera: Aphididae) (Gomez et al. 2006). Overall, insect feeding influences, both positively and negatively, the photosynthetic rates and efficiency in plants and also alters the quality and quantity of primary metabolites at the feeding sites (Duceppe et al. 2012). For example, a positive correlation occurs in the abundance of the immatures of *Ctenarytaina eucalypti* (Hemiptera: Psylloidea) and concentrations of methionine, valine, and threonine at the feeding sites on leaves of *Eucalyptus globulus* (Myrtaceae) (Steinbauer 2013).

Few insects attack *Eucalyptus* foliage because of low levels of dietary nitrogen and high levels of essential oils and phenolic compounds (Lawler and Foley 2002). Yet, about 400 species

of Psylloidea live on different species of *Eucalyptus* in Australia. The Aphalaridae is a highly diversified family in Australia with about 250 described species in 19 genera (Hollis 2004, Ouvrard 2014). The Aphalaridae live as free-living, lerp-forming, or gall-inducing taxa on various host plants, although a majority of them live on species of *Eucalytpus* and *Acacia* (Fabaceae). Free-living Aphalaridae generally feed on multiple plant taxa, whereas the gall-inducing taxa usually remain tied to a single plant species. Among the lerp-forming species, some are specific to particular hosts (e.g., *Glycaspis atkinsoni*) and a few others are not. This range of guilds evident in the Aphalaridae points to differences in their nutritional requirements and feeding patterns.

We know more about the lerp-forming Psylloidea than the free-living and gall-inducing taxa among the Australian Psylloidea. White (1969) and Taylor (1997) studied the lerp-forming *Cardiaspina densitexta* and *Cardiaspina albitextura* (both Aphalaridae) in South Australia and indicated that these species reach high populations through periodical cyclical outbreaks. White (2009) explains that population outbreaks of lerp-forming Aphalaridae feeding on mature—old leaves (described as 'senescence feeders') occur as a response to physiological changes in plants stressed by a variety of environmental factors. White (2009) further explains that the plant-growth vigor influences population build up in the Aphalaridae, which feed on tender leaves (described as 'flush feeders'). 'High' and 'very high' abundances of immatures of the lerp-forming *Cardiaspina* nr. *densitexta* living on *E. moluccana* leaves have been shown to be associated with elevated concentrations of amino N, whereas 'very high' abundance of immatures has been related to high levels of essential amino acids: isoleucine, leucine, lysine, tryptophan, valine, and threonine (Steinbauer et al. 2014). Steinbauer et al. (2014) further indicate that the immatures of Psylloidea benefit by mobilizing amino N at the feeding sites (nutrition sinks), which occurs when densities of the immatures are 'low' to 'moderate'/leaf, rather than 'high' (Steinbauer et al. 2014). (The terms 'immature' and its plural form 'immatures' and 'instars' instead of 'nymphal instars' have been used in this paper, following the new terminology proposed by Burckhardt et al. [2014].)

Little is known of the feeding biologies and nutritional requirements of the Psylloidea interacting with various species of *Eucalyptus*, except our previous work (Sharma et al. 2013, 2015a,b) and those of White (1969, 1970, 1986, 2009), Taylor (1997), Steinbauer (2013), and Steinbauer et al. (2014). Therefore, in this study, we evaluated the primary metabolites in the leaves of *Eucalyptus globulus globulus* (hereafter, *E. globulus*), *Eucalyptus sideroxylon* 'Rosea' (hereafter, *E. sideroxylon*), and *Eucalyptus macrorhyncha* to characterize the nutritional requirements of the immatures of three selected Aphalaridae, which live on species of *Eucalyptus*: the free-living *C. eucalypti*, an unnamed species of lerp-forming *Glycaspis* (*Glycaspis)* (hereafter, *Glycaspis* sp.), and an unnamed species of gall-inducing *Glycaspis* (*Synglycaspis)* (hereafter, *Synglycaspis* sp.). We report the quantity and quality of primary metabolites in the leaves of *E. globulus* (*C. eucalypti*), *E. sideroxylon* (*Glycaspis* sp.), and *E. macrorhyncha* (*Synglycaspis* sp.). The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio measurements have also been done to provide a comprehensive picture of C and N mobilization to feeding sites.

C. eucalypti, the species of *Glycaspis* and of *Synglycaspis* sp. chosen here occur in the Orange subregion of the South-eastern Highlands Bioregion of New South Wales (Government of New South Wales 2011). *C. eucalypti* live freely secreting micro quantities of honeydew. The generalist *C. eucalypti* principally feed on *E. globulus* and a few related taxa, such as *Eucalyptus bicostata*, *E. camaldulensis,* and *E. pulverulenta* (Hollis 2004). They complete 4–5 overlapping generations a year, feeding on juvenile leaves (0.5–2 cm long). The first and second instars feed on the mesophyll parenchyma, whereas the third, fourth, and fifth instars feed on phloem (Sharma et al. 2015a). *C. eucalypti* feed as groups (100–150 individuals of adults and immatures) on the same leaf and on new flushes of juvenile leaves of *E. globulus* (Sharma et al. 2015a). The species of *Glycaspis* construct 'lerps' using sugary exudates on the leaves of *E. sideroxylon* and *E. leucoxylon*. This specialist species complete 3–4 overlapping generations a year, feeding as solitary individuals. The first and second instars feed on parenchyma, whereas the third, fourth, and fifth instars feed on phloem (Sharma et al. 2013). The species of *Synglycaspis* induce spherical, ostiolate, pouch galls on the leaves of *E. macrorhyncha*. This specialist species completes two generations a year. The first instar initiates the gall on a young leaf (0.5–1 cm long; not a 'juvenile' leaf). The first and second instars feed on gall parenchyma, whereas the third, fourth, and fifth instars feed on gall phloem. All instars occur singly in galls. During occupation of galls by the second, third, and fourth instars this species secretes sugary exudates, which plug the gall ostioles (Sharma et al. 2015b).

Keeping the above context in view, we assayed the levels of primary metabolites (total carbohydrates, amino acids, fatty acids, C and N isotopic ratios [$\delta^{13}C$ and $\delta^{15}N$]) in the leaves of *Eucalyptus* hosting the group-feeding generalist *C. eucalypti*. We expected the leaf biochemistry to be different to that of the leaves of *Eucalyptus* hosting the solitary-feeding, lerp-forming specialist species of *Glycaspis,* and the solitary-feeding, gall-inducing specialist species of *Synglycaspis*. We further expected that the leaves infested by the lerp-forming *Glycaspis* sp. will be different in levels of primary metabolites compared with the leaves infested by the gall-inducing *Synglycaspis* sp. Therefore, we addressed the following research questions: 1) How do levels of primary metabolites vary in *Eucalyptus* leaves infested by the group-feeding *C. eucalypti* compared with the solitary-feeding *Glycaspis* sp. and *Synglycaspis* sp.? 2) How do levels of primary metabolites vary in *Eucalyptus* leaves infested by the free-living generalist *C. eucalypti* and the lerp-forming specialist *Glycaspis*, and gall-inducing specialist *Synglycaspis*? 3) Does any variation occur in the levels of primary metabolites in *Eucalyptus* leaves when infested by specialized habitat constructing *Glycaspis* (lerps) and *Synglycaspis* (galls)?

Materials and Methods

Study Site, Sampling Periods, and Numbers

Seeds of *E. globulus* and *E. sideroxylon* were collected from trees growing in recreation parks and along road verges in Orange (33° 17′ S, 149° 06′ E; elevation 860 m, annual mean rainfall 120 cm, annual mean summer [December–February] temperature 18°C and mean winter [June–August] temperature 5°C). The collected seeds were verified for their identity and confirmed by Colin Bower (Regional Botanist and Principal, *Flora*Search, Orange) before establishing them in the glasshouse. Plants were germinated in commercial seed-raising mix beds (Debco Pty Limited [Tyabb, Victoria, Australia]). Individual seedlings were subsequently transplanted into plastic pots (20 cm diameter) containing the commercial potting mix (Debco). One hundred individuals of 90-d old seedlings (~60 cm long shoots bearing 50–60 leaves) of *E. globulus* and *E. sideroxylon* were maintained at 23 ± 2°C and 60 ± 5% relative humidity (RH) under natural-light conditions in Charles Sturt University (Orange) [CSU–O]

glasshouse. Field-caught adults of *C. eucalypti* and *Glycaspis* sp. using a sweep net were released onto the established seedlings maintained in cages (BugDorm; BD2400 [MegaView Science Co., Ltd., Taichung, Taiwan]; L75 by W75 by H115 cm) to establish colonies. Because 4–5 overlapping generations of *C. eucalypti* occur in a year resulting in immatures and adults cohabiting, leaf material for *E. globulus* was pooled (uninfested vs. infested, each $n = 30$). Uninfested and infested leaves of *E. globulus* and *E. sideroxylon* were collected from seedlings raised in the glasshouse. The uninfested leaves were collected from seedlings maintained in separate cages, whereas the infested leaves were collected after 30 d, particularly after the aphalarid population numbers stabilized. Before biochemical evaluations, the collected leaves were cleaned using a hand-held sterile-water jet to remove insect secretions and the exuviae. Because immatures of *C. eucalypti* feed on the juvenile leaves of *E. globulus*, only juvenile leaves (0.5–2 cm long) were used in this study. The immatures and adults of the lerp-forming *Glycaspis* feed only on the mature leaves of *E. sideroxylon*, hence mature leaves (12–14 cm long) were used. Because the immatures of *Glycaspis* feed singly on leaves of *E. sideroxylon*, the leaf materials bearing actively feeding immatures (each developmental stage $n = 10$) and comparable uninfested leaf materials ($n = 10$) were used.

Galls induced by the species of *Synglycaspis* on the leaves of *E. macrorhyncha* were collected from Mullion Range State Conservation Area (33° 07′ S, 149° 07 ′E; elevation 870 m, Southeastern Highlands Bioregion) in October 2013–March 2014. The galls were classed into five categories using the developmental stage of the immature inhabiting the gall and size of the gall ($n = 100$). Uninfested leaves, without galls, occurring closest to gall-bearing leaves were used as control and care was exercised in their selection to avoid damaged materials. Because this species of *Synglycaspis* induces galls individually on the leaves of *E. macrorhyncha*, leaves bearing galls ($n = 10$, each developmental stage) and comparable uninfested leaf materials ($n = 10$) were used.

Sample Preparation

Leaf samples (juvenile leaves of *E. globulus* and mature leaves of *E. sideroxylon* from the glasshouse-reared seedlings, and young leaves of *E. macrorhyncha* from field) were collected in polyethylene bags and stored in an ice chest and transported to CSU–O, where they were stored immediately in 5–10 cm long ziplock polyethylene bags at −80°C in a deep-freezer. Samples were lyophilized (Labconco Freeze Drier, Labconco, Kansas), ground (Micro Hammer and Cutter Mill; Glen Creston ltd. London, United Kingdom), and stored in labelled glass vials. The dried samples were analyzed at the Department of Biochemistry, University of Nebraska, Lincoln, USA.

Nonstructural Carbohydrates

The quantitative determination of reducing carbohydrates was done following Somogyi–Nelson alkaline–copper method (Raman et al. 2006). Fifty milligrams of each of freeze-dried, uninfested, and infested leaves of *E. globulus*, *E. sideroxylon,* and *E. macrorhyncha* was transferred into a graduated measuring cylinder. Polysaccharides were hydrolyzed by adding 12.5 ml of 0.02 M benzoic acid and autoclaved for 20 min two times. The quantity of the solute lost due to evaporation during autoclaving was adjusted to 12.5 ml by adding deionized water. To standardize the quantity of amylase–amyloglucosidase mixture, 100, 250, and 500 µl from each sample (three replicates) were removed from the autoclaved solutions and diluted to 750 µl with 50 mM potassium acetate (pH 5) and 0.02 M benzoic acid. The samples were incubated at 42°C for 15 h after adding 250 µl of amylase–amyloglucosidase mixture (Muir and O'Dea 1992). Aliquots of 200, 250, and 500 µl from the three replicates of each sample were made up to 1 ml with deionized water. By adding appropriate reagents following Rose et al. (1991), the prepared samples were boiled for 10 min and cooled in an ice-cold water bath for 15 min. Absorbance of each sample was recorded at 600 nm in an UV-spectrophotometer (UV-1800, Shimadzu Scientific Instruments, Columbia, MD).

Isotopic Carbon and Nitrogen Ratios

$\delta^{13}C$, $\delta^{15}N$ concentrations were determined using dried leaf samples (please see 'Sample preparation' above) with an elemental analyzer (Carlo Erba EA—1108, CE Elantech, Lakewood, NJ) interfaced with an isotope-ratio mass spectrometer (Delta Plus, Thermo Electron Corporation, Waltham, MA) operating in continuous-flow mode. Both ^{13}C, ^{15}N ratios are presented in δ notation, following the equation:

$$\delta = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3$$

For $\delta^{13}C$ ratios, R_{SAMPLE} is the $^{13}C/^{12}C$ ratio of the sample and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard (Coplen 1996). For $\delta^{15}N$ ratios, R_{SAMPLE} is the $^{15}N/^{14}N$ ratio of the sample and R_{STD} is the $^{15}N/^{14}N$ ratio of the atmospheric dinitrogen (Mariotti 1983). Precision of duplicate measurements of $\delta^{13}C$ was ±0.1‰ and for $\delta^{15}N$ was <0.2‰. This analysis was done on single samples.

Amino Acids

Free amino acids were extracted from freeze-dried leaf tissue (100 mg) by vortexing in 2.5 ml of methanol–aqueous 0.01 N HCl (70:30 v:v) at 22°C for 60 min. The solids were pelletized by centrifugation at 748 × *g* (2,500 rpm) for 5 min. The supernatant containing the free-amino acids was transferred to clean, sterile glass tubes. The supernatants were added to 0.5 ml 50–100 mesh DOWEX 50x8 cation-exchange resin columns (Sigma-Aldrich, St. Louis, MO), following the method used by Gachomo et al. (2009). This was followed by two deionized water-washes (0.5 ml each wash) for eluting free amino acids with two subsequent washings each time using 0.5 ml of 3N aqueous NaOH. The eluate was dried in a speed-vacuum concentrator (Thermo Scientific, Waltham, MA) suspended in 100 µl of 0.01 N HCl to convert amino acids to chlorides and dried again using the speed-vacuum concentrator. The isolated amino acids were derivatized by heating at 100°C for 45 min in 50 µl of pyridine and 50 µl of N−tertiary–butyldimethylsilyl–N−methyl-trifluoroacetamide with 1% tertiary–butyldimethyl-chloro-silane (MSTFBA). The samples were subsequently evaporated in a nitrogen evaporator (Organomation, Berlin, MA) at 22°C until approximately 20 µl of pyridine remained in the 100 µl glass-microvolume inserts (Agilent Technologies, Santa Clara, CA), which were placed in crimp-top vials for analysis in the GC–MS (6890/5973 GC–MS system, MSD, 30 m by 0.25 mm DB5 column, Agilent Technologies). Inlet temperature of the GC–MS was 250°C and He (the carrier gas) was maintained at a constant flow of 0.7 ml min^{-1} for a total run time of 25 min. The oven temperature was initially maintained at 100°C for 1 min, then ramped at 15°C min^{-1} and held at 320°C (final temperature) for 10 min. The trimethyl-silyl derivatives of free amino acids were identified from the retention times of authentic standards and verified using a NIST (National Institute of Standards and Technology) compound-library database. This analysis was done on single samples.

Fatty-Acid Methyl Esters

Extracts of freeze dried and ground leaf-tissue samples (20 mg each) were obtained following Bligh and Dyer (1959) with modest modifications (explained in the following section). Dried samples were first rehydrated by treating with 1 ml of ice-cold water for 10 min retained on crushed ice followed by the removal of the 1 ml of water by centrifugation at 748 × *g* (2,500 rpm) for 5 min. Five milliliters aliquots of methanol–chloroform (2:1) containing 0.01% butylated hydroxytoluene (Sigma–Aldrich) were added, the headspaces replaced with Ar, and the samples were vortexed using a multi-vial vortexer (Fisher Scientific, Waltham, MA) at 30 g (500 rpm) for 60 min in polytetrafluoroethylene-lined screw-cap vials. An internal fatty-acid standard (17:1Δ10) was added as 100 µg of triglycerides (17:1/17:1/17:1) (triheptadecenoin; Nu-Check Prep, Elysian, MN,) per sample prior to vortexing. Lipids were isolated by solvent partitioning using 2 ml of aqueous KCl solution (0.08 %) and 2 ml chloroform. After vortexing for 10 s, the samples were centrifuged at 748 × *g* (2,500 rpm) for 5 min. The organic-solvent layer was separated using a disposable glass pipette (1 ml) and evaporated under a stream of N_2 at 45°C using the nitrogen evaporator. Fatty-acid methyl esters (FAMEs) were generated following Morrison and Smith (1964). The samples were first saponified in 1 ml of a 0.5 M methanolic KOH solution for 5 min on a heat block set to 100°C, followed by an additional 5 min incubation at 100°C with 1 ml BF_3 (12%) in methanol (Sigma–Aldrich). The addition of 0.5 ml hexane and 2 ml of saturated aqueous NaCl solution to the samples was followed by a 5-s vortexing and centrifugation at 748 × *g* (2,500 rpm) for 5 min. The hexane layers were separated using disposable glass pipettes (1 ml) transferred to crimp-top glass vials (5 ml) for analysis using a GC–MS.

GC–MS analysis of FAMES was performed using a gas-chromatograph system (7890A, Agilent Technologies) and a mass spectrometer (5975C VL Agilent Technologies) coupled with a triple-axis detector. Splitless 1 µl injections were volatized at 250°C and separated using a 'Select FAME' column (CP7421; 200 m by 271 µm by 0.25 µm; Agilent Technologies) using a constant He flow at an inlet pressure of 427.47 kilopascals. The oven temperature was ramped as follows: at 130°C held for 10 min and the temperature was raised by 10°C min^{-1} until 160°C; at 160°C held for 7 min and increased by 10°C min^{-1} until 190°C, which was held for 7 min; 220°C was reached with an increase of 10°C min^{-1} held for 22 min, and increased 10°C min^{-1} until the final temperature of 250°C was reached. This was held so for the next 17 min. Peak identity was assessed using NIST library and retention times of authentic FAME standards.

Statistical Analysis

Data pertaining to $\delta^{13}C$—$\delta^{15}N$ ratios, total-nitrogen content, amino acids, and fatty acids were analyzed using one-way ANOVA and comparisons among different stages and species were separated applying Tukey's separation to analyze the significance between the compared values. For overall comparisons between the three systems (*C. eucalypti–E. globulus*, *Glycaspis* sp.*–E. sideroxylon*, and *Synglycaspis* sp.*–E. macrorhyncha*), the host-plant data were pooled for the five instars of *Glycaspis* on *E. sideroxylon* and *Synglycaspis* on *E. macrorhyncha*, whereas the data for *C. eucalypti* were already combined because all developmental stages co-occur on the same juvenile leaf. To evaluate the relationship between tested primary-metabolite values in pooled data of the three systems, simple correlation values were generated. All analyses were carried out in GenStat (VSN International 2013), and graphs were prepared using Microsoft Office Excel 2007.

Table 1. Tukey's separation of TNC, $\delta^{13}C$, $\delta^{15}N$, amino acids, and fatty acids in *E. globulus–C. eucalypti* (free living), *E. sideroxylon–Glycaspis* sp. (lerp forming), and *E. macrorhyncha–Synglycaspis* sp. (gall inducing)

Host system	TNC	$\delta^{13}C$	$\delta^{15}N$	Amino acids	Fatty acids
E. globulus (U)	0.148^{b}	-30.03^{b}	-1.96^{b}	$1{,}568^{ab}$	0.212^{a}
E. globulus (I)	0.151^{b}	-27.98^{ab}	0.90^{b}	$6{,}415^{a}$	0.268^{a}
E. sideroxylon (U)	0.144^{b}	-29.99^{b}	5.68^{a}	485^{b}	0.105^{b}
E. sideroxylon (I)	0.164^{b}	-29.68^{b}	5.63^{a}	$1{,}258^{b}$	0.101^{b}
E. macrorhyncha (U)	0.202^{b}	-28.23^{ab}	-5.46^{c}	$2{,}989^{ab}$	0.117^{b}
E. macrorhyncha (I)	0.273^{a}	-27.23^{a}	-6.04^{c}	780^{b}	0.087^{b}

U: uninfested leaves; I: infested leaves.
Superscripted letters 'a', 'b', 'ab', 'c' represent Tukey's grouping.

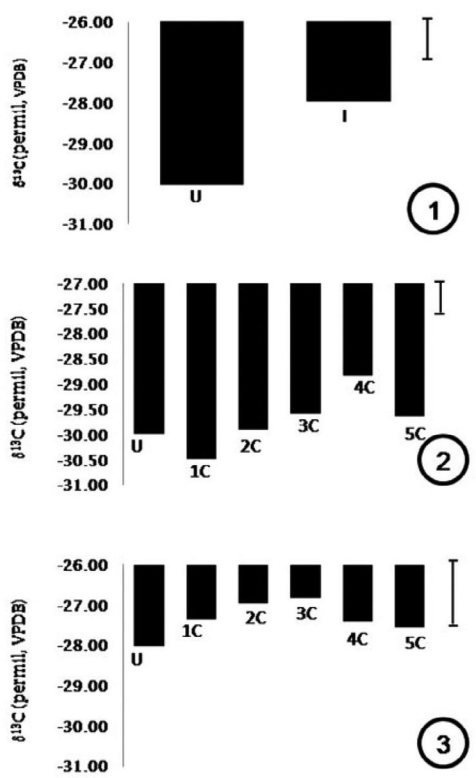
Results

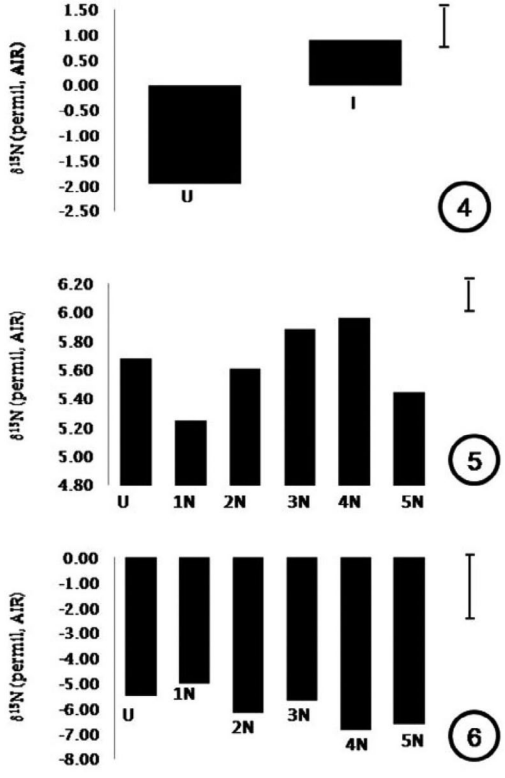
Total Nonstructural Carbohydrates

Overall, total nonstructural carbohydrates (TNC) values did not differ significantly in uninfested and infested leaves of *E. globulus* (*C. eucalypti*), *E. sideroxylon* (*Glycaspis*), and *E. macrorhyncha* (*Synglycaspis*) (Table 1). However a significantly consistent difference in TNC values occurs in *E. sideroxylon* when fed on by the second to fifth instars of *Glycaspis* ($P = 0.013$. $F_{1,4} = 18.05$, Table 2). TNC values rise significantly ($P \leq 0.06$; $F_{1,5} = 5.51$) in *E. macrorhyncha* particularly during metamorphosis of the third to the fourth instars of *Synglycaspis*, although, thereafter the values remain consistent (Table 2). Comparing the three systems, the TNC contents in the pooled-data populations of uninfested and infested leaves of *E. globulus*, *E. sideroxylon*, and *E. macrorhyncha* differ significantly ($P < 0.001$; $F_{5,8} = 32.25$; Table 1). TNC values in both uninfested and infested leaves of *E. macrorhyncha* are significantly greater than the infested and uninfested leaves of *E. globulus* and *E. sideroxylon*.

Isotopic Ratios

Negative $\delta^{15}N$ values indicate that the leaf samples generally have a lower ^{15}N–^{14}N ratio than the external atmosphere. Negative $\delta^{13}C$ value means a decrease in primary production and release of plant-based carbon (McCutchan et al. 2003). $\delta^{13}C$ values in uninfested and infested leaves of *E. sideroxylon* (*Glycaspis*) and *E. macrorhyncha* (*Synglycaspis*) do not differ significantly, but in *E. globulus* (*C. eucalypti*) the infested leaves are 2‰ more positive (Table 1 and Figures 1–3). When $\delta^{13}C$ values among uninfested and infested leaves of *E. globulus*, *E. sideroxylon*, and *E. macrorhyncha* are compared with each other, $\delta^{13}C$ values varied significantly ($P < 0.001$; $F_{5,8} = 17.72$). The overall values in the uninfested and infested leaves of *E. macrorhyncha* are greater than the comparable contexts of *E. globulus* and *E. sideroxylon*. Nitrogen-isotope ratios as indicated by $\delta^{15}N$ in uninfested and infested leaves of *E. sideroxylon* (*Glycaspis*), and *E. macrorhyncha* (*Synglycaspis*) do not differ significantly. However, in *E. globulus* (*C. eucalypti*) the infested leaves are 2‰ more positive (Table 1 and Figures 4–6). A comparison of $\delta^{15}N$ values among uninfested and infested leaves of *E. globulus*, *E. sideroxylon*, and *E. macrorhyncha* shows significant differences ($P < 0.001$; $F_{5,8} = 253.56$). However, $\delta^{15}N$ values do not present any discernible pattern among the three species.





Figures 1–3: $\delta^{13}C$ values in uninfested (U) and infested (I) leaves of *Eucalyptus globulus*, *E. sideroxylon,* and *E. macrorhyncha*. (**1**) *Eucalyptus globulus–C. eucalypti* (free living) system. (**2***) Eucalyptus sideroxylon–Glycaspis* (lerp forming) system. 1C–5C: five developmental stages of *Glycaspis.* (**3**) *Eucalyptus macrorhyncha–Synglycaspis* (gall inducing) system 1C—5C: five developmental stages of *Synglycaspis*.

Figures 4–6: **$\delta^{15}N$ values in uninfested (U) and infested (I) leaves of *Eucalyptus globulus*, *E. sideroxylon,* and *E. macrorhyncha***. (**4**) *Eucalyptus globulus–C. eucalypti* (free living) system. (**5**) *Eucalyptus sideroxylon–Glycaspis* (lerp forming) system. 1N—5N: five developmental stages of *Glycaspis*. (**6**) *Eucalyptus macrorhyncha–Synglycaspis* (gall inducing) system. 1N—5 N: five developmental stages of *Synglycaspis*.

Table 2. TNC (nmol/gm^{-1}) in *E. globulus–C. eucalypti* (free living), *E. sideroxylon–Glycaspis* (lerp forming), and *E. macrorhyncha–Synglycaspis* (gall inducing)

E. globulus–C. eucalypti		*E. sideroxylon–Glycaspis*						*E. macrorhyncha–Synglycaspis*					
U	I	U	I					U	I				
			1	2	3	4	5		1′	2′	3′	4′	5′
0.16	0.15	0.14	0.17	0.16	0.16	0.17	0.16	0.20	0.25	0.26	0.26	0.29	0.30

U: uninfested leaves; I: infested leaves.
1, 2, 3, 4, 5: leaves infested by populations of the 1, 2, 3, 4, and 5 instars.
1′, 2′, 3′, 4′, 5′: galls harboring populations of the 1, 2, 3, 4, and 5 instars.

Amino Acids

Compared with uninfested leaves of *E. globulus*, the number and total mass of free amino acids in the infested leaves of *E. globulus (C. eucalypti*) are not significantly different (Table 1). In the infested leaves of *E. globulus* the detected amino acids occur in the order: aspartic acid>alanine>serine>proline>threonine>leucine=phenyl alanine=glycine (Table 3). In the infested leaves of *E. sideroxylon* (*Glycaspis*) the detected free amino acids occur in the order: glycine>alanine>glutamine>serine>aspartic acid>valine>leucine> isoleucine=proline>leucine (Table 3). The types of free amino acids are more and their total mass is greater in the infested leaves of *E. sideroxylon* (Table 3). Particularly the total-mole percent of glycine increases in leaves infested by the first, second, and third instars, but decreases sharply in leaves infested by the fourth and fifth instars. In the infested leaves of *E. macrorhyncha* (*Synglycaspis*) the detected free amino acids are present in the order: glycine>leucine> alanine>serine>aspargine>glutamine> proline> isoleucine=tyrosine>lysine=valine>methionine (Table 3). Overall, a significant difference ($P < 0.1$; $F_{1,5} = 4.59$) occurs between uninfested and infested leaves of *E. macrorhyncha*.

When the three host systems are compared, a significant difference occurs in the total mass of free amino acids in *E. globulus* (*C. eucalypti*), *E. sideroxylon* (*Glycaspis*), and *E. macrorhyncha* (*Synglycaspis*) ($P \leq 0.05$; $F_{5,8} = 5.81$, Table 1). In the infested leaves of *E. globulus* and *E. sideroxylon* hosting *C. eucalypti* and *Glycaspis,* respectively, the total mass of free amino acids is greater when compared with uninfested leaves, whereas the total mass drops in infested leaves of *E. macrorhyncha*.

Fatty Acids

Total fatty-acid mass is not significantly different in infested and uninfested leaves of *E. globulus* (*C. eucalypti*) (Table 1). The total-mole percentage of saturated fatty acids is nearly the same in both uninfested and infested leaves of *E. globulus* and occurs in the following order: Arachidic acid=palmitoleic acid<stearic acid <oleic acid<linoleic acid<palmitic acid<α–linolenic acid. The mole percent of unsaturated fatty acids is elevated in infested leaves, with the exception of linoleic acid (Table 4). Significant differences are not apparent in uninfested and infested leaves of *E. sideroxylon* (*Glycaspis*) but, contrary to *E. globulus* (*C. eucalypti*) the total mass of fatty acids is lower in infested leaves (Table 1). Mole percent of saturated fatty acids is high in infested *E. sideroxylon* (*Glycaspis*) leaves. The mole percent values of unsaturated fatty acids are similar in both uninfested and infested leaves of *E. sideroxylon* (Table 4).

Total fatty-acids mass is not significantly different in uninfested and infested *E. macrorhyncha* leaves (*Synglycaspis*). However a steady decline in total fatty-acids mass in leaves hosting populations of the first to fifth instars occurs (Table 4). Total mass is significantly different in leaves infested by all of the instars ($P = 0.061$; $F_{2,4} = 6.09$). Among infested leaves of *E. macrorhyncha*, the mole percentages of oleic acid and linoleic acid are the greatest in leaves fed by the first instar, whereas the mole percent of palmitoleic acid is the lowest in leaves fed by the fifth instar. A significant difference occurs in the total mass of fatty acids in *E. globulus* (*C. eucalypti*), *E. sideroxylon* (*Glycaspis*), and *E. macrorhyncha* (*Synglycaspis*) ($P < 0.001$; $F_{5,8} = 27.53$; Table 1). The total mass increases in infested *E. globulus* leaves, whereas in the other two systems (*E. sideroxylon–Glycaspis*; *E. macrorhyncha–Synglycaspis*) the total mass of fatty acids decreases in infested leaves.

Correlation Among Tested Metabolites

TNC in uninfested and infested *E. globulus, E. sideroxylon*, and *E. macrorhyncha* leaves correlated positively and significantly with $\delta^{13}C$ and negatively—but significantly—with $\delta^{15}N$ values. A significant positive correlation also existed between total mass of fatty-acids and that of free amino acids; a significantly negative correlation occurred between $\delta^{13}C$ and $\delta^{15}N$ values (Table 5).

Discussion

In this article, we interpret the nutritional ecology of three species of Aphalaridae (*C. eucalypti*, unnamed species of *Glycaspis* and *Synglycaspis*) feeding on the leaves of three species of *Eucalyptus* in central-western New South Wales. *C. eucalypti* live freely and feed as groups, whereas species of *Glycaspis* and *Synglycaspis* construct lerps and induce galls, respectively, and feed as single individuals.

Primary Metabolites in Eucalyptus in Relation to Group-Feeding and Solitary-Feeding Aphalaridae

TNC levels in *E. globulus* infested by the group-feeding *C. eucalypti* increase negligibly (~2%), whereas in *E. sideroxylon* infested by the solitary-feeding *Glycaspis* and *E. macrorhyncha* infested by the solitary-feeding *Synglycaspis*, TNC values increase by 15 and 36%, respectively. The modest rise in TNC values in *C. eucalypti*-infested *E. globulus* matches with that shown in *G. hirsutum* when infested by *Aphis gossypii. A. gossypii* also are also known to feed in groups (Gomez et al. 2006). TNC reserves are essential for the growth and reproduction of Sternorrhyncha because these reserves are the most readily usable forms of energy (Richardson et al. 2013). Elevated levels of TNCs in leaves when

Table 3. Detected individual amino acids (mole %) in *E. globulus–C. eucalypti* (free living), *E. sideroxylon–Glycaspis* (lerp forming), and *E. macrorhyncha–Synglycaspis* (gall inducing)

Amino acids	*E. globulus–C. eucalypti*		*E. sideroxylon–Glycaspis*						*E. macrorhyncha–Synglycaspis*					
	U	I	U	I					U	I				
				1	2	3	4	5		1′	2′	3′	4′	5′
Isoleucine	...	...	9	...	...	...	...	11	43	2	3	...	...	...
Leucine	...	1	20	...	...	...	...	5	...	...	58	...	8	11
Lysine	...	...	24	...	...	...	...	...	...	4	...	...	...	...
Methionine	...	...	4	...	...	...	...	2	...	3	...	...	...	...
Phenylalanine	...	1	...	...	...	...	...	2	...	...	...	...	...	...
Threonine	...	7	0	...	...	...	...	2	...	11	...	4	...	2
Valine	...	...	...	...	...	...	24	...	3	2	2	...	...	...
Alanine	36	29	...	18	42	...	4	21	19	21	4	28	...	21
Asparagine	...	...	...	...	...	...	...	...	...	14	...	17	...	...
Aspartic acid	...	35	1	9	15	...	...	2	...	11	...	16	...	15
Glutamine	...	...	...	2	...	...	26	21	24	3	12	...	13	...
Glycine	64	1	24	64	24	100	3	4	...	7	2	1	79	30
Proline	...	8	...	...	...	...	6	5	5	8	4	8	...	6
Serine	...	19	1	6	19	...	...	14	6	15	16	21	...	15
Tyrosine	...	...	...	...	...	...	...	...	...	...	...	5	...	...

...: not detected; maybe in traces. U: uninfested leaves; I: infested leaves. 1, 2, 3, 4, 5: leaves infested by populations of the 1, 2, 3, 4, and 5 instars 1′, 2′, 3′, 4′, 5′: galls harboring populations of the 1, 2, 3, 4, and 5 instars.

Table 4. Detected individual fatty acids (mole %) in *E. globulus–C. eucalypti* (free living), *E. sideroxylon–Glycaspis* (lerp forming), and *E. macrorhyncha–Synglycaspis* (gall inducing)

Fatty acids	*E. globulus–C. eucalypti*		*E. sidereoxylon–Glycaspis*						*E. macrorhyncha–Synglycaspis*					
	U	I	U	I					U	I				
				1	2	3	4	5		1′	2′	3′	4′	5′
Palmitic acid (16:0)	23	22	23	21	18	16	12	11	26	19	15	14	12	11
Stearic acid (18:0)	3	3	4	3	11	2	3	2	4	3	14	2	4	3
Arachidic acid (20:0)	1	1	3	27	25	24	22	19	1	3	2	1	0	1
Palmitoleic acid (16:1 Δ7)	1	1	1	2	1	1	0	0	1	1	1	0	0	…
Palmitoleic acid (16:1 Δ9)	2	2	0	0	…	…	…	0	0	…	…	…	…	0
Oleic acid (18:1 Δ9)	8	9	10	6	6	6	5	4	5	5	5	5	4	4
Linoleic acid 18:2 Δ9,12)	15	20	17	13	9	7	5	12	12	22	15	12	11	9
α—Linolenic acid (18:3 Δ9,12,15)	48	43	28	16	15	13	15	14	52	23	19	17	16	14

…: not detected; may be in traces. U: uninfested leaves; I: infested leaves. 1, 2, 3, 4, 5: leaves infested by populations of the 1, 2, 3, 4, and 5 instars. 1′, 2′, 3′, 4′, 5′: galls harboring populations of the 1, 2, 3, 4, and 5 instars.

Table 5. Correlations between pooled values TNC, $\delta^{13}C$, $\delta^{15}N$, amino acids, fatty acids, and minerals in *E. globulus–C. eucalypti* (free living), *E. sideroxylon–Glycaspis* (lerp forming), and *E. macrorhyncha–Synglycaspis* (gall inducing)

	TNC	Fatty acids	Amino acids	$\delta^{13}C$	$\delta^{15}N$
TNC	—	—	—	—	—
Fatty acids	−0.519	—	—	—	—
Amino acids	−0.305	0.696*	—	—	—
δ13 C	0.831*	−0.170	0.140	—	—
δ15 N	−0.841*	0.099	0.047	−0.834*	—

* Values significant at 5% level (ignoring the symbol).

infested by Hemiptera show the plant's ability to replenish them with newly synthesized carbohydrates (Trumble et al. 1993). The feeding pressure imposed by the solitary-feeding species of *Glycaspis* and *Synglycaspis*, in the specific context of sugar utilization, appears more intense than that imposed by the free-living *C. eucalypti*; the feeding pressure is compensated by *E. sideroxylon* and *E. macrorhyncha* leaves by actively translocating photosynthates from uninfested host sites. Such a mobilization of sugar reserves to feeding sites neutralizes stress, enabling the plant tissue to recover from the physical and chemical damage caused by feeding action (Raman 2011). Several species of Aphididae locate sieve tubes because of their ability to detect high levels of sucrose (Hewer et al. 2010). This appears relevant in the context of the *Eucalyptus*-infesting Aphalaridae as well, although the feeding behaviors of the Aphalaridae are different from those of the Aphididae. Instars of Aphididae feed on the phloem tissue, whereas in the Aphalaridae, we have demonstrated that the first and second instars feed on mesophyll parenchyma and only the later instars feed on phloem (Sharma et al. 2013, 2015a,b). Increment in TNC levels in *E. sideroxylon* and *E. macrorhyncha*, especially during the development of later instars of the species of *Glycaspis* and *Synglycaspis*, which feed on phloem, explains the dramatic rise in the values of TNCs in leaves.

$\delta^{15}N$ signatures in *E. globulus* hosting the group-feeding *C. eucalypti* and in *E. sideroxylon* and *E. macrorhyncha* hosting the solitary-feeding glycaspids are distinctly different. With the infestation of *C. eucalypti*, $\delta^{15}N$ values in *E. globulus* leaves increase, whereas with the infestation of *Synglycaspis*, the $\delta^{15}N$ values in *E. macrorhyncha* leaves decrease. $\delta^{15}N$ values in *E. sideroxylon* leaves with the infestation of *Glycaspis* drop negligibly. Increase in $\delta^{15}N$ values in *E. globulus* infested by the group-feeding *C. eucalypti* is possibly influenced by NO_3-reductase activity as shown in the feeding physiology of *Myzus persicae* (Hemiptera: Aphididae) on different Brassicaceae (Wilson et al. 2011). In contrast, declining $\delta^{15}N$ values in *E. sideroxylon* and *E. macrorhyncha* leaves infested by the solitary-feeding *Glycaspis* and *Synglycaspis* could be because of mobilization of nitrogenous material to feeding sites as shown in the feeding action of the specialist, gall-inducing insects (Raman et al. 2006, Künkler et al. 2013), which mobilize nitrogenous materials to infestation sites and utilize them for their development and in generating specialized habitats.

Compared with uninfested leaves, the total free amino acid contents increase by three times in *E. globulus* leaves infested by *C. eucalypti* and by 1.6 times in *E. sideroxylon* leaves infested by *Glycaspis,* but decrease by 0.6 times in *E. macrorhyncha* leaves infested by *Synglycaspis*. Recanalization of amino acids explains the rise in total-amino acid mass (Giordanengo et al. 2010) in infested *E. globulus* and *E. sideroxylon* leaves. Increase in amino acids in the leaves of *E. globulus* hosting the free-living *C. eucalypti* is due to group-feeding behavior and also because these amino acids are not utilized. High abundance of immatures of *Cardiaspina* nr *densitexta* (Aphalaridae) is correlated with high concentrations of amino acid N in the leaves *E. moluccana*, but only very high abundance of immatures of *C.* nr *densitexta* is associated with greater concentrations of essential amino acids (Steinbauer et al. 2014). In this study, maximal increase in amino acids mass occurs in *E. globulus* leaves, when infested by group-feeding *C. eucalypti*. In the context of solitary feeders such as *Glycaspis* on *E. sideroxylon* and *Synglycaspis* on *E. macrorhyncha*, such a dramatic increment in the quality and quantity of amino acids does not occur.

High concentrations of the essential and nonessential amino acids enhance host-tissue palatability to insects (Saltzmann et al. 2008). Nonessential amino acids (e.g., glycine, alanine, arginine, and proline) are established phagostimulants in insect feeding (Renwick 1999). Against such an explanation, we infer that the generic increase in the levels of alanine, glycine, serine, proline, and aspartic acid in the three interacting systems explains the enhanced palatability of the host tissues and phagostimulation. However, the drop in the levels of alanine and glycine, both nonessential amino acids, in *E. globulus* leaves when infested by *C. eucalypti* indicates an increase in essential amino acids toward building of proteins.

Primary Metabolites in Eucalyptus Infested by Generalist and Specialist Aphalaridae

In general, $\delta^{13}C$ values in plant systems become less negative when either the stomatal diffusion becomes limited or the carboxylation capacity is enhanced. However, during insect feeding, $\delta^{13}C$ values increase consequent to selective feeding on the recently fixed and readily available carbon (McCutchan et al. 2003). $\delta^{13}C$ values increase in all three systems studied here. These results indicate an enhanced utilization of C materials by the specialist Aphalaridae. Less-negative values of $\delta^{13}C$ indicate an enrichment of C in host plants, emphasizing the utilization of higher levels of CO_2 in leaves to generate more sugars (Damesin and Lelarge 2003). The negligible change in $\delta^{13}C$ values in *E. sideroxylon* and *E. macrorhyncha* leaves is possibly due to the innate physiological differences in species of *Eucalyptus* and patterns of carbon allocation to their foliage. However, the modest change in $\delta^{13}C$ values occurring during feeding by the second and third instars of *Glycaspis* on *E. sideroxylon* correlates with the TNC values during comparable phase of development in *Glycaspis*. In *E. macrorhyncha* leaves infested by *Synglycaspis* where $\delta^{13}C$ values increase in leaves fed by the first, second, and third instars which plateau in leaves fed by the fifth instar. This finding corroborates with the TNC data shown in this article.

Infestations by generalist species of Aphididae and Aleyrodidae on leaves of *Pisum sativum* (Fabaceae) and *G. hirsutum*, respectively, redirect amino acids and enhance amino acid biosynthesis at the infestation sites (Sempruch et al. 2011, Dubey et al. 2013). Such an increment in the amino acid mass in host tissues is indicated vital for insect growth and development (Sempruch et al. 2011), whereas Dubey et al. (2013) indicate that feeding by Hemiptera intensifies amino acid biosynthesis as a plant response to ward off other insects via decarboxylation of amino acids. High levels of essential amino acids, such as leucine, isoleucine, and threonine occur in young leaves of *E. macrorhyncha*, which are utilized by the early instars of the specialist gall-inducing *Synglycaspis*. These essential amino acids decline in quantity as the insects metamorphose into later instars.

Fatty acids increase by 0.3 times in leaves of *E. globulus,* but decrease by 0.04 times in *E. sideroxylon*, and by 0.26 times in *E. macrorhyncha*. In galls induced by *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae) on *Solidago altissima* (Asteraceae) both linoleic and linolenic acids increase (Tooker and De Moraes 2009), whereas in the gall induced by *Synglycaspis* only the linoleic acid increases during feeding by the first and second instars. This could be due to variations in the feeding behaviors of the respective gall-inducing arthropods: *G. gallaesolidaginis* inflicts marked damage to stem parenchyma during its feeding using its relatively large mandibles, whereas the early instars of *Synglycaspis* insert their delicate stylets intercellularly inflicting minimal damage to host tissues (Sharma et al. 2015b). Feeding action of sap-sucking insects enhances the biosynthesis of fatty acids in infested plant parts (Kanobe 2012).

Primary Metabolites in Eucalyptus Infested by the Lerp-Constructing Glycaspis and Gall-Inducing Synglycaspis

When comparing *Glycaspis* and *Synglycaspis* a noticeable variation occurs in the levels of TNC and $\delta^{13}C$ values. TNC levels increase in leaves of *E. sideroxylon* infested by *Glycaspis* and those of *E. macrorhyncha* infested by *Synglycaspis*, but a greater level of increase occurs in the leaves of *E. macrorhyncha*. This variation indicates greater feeding pressure imposed by the gall-inducing *Synglycaspis*. Feeding action of both glycaspid species makes $\delta^{13}C$ values increase in the infested leaves of *E. sideroxylon* and *E. macrorhyncha*, although the greatest level of changes occurs in the leaves of *E. macrorhyncha,* which indicates greater utilization of carbon-based materials by the gall-inducing *Synglycaspis*. $\delta^{15}N$ values too appear similar in *E. sideroxylon* and *E. macrorhyncha* leaves, suggesting mobilization of nitrogenous material to feeding sites (Raman et al. 2006, Künkler et al. 2013).

Conclusion

The three systems investigated here, treated as representative guilds of the Australian Aphalaridae, demonstrate a pattern. The lerp-forming taxon, the species of *Glycaspis*, occurs at an intermediate position between the free-living and the gall-inducing Aphalaridae. Overall, considering the levels and quantities of various metabolites measured in this study, the free-living *C. eucalypti* stress leaves of *E. globulus* more intensely by its group-feeding behavior, whereas the lerp-forming *Glycaspis* and the gall-inducing *Synglycaspis* impose an intense stress level draining more of sugars from their respective host leaves, principally because of their requirements to build characteristic habitats, viz., the gall and the lerp.

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References

Bligh E. G., Dyer W. J.. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911–917.

Burckhardt D., Ouvrard D., Queiroz D., Percy D. 2014. Psyllid host-plants (Hemiptera: Psylloidea): Resolving a semantic problem. *Fla. Entomol.* 97: 242–246.

Coplen T. B. 1996. New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. *Geochim. Cosmochim. Acta 60: 3359–3360.*

Damesin C., Lelarge C. 2003. Carbon isotope composition of current-year shoots from Fagus sylvatica in relation to growth, respiration and use of reserves. *Plant Cell Environ.* 26: 207–219.

Dubey N. K., Goel R., Ranjan A., Idris A., Singh S. K., Bag S. K., Chandrashekar K., Pandey K. D., Singh P. K., Sawant. S. V. 2013. Comparative transcriptome analysis of *Gossypium hirsutum* L. in response to sap sucking insects: Aphid and whitefly. *BMC Genomics* 14: 241. doi:10.1186/1471-2164-14-241.

Duceppe M.-O., Cloutier C., Michaud D. 2012. Wounding, insect chewing and phloem sap feeding differentially alter the leaf proteome of potato, *Solanum tuberosum* L. *Proteome Sci.* 10: 73. doi: 10.1186/1477-5956-10-73.

Gachomo E., Allen J. W., Pfeffer P. E., Govindarajulu M., Douds D. D., Jin H., Nagahashi G., Lammers P. J., Shachar-Hill Y., Bücking H. 2009. Germinating spores of *Glomus intraradices* can use internal and exogenous nitrogen sources for *de novo* biosynthesis of amino acids. *New Phytol.* 184: 399–411.

Giordanengo P., Brunissen L., Rusterucci C., Vincent C., van Bel A., Dinant S., Girousse C., Faucher M., Bonnemain J. L. 2010. Compatible plant—aphid interactions: How aphids manipulate plant responses. *C. R. Biol.* 333: 516–523.

Gomez S. K., Oosterhuis D. M., Hendrix D. L., Johnson D. R., Steinkraus D. C. 2006. Diurnal pattern of aphid feeding and its effect on cotton leaf physiology. *Environ. Exp. Bot.* 55: 77–86.

Government of New South Wales. 2011. *Bioregions of NSW—Biodiversity.* (http://www.environment.nsw.gov.au/bioregions/Bioregions.htm) (accessed July 2014).

Hewer A., Will T., van Bel J. E. 2010. Plant cues for aphid navigation in vascular tissues. *J. Exp. Biol.* 213: 4030–4042.

Hollis D. 2004. *Australian Psylloidea: jumping plant lice and lerp insects*. Australian Biological Resources Study, CSIRO, Canberra, Australia.

Kanobe C. 2012. Fatty acid changes in soybean (*Glycine max*) under soybean aphid (*Aphis glycines*) infestation and their implications on plant defense against insects, Ph.D. Dissertation, Iowa State University, Ames, pp. 1–135.

Künkler N., Brandl R., Brandle M. 2013. Changes in clonal poplar leaf chemistry caused by stem galls alter herbivory and leaf litter decomposition. *PLoS One* 8: e79994. doi: 10.1371/journal.pone.0079994.

Lawler I. R., Foley W. J. 2002. Chemical ecology of herbivory in Eucalyptus: Interactions between insect and mammalian herbivores and plant essential oils, pp. 324–344. In Coopen J. J. W. (ed.), *Eucalyptus: the genus Eucalyptus. Medicinal and Aromatic Plants—Industrial Profiles.* vol. 22. CRC Press, Boca Raton, FL.

Mariotti A. 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 303: 685–687.

McCutchan J. H., Lewis W. M., Kendall C., McGrath C. C. 2003. Variation in trophic shift for stable-isotope ratios of carbon, nitrogen and sulfur. *Oikos* 102: 378–390.

Morrison W. R., Smith L. M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride—methanol. *J. Lipid Res.* 5: 600–608.

Muir J. G., O'Dea K. 1992. Measurement of resistant starch: Factors affecting the amount of starch escaping digestion in vitro. *Am. J. Clin. Nutr.* 56: 123–127.

Offor E. 2010. The nutritional requirements of phytophagous insects: Why do some insects feed on plants? http://papers.ssrn.com/sol3/papers.cfm?abstract_id=1535274 (accessed 10 July 2015).

Ouvrard D. 2014. *Psyl'list—The world Psylloidea database.* (http://www. hemiptera-databases.com/ psyllist) (accessed 9 September 2014).

Raman A. 2011. Morphogenesis of insect-induced plant galls: Facts and questions. *Flora* 206: 517–533.

Raman A., Madhavan S., Florentine S. K., Dhileepan K. 2006. Stable-isotope ratio analyses of metabolite mobilization in the shoot galls of *Parthenium hysterophorus* (Asteraceae) induced by *Epiblema strenuana* (Lepidoptera, Tortricidae). *Entomol. Exp. Appl.* 119: 101–117.

Renwick J. A. A.1999. Phytochemical modification of taste: Insect model, pp. 221–230. In Cutler H. G., Cutler S. J. (eds.), *Biologically active natural products*. CRC Press, Boca Raton, FA.

Richardson A. D., Carbone M. S., Keenan T. F., Czimczik C. I., Hollinger D. Y., Murakami P., Schaberg P. G., Xu X. 2013. Seasonal dynamics and age of stemwood non-structural carbohydrates in temperate forest trees. *New Phytol.*197: 850–861.

Rose R., Rose C. L., Omi S. K., Forry K. R., Durall D. M., Bigg W. L. 1991. Starch determination by perchloric acid vs enzymes: Evaluating the accuracy and precision of six colorimetric methods. *J. Agric. Food Chem.* 39: 2–11.

Saltzmann K. D., Giovanini M. P., Zheng C., Williams C. E. 2008. Virulent Hessian-fly larvae manipulate the free amino acid content of host wheat plants. *J. Chem. Ecol.* 34:1401–1410.

Schmidt L., Schurr U., Rose U. S. 2009. Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. *Plant Cell Environ.* 32: 893–903.

Schwachtje J., Baldwin I. T. 2008. Why does herbivore attack reconfigure primary metabolism? *Plant Physiol.* 146: 845–851.

Sempruch C., Michalak A., Leszczyński B., Chrzanowski G. 2011. Effect of *Sitobion avenae* (Fabricius, 1775) feeding on the free amino acid content within selected parts of Triticale plants. *Aphids and Other Hemipterous Insects* 17: 137–144.

Sharma A., Raman A., Taylor G., Fletcher M. 2013. Nymphal development and lerp construction of *Glycaspis* sp. (Hemiptera: Psylloidea) on *Eucalyptus sideroxylon* (Myrtaceae) in central-west New South Wales, Australia. *Arthropod. Struct. Dev.* 42: 551–564.

Sharma A., Raman A., Taylor G. S., Fletcher M. J., Nicol H. 2015a. Development, feeding and oviposition behaviour of *Ctenarytaina eucalypti* (Maskell) (Hemiptera: Psylloidea: Aphalaridae) on *Eucalyptus globulus* (Myrtaceae) in the central tablelands of New South Wales. *Aust. Entomol.* 54: 159–171.

Sharma A., Raman A., Taylor G. S., Fletcher M. J., Nicol H. 2015b. Feeding and oviposition behaviour of a gall-inducing species of *Glycaspis* (Syngycaspis) (Hemiptera: Psylloidea: Aphalaridae) and development of galls on the leaves of *Eucalyptus macrorhyncha* (Myrtaceae) in central western New South Wales, Australia. *Eur. J. Entomol.* 112: 75–90.

Steinbauer M. J. 2013. Shoot feeding as a nutrient acquisition strategy in free-living psylloids. *PLoS One* 8: e77990. doi: 10.1371/journal.pone.0077990.

Steinbauer M. J., Burns A. E., Hall A., Riegler M., Taylor G. S. 2014. Nutritional enhancement of leaves by a psyllid through senescence-like processes: Insect manipulation or plant defence? *Oecologia* 176: 1061–1074.

Taylor G. S.1997. Effect of plant compounds on the population dynamics of the lerp insect, *Cardiaspina albitextura* Taylor (Psylloidea: Spondyliaspididae) on eucalypts, pp. 37–57. In Raman A. (ed.), *Ecology and evolution of plant-feeding insects in natural and manmade environments.* International Scientific Publications, New Delhi, India and Backhuys publishers, Leiden, the Netherlands.

Tooker J. F., De Moraes C. M. 2009. A gall-inducing caterpillar species increases essential fatty acid content of its host plant without concomitant increases in phytohormone levels. *Mol. Plant Microbe Interact.* 22: 551–559.

Trumble J. T., Kolodny-Hirsch D. M., Ting I. P. 1993. Plant compensation for arthropod herbivory. *Annu. Rev. Entomol.* 38: 93–119.

VSN International. 2013. *GenStat for windows.* VSN International, Hempel Hempstead, Hertfordshire.

White T.C.R. 1969. An index to measure weather induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* 50: 905–909.

White T.C.R 1970. Some aspects of the life history, host selection, dispersal and oviposition of adult *Cardiaspina densitexta* (Homoptera: Psyllidae). *Aust. J. Zool.* 18: 105–117.

White T.C.R 1986. Weather, Eucalyptus dieback in New England, and a general hypothesis of the cause of dieback. *Pac. Sci.* 40: 58–78.

White T.C.R. 2009. Plant vigour versus plant stress: A false dichotomy. *Oikos* 118: 807–808.

Wilson A. C. C., Sternberg L. da S. L., Hurley K. B. 2011. Aphids alter host-plant nitrogen isotope fractionation. *Proc. Natl. Acad. Sci. USA.* 108: 10220–10224.