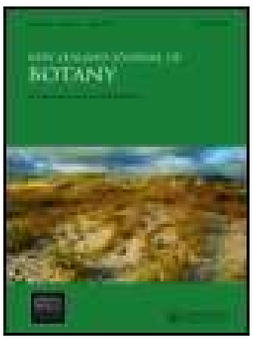

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RESEARCH ARTICLE

Developmental and compositional changes in *Leptospermum scoparium* nectar and their relevance to mānuka honey bioactives and markers*

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ABSTRACT

Mānuka (*Leptospermum scoparium*) is valued for its unique bioactive honey, but relatively little is known about mānuka nectar and the factors modulating its production. In this study the compositional variability of nectar collected throughout mānuka floral development was measured in glasshouse-cultivated plants. The total yield of nectar was estimated by rinsing flowers with water and measuring total sugars (°Brix). The concentration of total sugars and of dihydroxyacetone (DHA), the precursor to methylglyoxal and the key mānuka honey bioactive, increased through floral development and maximised just prior to initiation of flower degradation. Similarly, the patterns of several mānuka honey authenticity markers including lepteridine, leptosperin and 3-phenyllactic acid also followed a similar trend through flower development, suggesting that their biosynthesis was associated with nectar production. Leaf exudates produced as a result of scale insect infestation of some mānuka plants were deficient in the key markers DHA, leptosperin and lepteridine, but did contain 4-hydroxyphenyllactic acid. This study suggests that the predominant key markers of genuine mānuka are biosynthesised in the floral tissues of the plant rather than elsewhere. For robust analysis and comparison of mānuka nectar yield and composition, sampling needs to take into account the floral developmental stage of the flowers sampled.

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Introduction

Honey, the sweet syrup made by bees foraging nectar from a range of botanical sources, has been collected by humans for thousands of years for both its nutritional and medicinal value (Bogdanov et al. 2008). In New Zealand, the honey produced from the native plant *Leptospermum scoparium* J.R. & G.Forst. (Myrtaceae), known as mānuka honey, has become particularly valuable in recent years (Anon. 2016). This is largely because of its non-peroxide antibacterial activity, which is due to the accumulation of methylglyoxal

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(MGO) in the mature honey (Adams et al. 2008; Mavric et al. 2008). Subsequent studies showed that dihydroxyacetone (DHA) is the precursor of MGO and that it accumulates in relatively high concentrations in the floral nectar of mānuka and some other *Leptospermum* species (Adams et al. 2009; Norton et al. 2015).

However, DHA has not been reported from the nectar of kānuka (*Kunzea ericoides*), the morphologically similar myrtaceous plant that often co-exists with mānuka (and regularly confused with), and can have overlapping flowering times. In addition to DHA and MGO, several other phytochemicals (Figure 1) have been proposed as being unique to mānuka nectar, including leptosperin (Kato et al. 2014) and lepteridine (Beitlich et al. 2016). Recent research for the New Zealand Ministry for Primary Industries (MPI), proposing a regulatory definition to provide confidence in the authenticity of mānuka honey products, has found 3-phenyllactic acid, 2′-methoxyacetophenone, 2-methoxybenzoic acid and 4-hydroxyphenyllactic acid as characteristic markers of mānuka nectar (Anon. 2017). However, relatively little is known about mānuka nectar and the factors modulating its production.

The biology and chemical interactions involved in nectar production and regulation are complex, with only generalised models described in the literature (Heil 2011). Plants produce nectar to attract pollinators that are influenced by its composition (Stevenson et al. 2017). The volume of nectar, concentration of sugars and other metabolites vary enormously between plant species and are also influenced by microbial communities (Roy et al. 2017). In terms of optimising the value of mānuka honey production, apiarists need to maximise standing sugar on a plant while maintaining a high DHA content. This can be achieved by either having plants with more flowers or flowers with greater nectar volume. Environmental conditions can influence the number of initiated flower buds, plus photosynthetic limitations (through leaf area and temperature) may dictate a maximum rate of sugar production. However, data on nectar from Australian *Corymbia gummifera* (Myrtaceae) indicate little temporal pattern to nectar secretion, with variation between trees and sample periods (Goldingay 2005), suggesting that this species had a generalised pollination system. Once a flower has been pollinated there seems little point in the flower continuing to supply nectar, but for some *Eucalyptus* (also Myrtaceae) species it has been shown that exposure to visitors, such as bees, had a negligible effect on nectar carbohydrate composition (Davis 1997). To complicate this further, the foraging bees may also have a preference for a selected range in nectar sugar concentration and composition (Singaravelan 2010; Good et al. 2014).

It is difficult to sample nectar directly from mānuka flowers accurately, particularly if the nectar yield is low or if late in the day after sequestration by insects. A useful review (Morrant et al. 2009) on sampling nectar from flowers with low nectar volumes compared washing flowers (excised whole flowers agitated in 2 mL water) with rinsing flowers (micropipetting water directly over the nectaries in situ). The results from comparison of sampling with microcapillary tubes, blotting up with filter paper, washing and rinsing suggested that the last two mentioned treatments offered the best recovery. The preservation of the nectar wash samples was also considered, but published information on sugar stability with the addition of benzyl alcohol to nectar samples suggested that this made little difference to the composition compared with refrigeration (Morrant et al. 2009).

There is only limited published information on the variation in mānuka nectar yield. It has been shown that, for East Cape mānuka (bagged to exclude insects), the standing

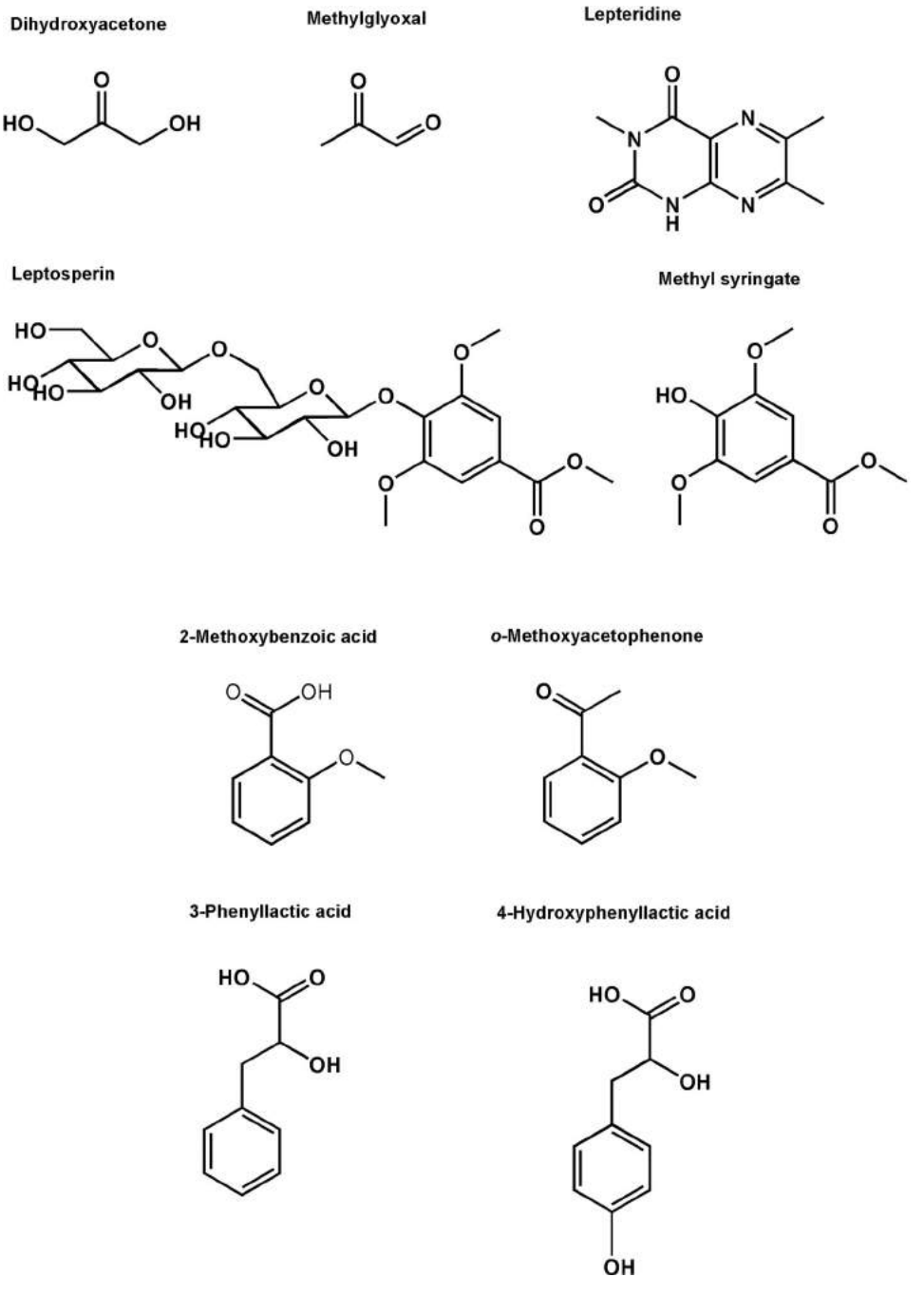

Figure 1. Key metabolites in mānuka nectar.

nectar volume between 0730 h and 1730 h increased 4-fold and could also vary by 2- to 11-fold over 3 days (G. K. Burge, The New Zealand Institute for Plant & Food Research Limited [PFR], unpubl. data). That research also showed that nectar volume varied with flower age, peaking at 6 days. A similar pattern has also been shown for native

pōhutukawa (*Metrosideros excelsa*: Myrtaceae), but the time of peak production varied between trees (Schmidt-Adam et al. 1999).

Quantification of mānuka nectar DHA was first reported by Adams and co-workers, suggesting a '… crude calculation afforded a value for dihydroxyacetone in the nectar as equivalent to ~13,600 mg/kg in honey …' (Adams et al. 2009, p. 1052). Williams et al. (2014) broadly classified DHA distribution in nectar as low (< 1 mg/g), medium (1–2 mg/g) and high (> 2 mg/g) depending on geographic source and/or cultivar. Nickless and co-workers developed spectroscopic methods and chemometrics to quantify nectar sugars and DHA in a range of *L. scoparium* cultivars (Nickless et al. 2014, 2016), but used a better-established derivatisation and high performance liquid chromatography (HPLC) method to quantify nectar DHA and show that cultivar variation had a greater impact than environmental variables on nectar yield (Nickless et al. 2017). Recently, in contrast to the proposed MPI markers (Anon. 2017), Bong and co-workers profiled a range of nectars using chromatographic and spectroscopic techniques, to fingerprint and authenticate honey from New Zealand, finding that only leptosperin, lepteridine and 2-methoxyacetophenone are exclusive to mānuka (Bong et al. 2017). A recent study has shown that mānuka nectar yield and composition are strongly influenced by plant genotype, flower age and the environment (Clearwater et al. 2018).

A better understanding of the basis of mānuka nectar production could allow an improvement in hive management, honey production and authentication. In this study, we report the collection analysis and quantification of key quality attributes of mānuka nectar at different floral development stages (FDS).

Materials and methods

Plant material

Mānuka (*Leptospermum scoparium*) foliage, including seed capsules, was collected in March 2013 from a roadside in Opito Bay, Coromandel, New Zealand (Plant Extracts Research Unit herbarium voucher number 130330-01). Seedlings were established and grown at PFR, Lincoln. They were cultivated in an unheated greenhouse with the controlled release fertiliser Osmocote Plus (Scotts Europe BV). The greenhouse was not heated and an exhaust fan operated for cooling when the temperature was above 18 °C and an evaporative cooler was set to 23 °C. Flying insects were excluded by mesh insect screens and no insects were observed on the plants. Plants were watered via microjet sprinklers for 3 min daily. In their second growing season, six plants flowered, allowing nectar collection. Flowers collected from these plants were classified based to eight developmental stages (see Figure 2): 1. immature bud with corolla development just visible inside bud scale; 2. expansion of corolla, bud scales have disappeared; 3. corolla expanding rapidly, but still closed; 4. first sign of open flower with individual petals within the corolla visible; 5. petals completely open, green hypanthium with first sign of visible nectar, stamens tightly folded over; 6. stamens begin to unfold, hypanthium begins to change colour, nectar visible; 7. hypanthium now rich in nectar and crimson coloured, anthers are mature on the unfolded stamens; 8. anthers have released pollen and stamen shrivelling has started, dark hypanthium rich in nectar.

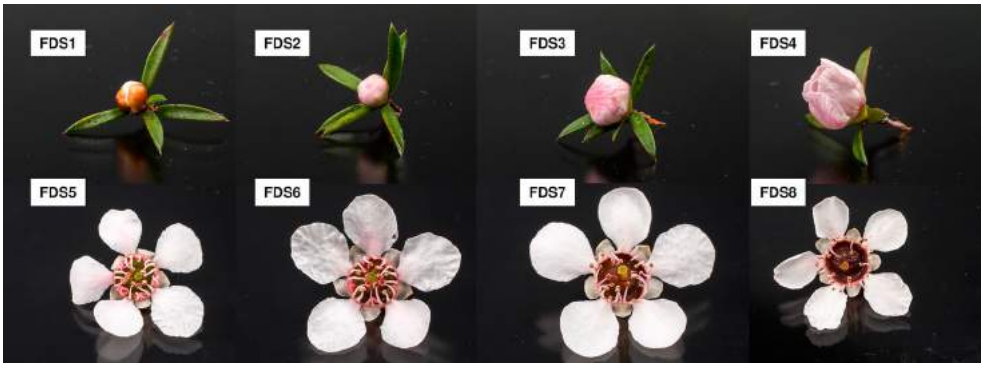

Figure 2. Mānuka flower development stages (FDS).

Nectar sampling

A standardised protocol was developed for nectar collection from individual flowers removed from the plant stems, using a method modified from Morrant et al. (2009). Nectar washes were not undertaken from the compact tissues of FDS 1 and 2 samples, and the petals were removed from FDS 3 and 4 samples to allow access to the hypanthium surface. Water (10 µL) was dropped onto the hypanthium surface using a disposable pipette tip. The sample was washed around the flower hypanthium several times before removal into an Eppendorf tube and freezing prior to analyses. Nectar washes were pooled from between three and six flowers, to give a composite sample for analysis. Six of these composite samples were collected from FDS 3, 4 and 5, while nine composite samples were collected from FDS 6, 7 and 8, giving a total of 45 nectar samples for analysis. Samples were stored at −20 °C until thawing and centrifuging immediately before derivatisation for HPLC analysis. Subsamples of nectar wash samples from FDS 5 to FDS 8 were also dried to constant weight over silica gel to compare the masses recovered with those estimated by °Brix measurements. As a measure of total sugars, the °Brix value of each nectar wash sample was measured using a hand-held refractometer (ADE Advanced Optics).[1] After the plants had finished flowering, a sticky clear liquid coated the leaves and stems of branches of several plants that were infested with a scale insect, common on mānuka (van Epenhuijsen et al. 2000). These stem exudates were collected using a pipette with a 10 µL tip and bulked from four individual plants into four sample vials for analysis.

Derivatisation and quantitative HPLC analyses for DHA

This method, which measures DHA and MGO, was modified from that reported previously by Windsor et al. (2012). A subsample (20 µL) of nectar wash was combined with internal standard solution (ISS; 200 µL, 0.1 M citrate buffer pH 4, containing 0.1 µL/mL hydroxyacetone [HA]), pentafluorobenzylhydroxylamine (PFBHA; 30 µL, 10 mg/mL) in an Eppendorf tube and centrifuged (10,000 rpm, 5 min). The solution was incubated at room temperature for at least 60 min, MeCN (350 µL) added and centrifuged again prior to subsampling for HPLC analysis. Analyses were performed on an Agilent 1100 HPLC (Agilent Technologies) fitted with a diode array detector, using a

Phenomenex Kinetex (100 × 3.0 mm RP-18, 2.6 μm) column and Phenomenex guard column (SecurityGuard 4 × 2 mm RP-18) at 30 °C. Peaks were monitored at 263 nm. The mobile phase was CH_3CN in H_2O, both containing formic acid (0.1%), with a linear programme: 30% MeCN at 0 min, 100% at 4 min, 30% at 5 min and 30% at 10 min. The flow rate was 0.5 mL/min with injection volumes of 5 μL. DHA was expressed as mg/g nectar sugar as estimated from the °Brix measurements.

Liquid chromatography–mass spectrometry analyses of marker compounds

The *liquid chromatography–mass spectrometry* (LCMS) system consisted of a Thermo Scientific Q Exactive Plus Orbitrap (HR/AM) LCMS/MS coupled with a Vanquish UHPLC system (Binary Pump H, Split Sampler HT, DAD HL, Dual Oven). A 3 μL aliquot of each prepared extract (5 μL nectar was diluted in 95 μL H_2O) was separated with a mobile phase consisting of 0.1% formic acid in type 1 water (A) and 0.1% formic acid in acetonitrile (B) by reverse phase chromatography (Hypersil GOLD Vanquish aQ 1.9 μm, 100 mm × 2.1 mm, P/N: 25302-102130-V, Thermo Scientific) maintained at 40 °C with a flow rate of 400 μL/min. A linear gradient was applied: as 0–1 min/0% B, 10 min/50% B, 13–16 min/100% B and 17–20 min/0% B. The eluent was scanned from 1–17 min by API-MS (Orbitrap) with electrospray ionisation (ESI) in both negative and positive ion modes. Data were acquired for precursor masses from *m/z* 110–1200 amu at 70K resolution with data-dependent ms/ms for product ions generated by normalised collision energy (NCE:30) at 17.5K resolution. Compounds were identified based on their mass spectra, fragmentation patterns and the retention times of reference standards where available. The standards included: 3-phenyllactic acid (Aldrich, ≥ 98%), 2′methoxyacetophenone (Aldrich, 99%), 4-hydroxyphenyllactic acid (Sigma, 97%) and 2-methoxybenzoic acid (Sigma, 99%). Leptosperin (analytically pure by ^{1}H NMR) was obtained from Associate Professor David Greenwood (University of Auckland). Lepteridine was tentatively identified by its high resolution mass spectra and UV maxima. Data were processed with the aid of Xcalibur 4.0 and Compound Discoverer 2.0 SV1 (Thermo Electron Corporation). Integrated peak areas for each compound were normalised relative to the total sugar (°Brix) contents of the nectar samples.

Liquid chromatography–mass spectrometry analyses of stem exudates for carbohydrates

Nectar samples were reconstituted in water (100 μL) mixed and an aliquot (50 μL) diluted to 400 μL and filtered with a Single Step vial 0.22 μm PVDF (Thompson, Part No. 65531-200) filter. The LCMS system consisted of a Thermo Electron Corporation Finnigan Surveyor MS pump, Thermo Accela Open Auto sampler (PAL HTC-xt with DLW), Finnigan Surveyor PDA plus detector and a ThermaSphere TS-130 column heater (Phenomenex). An aliquot (2 μL) of each sample was separated with a mobile phase consisting of 10 mM (770 mg/L) ammonium acetate in water (A) and acetonitrile (B) by normal phase chromatography (ZIC-pHILIC stationary phase, 5 μm, PEEK 100 mm × 2.1 mm id; SeQuant, Part No.1.50462.001) maintained at 40 °C with a flow rate of 300 μL/min. A linear gradient was applied: 0–2 min, 10% A; 30–34.5 min, 50% A; 40–45 min, 10% A. Data were acquired by PDA (200–600 nm) and API-MS (LTQ, 2D linear ion-trap, Thermo-Finnigan) with ESI in both negative and positive ion modes for parent masses in the range

m/z 150–1000 amu with MS^3. Data were processed with the aid of Xcalibar 4.0 (Thermo Electron Corporation).

Results

We defined eight FDS (Figure 2), six of which (see Materials and methods) contained extractable nectar sugars. Standing nectar concentrations increased from FDS 5 until 8 (Figure 3). Based on °Brix measurements as a proxy for total sugars, nectar production increased from FDS 3 to 8 (Figure 4). The nectar DHA concentrations, relative to total sugars, increased from FDS 3 to FDS 7 before decreasing slightly at FDS 8 (Figure 5). We did not detect any MGO in any of the samples.

LCMS analyses of the nectar solutions identified a range of phytochemicals (Figure 1), including the proposed (Anon. 2017) MPI markers (Figure 6). Of these, 2′-methoxyacetophenone peaked (c. FDS 5) early, whereas 2-methoxybenzoic acid continued to increase over the FDSs. Several other compounds that have potential as markers of genuine mānuka were also identified (Figure 7). Of these, leptosperin and lepteridine peaked earlier in the FDS than methyl syringate and 2-methoxybenzoic acid.

Analysis of several phloem exudate samples by HPLC and LCMS showed the exudates to be deficient in DHA, leptosperin and lepteridine. The exudates did not contain the other proposed markers 2′-methoxyacetophenone and 2-methoxybenzoic acid. However, the exudates showed traces of 3-phenyllactic acid, plus 4-hydroxyphenyllactic acid at concentrations higher than in nectars.

Discussion

A Coromandel mānuka seed line was chosen to examine the influence of FDS on nectar yield and composition. These mānuka plants were the subject of a previous study on the

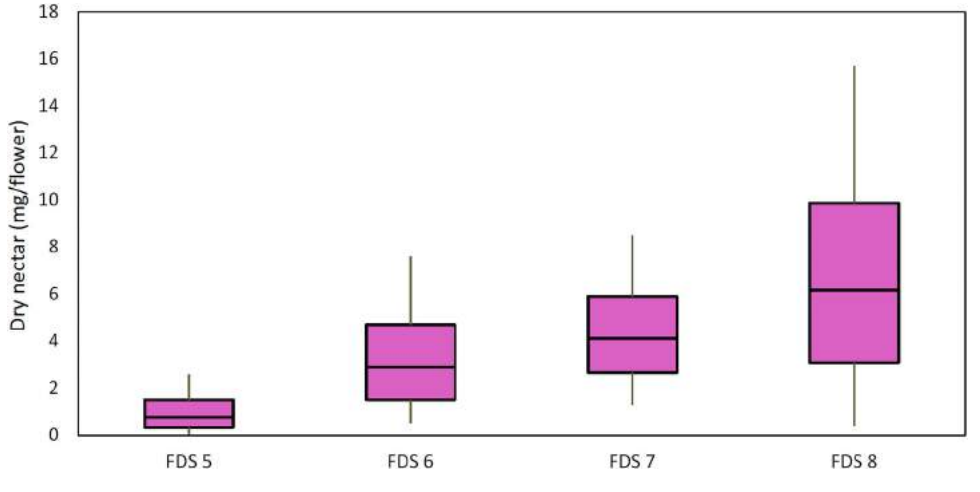

Figure 3. Measured standing nectar from glasshouse-grown mānuka flowers. Box is interquartile range, intercepted by mean, whiskers represent minimum and maximum values. FDS, flower development stage.

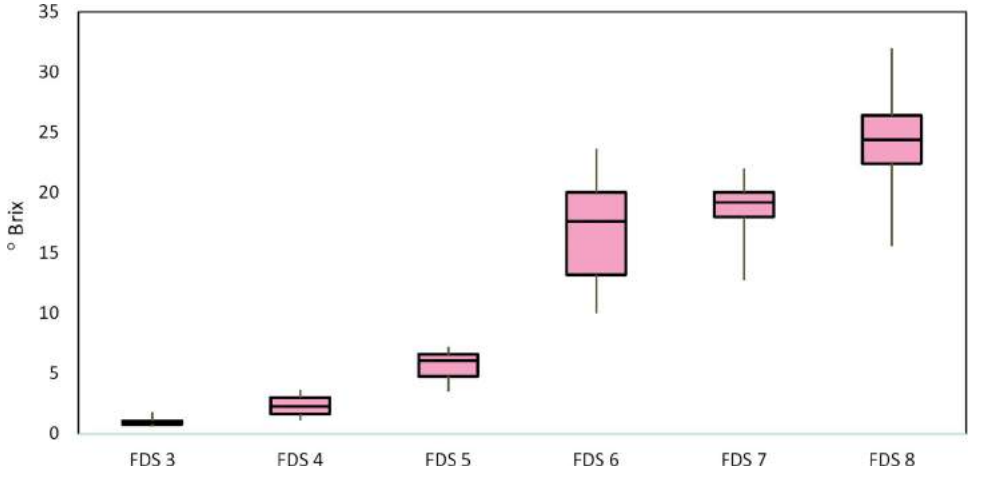

Figure 4. Mānuka nectar total sugar (°Brix) development in glasshouse-grown mānuka flowers. Box is interquartile range, intercepted by mean, whiskers represent minimum and maximum values. FDS, flower development stage.

identification of antibacterial trimethyl nortriketones (Killeen et al. 2016). Initial studies on the flowers of the Coromandel-sourced mānuka showed it produced a moderate amount of nectar containing DHA, alongside the expected monosaccharides glucose and fructose, typical of mānuka nectar (Williams et al. 2014).

Sampling and FDS protocols

The plants were kept in a greenhouse primarily to minimise the influence of foraging insects. Davis (1997) defined five stages of floral development in a study on *Eucalyptus*

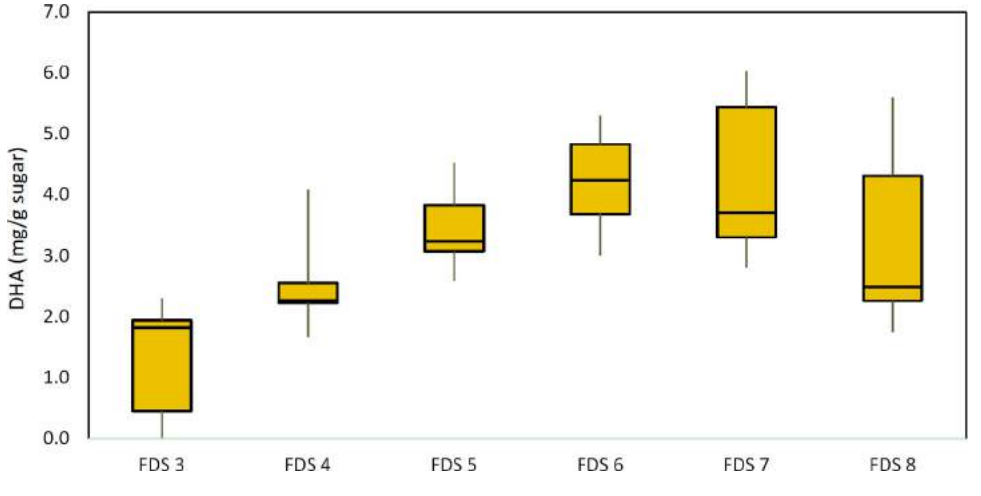

Figure 5. Mānuka nectar dihydroxyacetone (DHA) production (mg/g sugar) over flower development stages (FDS) in glasshouse-grown mānuka flowers. Box is interquartile range, intercepted by mean, whiskers represent minimum and maximum values.

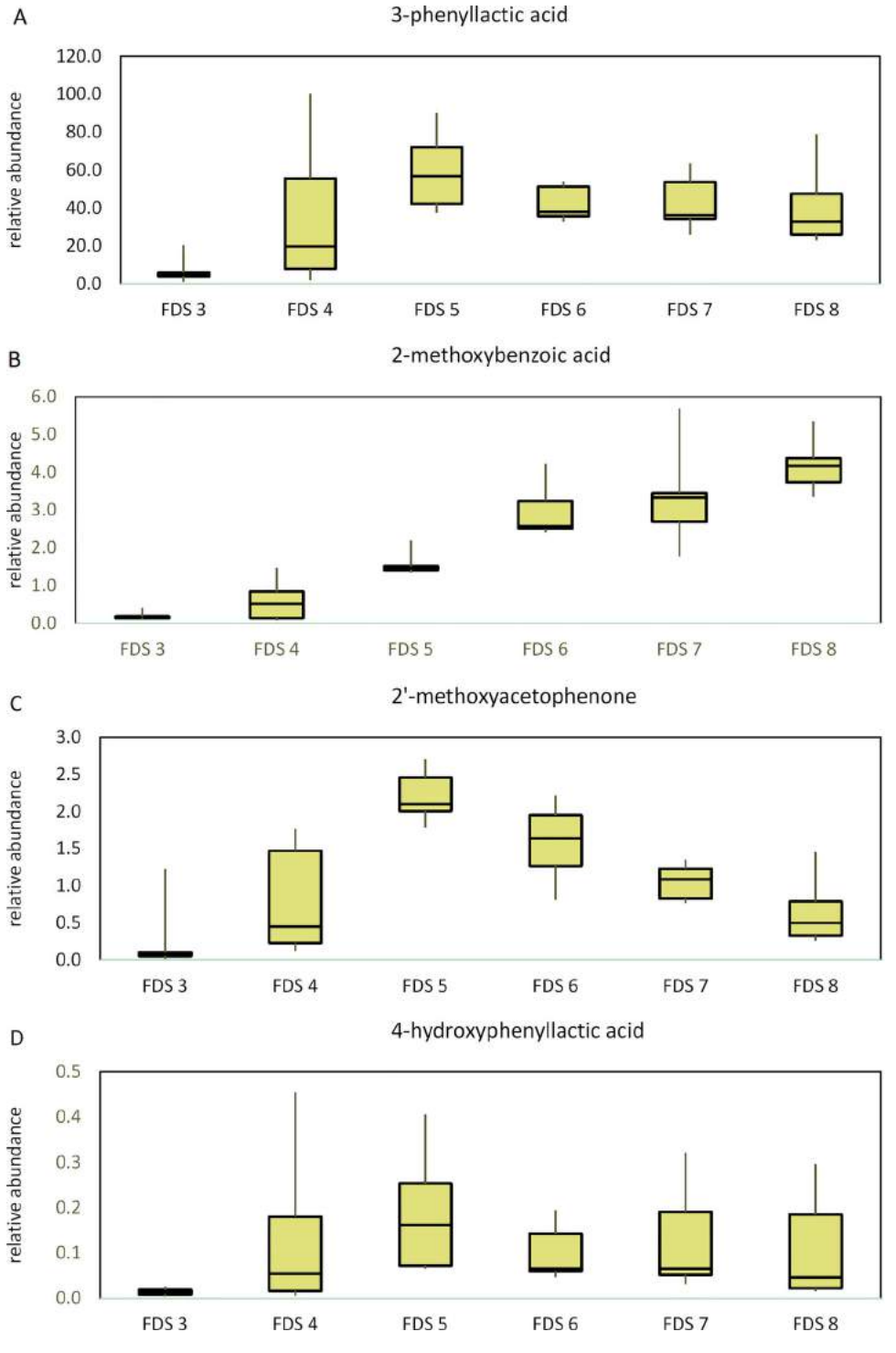

Figure 6. Trends in MPI proposed mānuka nectar markers (normalised to °Brix) over flower development stages (FDS) of glasshouse-grown mānuka flowers (see Discussion). Box is interquartile range, intercepted by mean, whiskers represent minimum and maximum values.

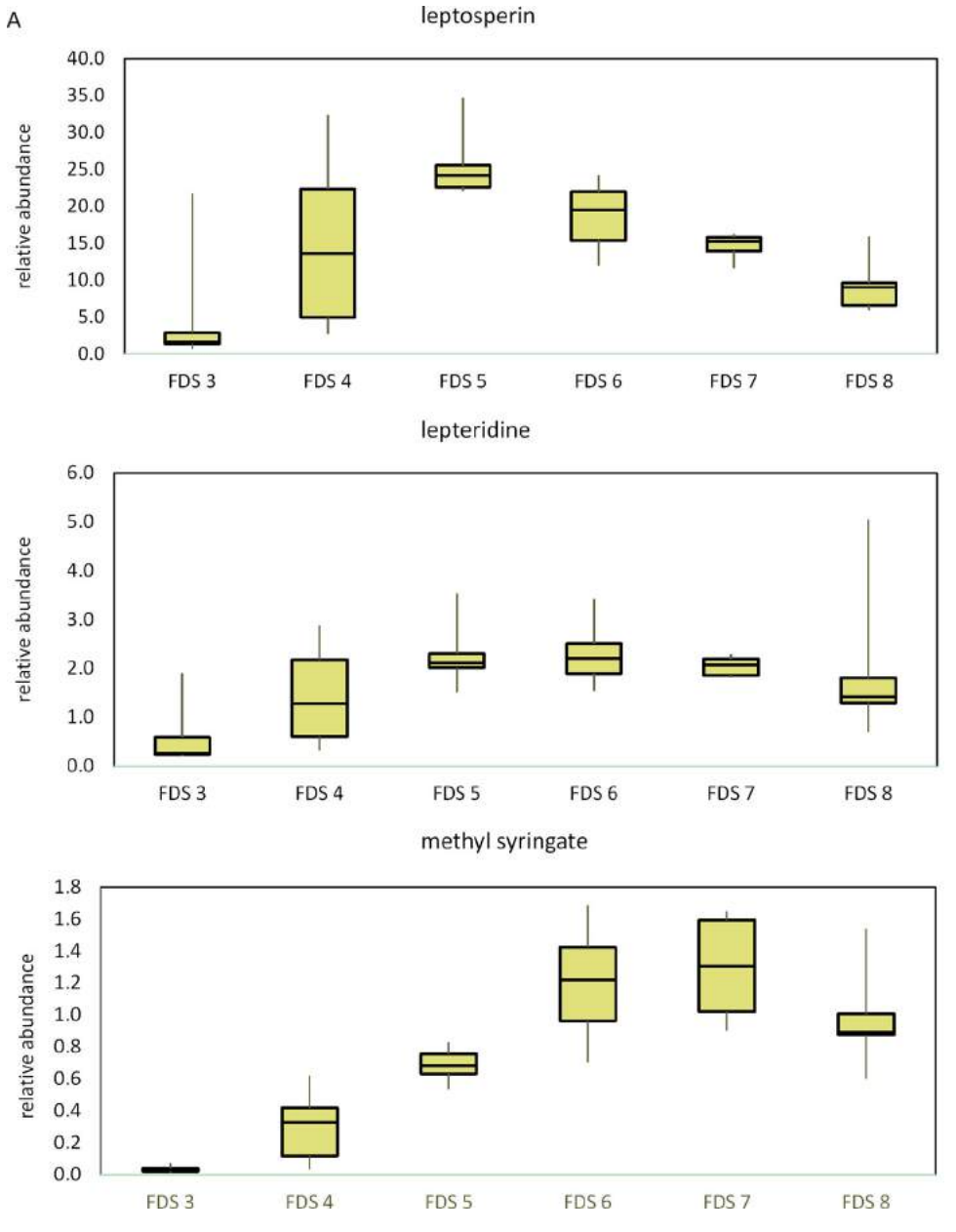

Figure 7. Trends in key mānuka nectar markers (normalised to °Brix) over flower development stages (FDS) in glasshouse-grown mānuka flowers. Box is interquartile range, intercepted by mean, whiskers represent minimum and maximum values.

flowers, ranging from closed buds to abscised petals and hardening of the capsule. However, in order to map the changes in nectar and chemistry in detail, we defined eight FDS (Figure 2). In the glasshouse, the overall timeframe from petal opening until flower abscission was about 2 weeks, with flowers opening more rapidly with higher temperatures. Standing nectar concentrations were obtained by collecting nectar from FDS 5–8

using a micropipette and then removing the water by drying (Figure 3). The amount of nectar produced by each flower was measured by recording the dry matter content of samples collected and by proxy through measurement of total nectar sugars (°Brix; USDA 1981 [January]). There was a positive correlation ($R^2 = 94$) between these values, showing that nectar °Brix measurements were a good approximation of total nectar.

For sampling mānuka nectar, excised individual flowers were washed with water around the flower hypanthium surface several times prior to removing the sample. Because of the variability in both yield and composition, the washings of at least four individual flowers were pooled for each sample analysed. Under glasshouse containment, nectar production was clearly visible from FDS 5 when the hypanthium surface was still green and the stamens were tightly curled inwards. These results are in contrast to a recent report (Nickless et al. 2017, p. 103) that suggested that *L. scoparium* flowers 'do not start secreting nectar until the anthers are mature and fully extended from the hypanthium edge'. This discrepancy may in part be explained by our study having the benefit of glasshouse containment that excluded exposure to foraging insects. Nectar production in our study continued to increase until FDS 8 (Figure 4). However, our observations (data not shown) are that, for field-grown plants, nectar production peaks at FDS 7 and starts to diminish by FDS 8.

Flower development and nectar DHA content

To measure DHA variation, nectar wash samples were combined with an internal standard hydroxyacetone (HA) and were derivatised using PFBHA. This converted the endogenous DHA and the HA internal standard into stable oximes, which were separated by HPLC and quantified by UV detection. The DHA concentrations are reported relative to total sugars, as estimated by °Brix measurements of the nectar wash solutions. We found the ratio of DHA to total sugar had a broad range of values, from undetectable in early developing flowers to more than 5 mg/g in mature flowers. The mean value of > 4 mg/g (Figure 5), by the Williams et al. (2014) definition, would classify this Coromandel line of mānuka as a high (> 2 mg/g DHA/total sugar) DHA producer. It was not always possible to clearly see visible nectar on the hypanthium surface of the FDS 3 and 4 flowers, but the sampling method used showed that the surface wash from these samples contained both sugars and DHA.

Flower development and nectar metabolites

The nectar solutions from different mānuka FDS analysed using LCMS showed (Figures 6–7) that they contained a range of potential genuine mānuka marker compounds. 3-Phenyllactic acid was the most abundant nectar marker (Figure 6), in agreement with the MPI (Anon. 2017) proposed specification. However, Bong et al. (2017), suggest that 3-phenyllactic acid and 4-hydroxyphenyllactic acid are also present in kānuka nectars. These authors also claim that 2-methoxybenzoic acid and 2′-methoxyacetophenone are not stable with prolonged storage and therefore not ideal markers of genuine mānuka. Our study showed 2′-methoxyacetophenone concentrations peaked (Figure 6) relatively early in the FDS before decreasing, which may be a reflection of either their stability or a change in biosynthesis. Of the alternative marker compounds (Figure 7), and excluding

DHA and MGO, there are two phytochemicals, leptosperin and lepteridine (Figure 1), that have also been suggested as reliable markers of authentic mānuka (Bong et al. 2017). In our FDS study, leptosperin and lepteridine peaked earlier than methyl syringate and 2-methoxybenzoic acid. The former compound may be produced in part from the degradation of leptosperin.

Mānuka phloem metabolites

We wanted to know whether the marker compounds were only produced in the floral nectar or whether they were systemic and found in other vascular tissues (e.g. phloem) and then translocated. Phloem sampling is difficult to manage because of elevated hydrostatic pressures (Palmer et al. 2013). However, because of scale insect (van Epenhuijsen et al. 2000) infestation, several of the mānuka plants in the glasshouse had a clear exudate material accumulating on stems and undersides of leaves. An example of this is provided in the photograph in Figure S1 (Supplementary Material), alongside an electron micrograph (Figure S2) of a scale insect attached to a stem. The insect uses a stylet to penetrate the stem and access the sugary phloem. It excretes a 'honeydew' that collects and runs down the stem and accumulates as a sticky exudate.

The analysis of the phloem exudate samples showed them to be lacking DHA, leptosperin and lepteridine, indicating that the biosynthesis of these compounds is closely linked to nectar production. Furthermore, the exudates did not contain the other proposed markers 2′-methoxyacetophenone and 2-methoxybenzoic acid, but their absence may be partly attributable to their stability since Bong et al. (2017) found them to be chemically unstable over prolonged storage. The exudates showed traces of 3-phenyllactic acid, plus 4-hydroxyphenyllactic acid at concentrations higher than in nectars. Both of these compounds have been found in kānuka nectars (Bong et al. 2017), so it would be interesting to know if they are also found in kānuka phloem extracts. Forty-five FDS nectars were analysed, but only four composite exudates were available, so further studies are needed to confirm these results. Flow of metabolites in and out of phloem is thought to be mostly regulated by plasmodesmata and specific transmembrane transporters (Turgeon & Wolf 2009), so it would be interesting to determine where the phenyllactic acid-derived compounds are actively transported in mānuka. Analysis of the composite exudate samples for carbohydrates showed a range of compounds (data not presented) with relatively low concentrations of the key nectar metabolites, fructose and glucose. The remaining carbohydrates (based on their molecular ions) were di-, tri-, tetra- and penta-saccharides. The carbohydrate profile of mānuka honey has been reported by several research groups who found a range of di- and tri-saccharides, but no reports of tetra-saccharides (Weston & Brocklebank 1999; Goss 2009). Our results suggest that the predominant key markers of genuine mānuka honey are biosynthesised in the floral tissues rather than translocated from elsewhere in the plant through the phloem.

To conclude, we have examined the changes in total sugars, the bioactive precursor DHA and selected phytochemical marker compounds in mānuka flower nectars as they developed from buds to fully open flowers. These measured compounds were highly variable across different FDS, which partially explains why reports of nectar composition are so variable from flowers where FDS has not been clearly documented. We observed the same general patterns and trends in other mānuka lines (data not presented), both in

the glasshouse and field environments, with the latter resulting in less nectar accumulation because of nectar removal by foraging insects. For robust analysis and comparison of mānuka nectar yield and composition, sampling needs to take into account FDS of the flowers sampled.

The intricate interactions involved in nectar production are multifaceted and require further research from a range of scientific disciplines. A recent study found that variation in nectar yield was correlated with temperature prior to nectar sampling, but discounted the effects of other environmental variables on nectar yield and DHA concentration (Clearwater et al. 2018). The amount of sugars and DHA varied considerably between genotypes, but given the differences in defined flower stages, the trends that Clearwater and colleagues found for total sugars and DHA content were in agreement with those we report. Other studies suggest that microbial species can influence nectar composition and thereby affect the floral reward offered to plant pollinators (Vannette et al. 2013; Good et al. 2014). The mānuka genome has recently been mapped providing a new set of tools for probing mānuka genetic diversity (Chagné et al. 2017), including potential microbial influences on flower nectar production and composition. Nectar production is one of the main processes that needs to be understood to maximise mānuka honey yield. Although it is difficult to control which plants the bees will visit, developing knowledge of both the genetics and the chemistry influencing flower attractiveness, in terms of volatiles and/or other nectar compositional rewards, is an area of research that warrants further investigation.

Note

1. When a solution contains dissolved solids other than pure sucrose, then the °Brix value only approximates the dissolved solid content, but for glucose and fructose solutions these are within 2% of that for sucrose, based on their relative densities (see ICUMSA 1998).

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