Unravelling the secrets of nectar production for bioactive honey

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Forward

Bioactive honey holds highest value of the honey bee products produced from this region of the world. Driven by the discovery of the non-enzymatic conversion of nectar dihydroxyacetone (DHA) to methylglyoxal (MGO) once the honey is formed, the focus has been on honeys that contain measurable amounts of MGO as this is directly related to their value.

DHA is an unusual substance to be produced by a plant and thus the first question to be resolved is if it is produced by the plant, or an organism associated with the plant. A mutualistic relationship between the plant and an organism could achieve a similar result as the plant itself producing the substance. The appearance of DHA is linked to the production of the carbohydrates in the nectar.

The amount of DHA produced ranges from nil to the highest levels of over 20,000 mg/kg. Understanding the detail of how this DHA is produced can only be resolved by unravelling the biosynthetic pathway. This entails finding the genes that make the enzymes for DHA production. This is slowly being unpicked and this project added to the understanding of this mechanism.

What is impressive is the ability of the plant to reabsorb the nectar, and then replace it continuously.

Unexpected was to find DHA appearing in nectar from a wider range of plants and across a wider range of genera than expected. Whereas the marketing has been for Manuka honey (MGO-based, non-peroxide bioactive honey) from a single species *Leptospermum scoparium*, previous work by Dr Peter Brooks and Dr Simon Williams showed a wide range of Australian *Leptospermum* species produced DHA, and now this project has broadened this plant group to other genera of Myrtaceae.

Australia has a reputation of producing bioactive honeys and perhaps the cause of this status is only now becoming clearly understood as we look further into the nectar chemistry of our native flora.

Dr Liz Barbour
CEO

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Abbreviations

DHA	Dihydroxyacetone
Tsugar	Total Sugar
CWIN	Cell wall invertase
SWEET9	Sugar Will Eventually be Exported Transporters 9

About the Author(s)

Patrick Finnegan is an Associate Professor in the School of Biological Sciences, University of Western Australia, Perth, Western Australia. He is a plant molecular physiologist interested in the genetics underlying important physiological traits. He has researched, and supervised Honours, Masters and PhD students in aspects of the enzymology of C4 photosynthesis, plant respiration in crops and model plants, adaptations for high nutrient use efficiency in Proteaceae, along with this work to understand the presence of dihydroxyacetone in the nectar of plants within the Myrtaceae, with an emphasis on species of *Leptospermum*. For more information, please visit https://orcid.org/0000-0001-5021-1138.

Sylvester A. Obeng-Darko grew up in Assin Manso, but also places like Accra, Cape Coast and Takoradi, all in Ghana as his home. He attended West African Senior Secondary School in Accra, Ghana. He received his Bachelor of Science in Molecular Biology and Biotechnology from the University of Cape Coast, Ghana, and his Master of Science in Biotechnology from the Kwame Nkrumah University of Science and Technology, Kumasi also in Ghana. He later pursued a Master by Research (Biological Science) from the University of Waikato, New Zealand where his research focused on the molecular aspects of nectar production and the anatomy of the floral nectaries of Leptospermum scoparium in an attempt to understand and explain the mechanism involved in the production of nectar and its components. He has over a decade of experience in plant pathology research particularly in coconut palm, where he spent a substantial part of his early research career with the Council of Scientific and industrial Research – Oil Palm Research Institute, Ghana in the development of molecular diagnostics tools for detecting coconut wilt diseases. Sylvester A. Obeng-

Darko has several publications to his credit on topics related to nectar production and coconut palm diseases. For more information, visit https://www.researchgate.net/profile/Sylvester-Obeng-Darko

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Acknowledgments

The project was delivered as a collaboration between the University of Western Australia and the University of the Sunshine Coast. We are grateful to Gather By Pty Ltd for making their facility and planting material available for use in this project.

A number of individuals helped in the work based in south-west of Western Australia. We thank Jean Sloan for identifying sites of *Leptospermum nitens* and collecting the nectar together with Ivan Iozada Lawag, Toby Bird and Shutong Liu.

In Queensland, we thank the staff of Gather By, Martin Brown, Jenna Ford, Renata Lucia Grununnvaldt, Simon Williams and the staff of USC for their help with nectar sampling.

Executive Summary

Manuka honey, derived from the nectar of *Leptospermum* spp., is famous for its strong bioactivity. The high bioactivity of this honey is due to its methylglyoxal (MGO) content. Methylglyoxal in honey arises from dihydroxyacetone (DHA) in nectar through autocatalyic conversion during honey maturation. That is, DHA is found in floral nectar of *Leptospermum scoparium* and other species of *Leptospermum*, while MGO only appears in honey. Intriguingly, DHA is not a normal plant metabolite. It is not yet certain that DHA in floral nectar is produced by the plant, or if it is produced by another process. The first objective of this research was to determine the timing of the appearance of DHA in floral nectar of *L. polygalifolium* and *L. nitens*, species of *Leptospermum* endemic to Queensland / NSW and Western Australia, respectively. The second objective was to link gene transcript patterns within floral nectaries to DHA appearance in nectar to create a hypothetical plant-derived pathway to explain the appearance of DHA in nectar. The goal was to provide industry with advanced knowledge of key traits to predict DHA appearance in nectar to supply growers and apiarists with management tools to increase DHA levels in nectar, thus increasing MGO levels and bioactivity in mature honey.

Nectar was collected from flowers of wild and cultivated *L. polygalifolium* and wild *L. nitens* across flower life span. Nectar levels of DHA were determined by High Performance Liquid Chromatography. Flowers were collected from *L. nitens* across flower development to determine phosphatase activity and to isolate RNA from samples enriched in nectary tissue. The RNA population

was sequenced to generate a transcriptome for *L. nitens*. The transcriptome was used to determine the transcript patterns for the enumerated genes and how these patterns changed as flowers aged.

The appearance of DHA in floral nectar of both *L. polygalifolium* and *L. nitens* followed the profile of total sugar. Both DHA and total sugar concentrations peaked early in flower development, and then declined as flowers aged. The timing, but not the amounts, of DHA and total sugar appearing on *L. polygalifolium* flowers was similar. The timing of peak DHA was earlier in *L. nitens* than in *L. polygalifolium*. The dynamics of the ratio of DHA to total sugar in *L. polygalifolium* indicated that nectar is continually secreted and reabsorbed by flowers in this species. Apiarists now know the best time for bees to forage on *L. polygalifolium* and *L. nitens* flowers to optimise the harvest of DHA.

It was also discovered that other species of Myrtaceae outside of the genus *Leptospermum* accumulate DHA in their nectar. The species identified to accumulate DHA are *Ericomyrtus serpyllifolia* and *Verticordia chrysantha*. This knowledge provides apiarists with alternative opportunities outside the genus *Leptospermum* to harvest nectar that will potentially yield bioactive honey.

Introduction

Floral nectar is essentially a solution of sugar produced by specialized structures known as nectaries. Nectar has several ecological functions; for example, it acts as a signalling substance in the chemical communication with animal pollinators, protects flower gynoecium against microorganisms, and serves as a food reward for pollination services to pollen vectors, thereby assisting plant fecundity. Importantly, nectar is the primary resource for honey production from honeybee foraging activities. The composition of floral nectar differs among species. Nectar quality (e.g., volume, constituents and their amounts) is correlated with the foraging behaviour of pollinators. In the case of honeybees, nectar quality impacts on the composition of the resulting honey. Most honey exhibits antimicrobial activity due to the enzymatic production of hydrogen peroxide and/or the presence of flavonoids.

Manuka honey is famous for its medicinal properties in wound healing. Honey produced from the foraging activities of honeybees on floral nectar of some *Leptospermum* spp. results in manuka honey. Dihydroxyacetone (DHA) in *Leptospermum* floral nectar is the precursor compound for the antimicrobial agent MGO (Adams et al. 2008) present in manuka honey. MGO is present only in the honey upon conversion from DHA during honey maturation. Currently, DHA appearance in nectar is thought to be exclusive to *Leptospermum* spp. Although DHA is a nectar constituent in *Leptospermum* spp. floral nectar, the mechanism, and benefit of DHA to *Leptospermum* spp. flower is enigmatic considering that its production is not common among plants. Presently more than 87 *Leptospermum* spp. are recognised of which 84 are endemic to Australia. Many of the species in Australia have been tested to determine the DHA concentration in their nectar (Williams et al. 2018) providing a basis to explore the value of a manuka honey industry.

Whilst there is an urgent need to search for alternative antibacterial agents due to increasing antibiotic resistance, natural precursor compounds like DHA may be pivotal to providing non-resistance-building alternatives for future health management. Understanding how DHA appears in floral nectar of *Leptospermum* spp. thus forms the basis for this research.

Objective(s)

The objective of this project was to understand the genetic control of *Leptospermum* nectar production and its relationship with dihydroxyacetone (DHA) appearance so that plant material can be selected and manipulated to optimise production of high-quality nectar.

Key activities

Leptospermum species were monitored for their nectar production cycle in relation to DHA production

The genetic control of DHA production was explored using transcriptome analysis by RNA sequencing.

Impact(s)

Production of Australian *Leptospermum* honey will be optimised through understanding of DHA production within the nectar of different species/provenances and the underlying gene expression profiles responsible for DHA production.

Academic outputs

Sylvester Obeng-Darko was the resource facilitator in a student workshop entitled *From nectar to Honey; A healthy choice for mankind* for international students from Japan, 10th March 2020, hosted by UWA student exchange.

Obeng-Darko, S.A., Brooks, P.R., Veneklaas, E.J. and Finnegan, P.M. (2022). Sugar and dihydroxyacetone concentrations in floral nectar suggest continuous exudation and reabsorption in *Leptospermum polygalifolium* Salisb. (Submitted to Plant Science 14th March 2022)

Manuscript in preparation describing dynamics of DHA production in *L. nitens,* linked to transcriptome analysis of nectary-enriched tissues.

Manuscript in preparation describing the discovery of DHA in nectar of *Ericomyrtus serpyllifolia* (Myrtaceae) and *Verticordia chrysantha* (Myrtaceae).

PhD thesis being compiled by Sylvester Obeng-Darko.

Industry outputs

Industry engagement with beekeepers of the Greater Bunbury Regional Chapter of the WA APIARISTS' SOCIETY, 18th August 2021. A talk was presented on floral nectar compositions and nectar quality of *Leptospermum* spp.

Project Activities

Sites

- Queensland and New South Wales – *Leptospermum polygalifolium*
- South-west Western Australia – *Leptospermum nitens* and species within the genera *Ericomyrtus*, *Chamelaucium*, *Kunzea*, and *Verticordia*

PART 1: Sugar and dihydroxyacetone concentrations in floral nectar suggest continuous exudation and reabsorption in *Leptospermum polygalifolium* Salisb.

Published paper

Obeng-Darko, S.A., Brooks, P.R., Veneklaas, E.J. and Finnegan, P.M. (2022). Sugar and dihydroxyacetone concentrations in floral nectar suggest continuous exudation and reabsorption in *Leptospermum polygalifolium* Salisb. (Submitted to Plant Science Volume 323, 111378)

Introduction

Floral nectar accumulation and exudation over flower lifespan are dependent on internal and external factors, invariably impacting nectar quality. Current nectar quality estimation models overlook time of day, daily (24 h), and long-term dynamics of nectar exudation and accumulation over flower lifespan. *Leptospermum polygalifolium* Salisb. can accumulate high concentration of dihydroxyacetone (DHA), precursor of the antimicrobial compound methylglyoxal found in honey obtained from Leptospermum spp. floral nectar.

Methodology

To explain the dynamics of nectar quality over flower lifespan, we collected standing nectar from flowers of *L. polygalifolium* clones at different flower age and then re-collected 24 h later from the same flower. High-Performance Liquid Chromatography was used to quantify DHA amount and total equivalents of glucose + fructose (Tsugar) per flower in the nectar.

Key Results

DHA and Tsugar amount per flower differed between clones and with flower age. In standing nectar, the amount of DHA and Tsugar per flower rose to a broad peak post-anthesis before decreasing. Immediately after peaking, DHA declined more quickly than Tsugar in standing nectar due to a decrease in the exudation potential for DHA than that of Tsugar, suggesting reabsorption of these components.

Figure 1: Flowers of cultivated *Leptospermum polygalifolium* in the field. (A) A bush of *L. polygalifolium*; (B) *Leptospermum polygalifolium* flowers differing in developmental age: (a) Popcorn stage of the flower, petals about to open; (b) Freshly opened flower with all petals relaxed, and (c) Open flower with fully relaxed petals. Arrowheads show the nectary surface where nectar readily accumulates; (C) Selected flowers concealed in net bags to reduce nectar removal by robbers and pollinators.

Figure 2 *Leptospermum polygalifolium* flower development beginning approximately 36 h before anthesis (-36 h bud) to flower senescence at day 14. Flowers at day 14 was not significantly different from day 19 flowers. Flowers at approximately -12 h were termed the popcorn stage. On day 1, there was no detectable nectar. Nectar was visible from day 2. Arrowheads show nectary surface on day 4, and accumulated nectar on day 6 and hypanthium on day 8.

Table 1 Two-way ANOVA on data comparing *Leptospermum polygalifolium* clones M09-005 and M10-001 for dihydroxyacetone (DHA), Tsugar (fructose plus glucose) and DHA : Tsugar. Accumulated nectar and freshly exuded nectar within 24 h across post-anthesis flower development. P values < 0.05 (bold) are considered significant.

			Accumulated nectar				Freshly Exuded within 24 h			
Nectar trait	Source of variation	DF	Sum of squares	Mean squares	F	*P value*	Sum of squares	Mean squares	F	*P value*
DHA	Clone	1	0.34	0.34	6.23	**0.0152**	0.02	0.02	6.01	**0.0115**
	Flower age	7	2.09	0.29	5.49	**< 0.0001**	0.59	0.08	29.69	**< 0.0001**
	Interaction	7	0.43	0.06	1.12	0.3624	0.04	0.01	1.87	0.09
Tsugar	Clone	1	965	965	3.2	0.0784	7.45	7.45	0.06	0.8081
	Flower age	7	15077	2153	7.15	**< 0.0001**	10513	1501	12.01	**< 0.0001**
	Interaction	7	1495	213	0.71	0.6646	1858	265	2.12	0.0543
DHA : Tsugar	Clone	1	205	205	35.78	**< 0.0001**	76	76	33.9	**< 0.0001**
	Flower age	7	620	88	15.43	**< 0.0001**	341	48	21.68	**< 0.0001**
	Interaction	7	72	10	1.81	0.1014	36	5.18	2.29	**0.0379**

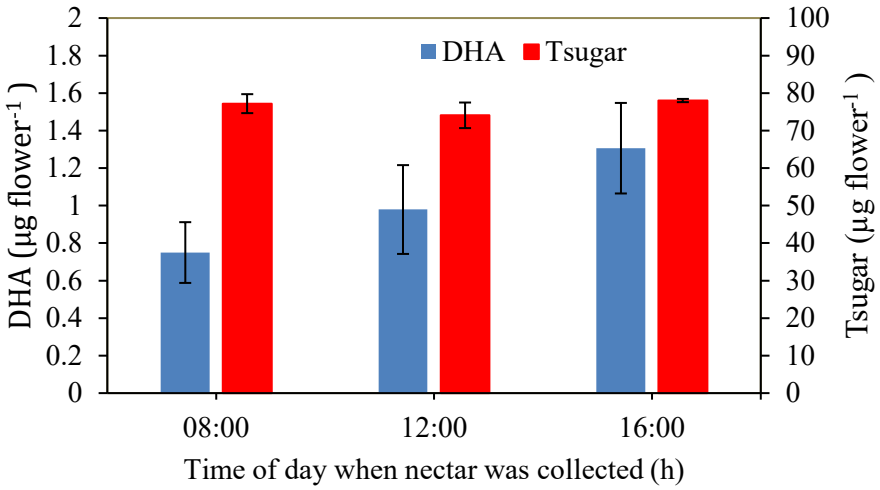

Figure 3: DHA and Tsugar amount in nectar versus time of day when nectar samples were collected for clone M09-005 of *Leptospermum polygalifolium*. Standing nectar that had accumulated since anthesis in day 07 flowers was collected at the indicated times. Different sets of flowers were sampled at each time. Means ± s.e.m. (n = 5) are presented. One-way ANOVA (p<0.05) was used to compare DHA (p = 0.229) and Tsugar (p = 0.511) versus time of day.

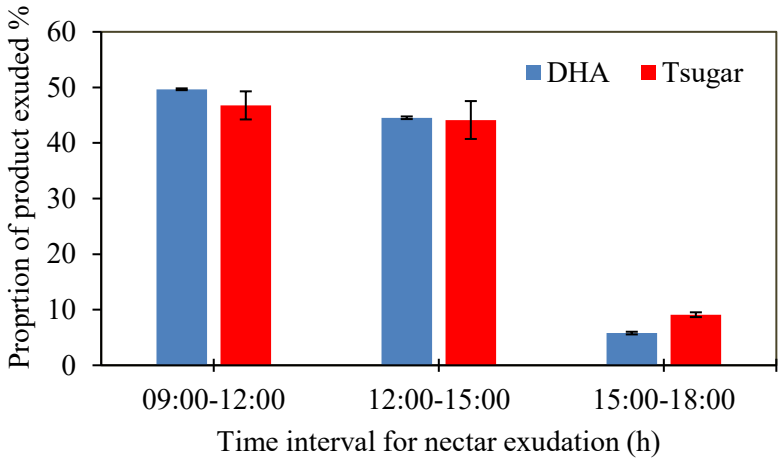

Figure 4 : Proportion of DHA and Tsugar exuded into nectar of the same flowers of clone M10-001 during the course of day 7. Standing nectar was removed at 09:00 h and discarded. Freshly exuded nectar within each 3 h block was analysed. Outcome was expressed as a percentage of the total DHA (0.348 µg ± 0.081) and sugar 68.4 µg ± 10.5) that was exuded during the 9 h experiment. Means ± s.e.m. (n = 4) are shown.

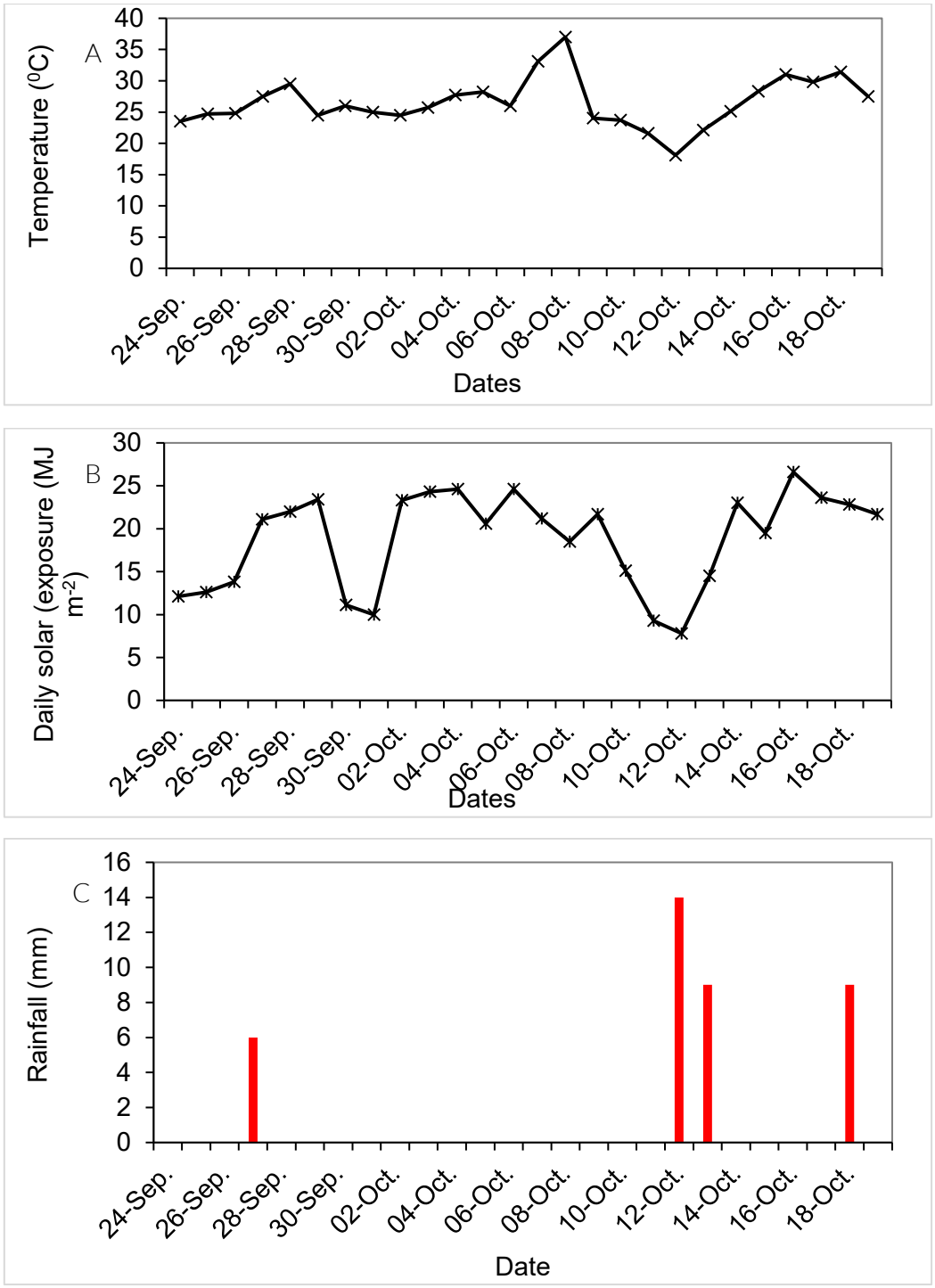

Figure 5: Mean weather variables reported before and during the experimental period. Data were obtained from weather station numbers 058158 (28. 3395^0 S, 153.3809^0 E) and 058036 (28.31^0 S, 153.2229^0 E) Australian Bureau of Meteorology (http://www.bom.gov.au). (A) Mean air temperature reading on each day recorded at weather station number 058036; (B) Daily average solar exposure data recorded at station number 058036; and (C) Rainfall measurement data recorded at station number 058036. Experimental period was 2 October to 18 October 2019.

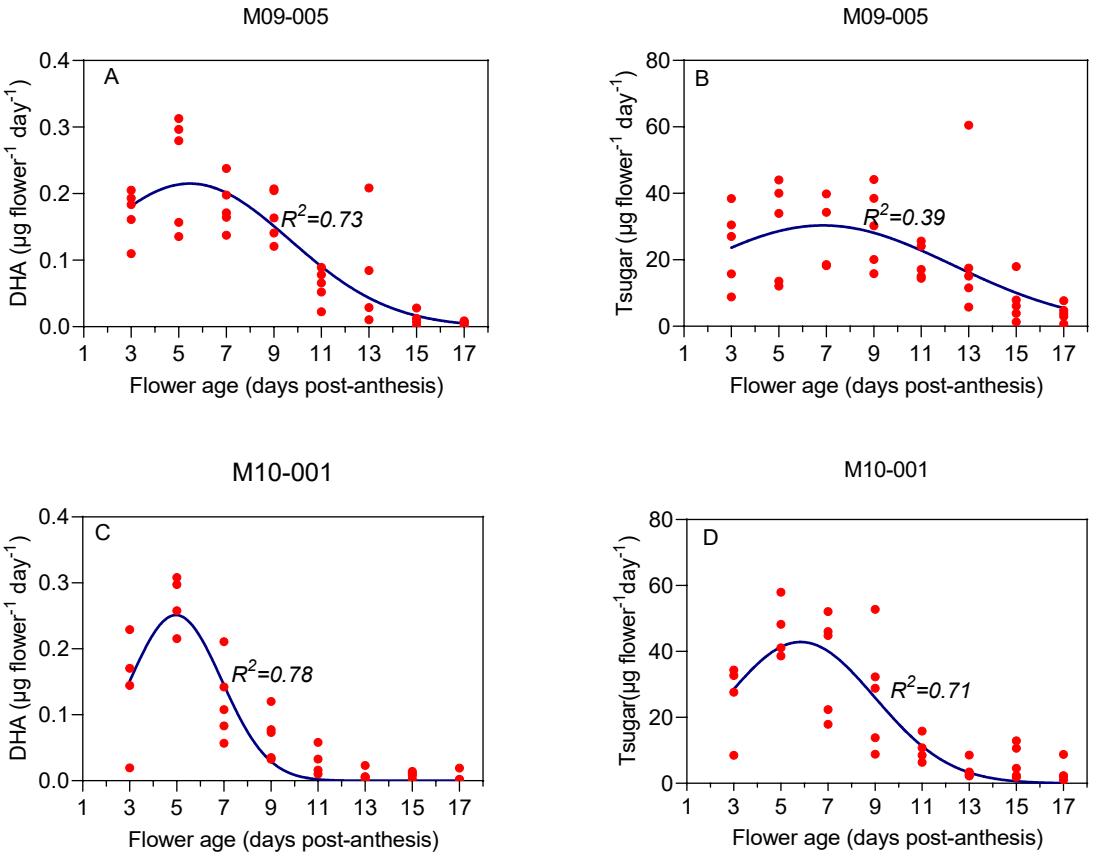

Figure 6: Nonlinear regression models of data for freshly exuded *Leptospermum polygalifolium* nectar. Curve fitting and smoothing was based on the Gaussian distribution.

Figure 1. (A) DHA model for flowers from clone M09-005, $E = 0.22 \times exp\left\{-0.5 \times \left(\frac{d-5.5}{4.2}\right)^2\right\}$

Figure 2. (B) Tsugar model for flowers from clone M09-005, $E = 30.34 \times exp\left\{-0.5 \times \left(\frac{d-6.9}{5.5}\right)^2\right\}$

Figure 3. (C) DHA model for flowers from clone M10-001, $E = 0.25 \times exp\left\{-0.5 \times \left(\frac{d-4.9}{1.9}\right)^2\right\}$

Figure 4. (D) Tsugar model for flowers from clone M10-001, $E = 42.87 \times exp\left\{-0.5 \times \left(\frac{d-5.8}{3.2}\right)^2\right\}$

Conclusions

Dynamics of DHA: Tsugar ratio across flower development between standing nectar and nectar exuded over the next 24 h was similar, indicating that exudation and reabsorption occurred concomitantly hence establishing a balance between accumulation and exudation. A quantitative model suggested that flowers have the potential to accumulate more DHA and Tsugar in nectar than was measured.

Part II: Dihydroxyacetone in floral nectar of Myrtaceae genera outside *Leptospermum*

Introduction

A molecular study of the *Leptospermum* genus found that the genus, as presently defined, is not a single evolutionary lineage (Binks et al., 2022)[1]. They recommended dividing *Leptospermum* into five genera, and this is currently being worked on by taxonomists. Noted was that there were a number of other genera found to be closely related to *Leptospermum*, and this project questioned whether species within these genera also contained DHA.

Methodology

Floral nectar samples from five species in four genera within the Myrtaceae family (Figure 8) were collected and analysed for DHA and Tsugar levels (Table 2). The genera from which nectar was collected were *Ericomyrtus, Chamelaucium, Kunzea*, and *Verticordia* all in the area of Kulin, south-west Western Australia (Figure 7).

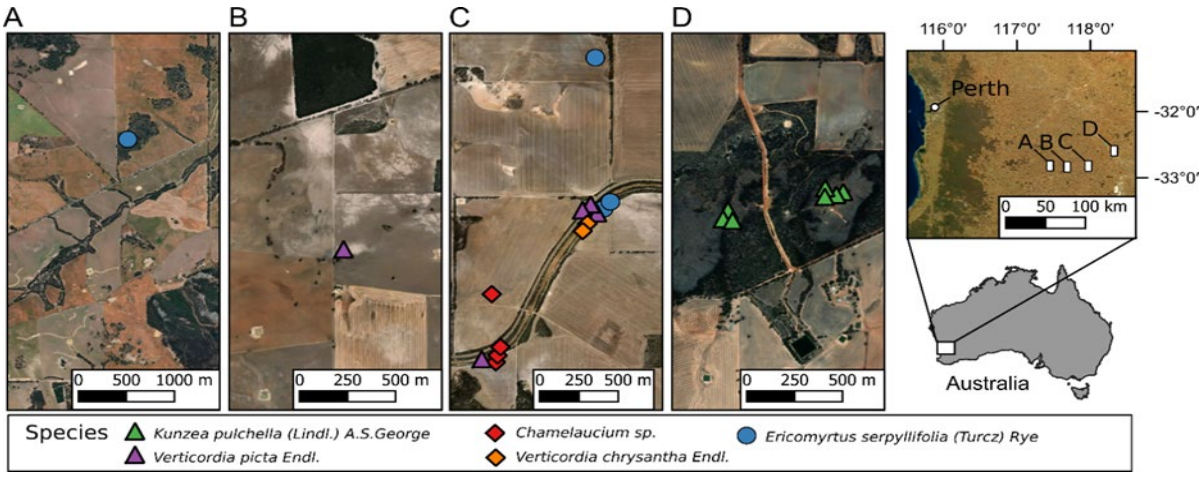

Figure 7: The study locations of the plant species whose floral nectar samples were collected within the Kulin region of Western Australia.

Results

DHA was detected in the nectar of *E. serpyllifolia* and *V. chrysantha* (Table 2), demonstrating that these two genera have the capacity to produce DHA. The amount of the DHA detected varied between *E. serpyllifolia* and *V. chrysantha* ($p < 0.05$). DHA was not detected in the nectar of *V. picta*, *Chamelaucium* sp. and *Kunzea pulchella* (Table 2).

[1] Binks RM, Heslewood M, Wilson PG & Byrne M (2022) Phylogenomic analysis confirms polyphyly of Leptospermum and delineates five major clades that warrant generic recognition. Taxon 71.2: 348-359.

Figure 8: The different species of Myrtaceae plants flowers from which nectar samples were collected: **A** *Chamelaucium* sp. Bendering (T.J. Alford 110), **B**. *Ericomyrtus serpyllifolia* (Turcz) Rye, **C**. *Verticordia picta* Endl , **D**. *Verticordia chrysantha* Endl, **E**. *Kunzea pulchella* (Lindl.) A.S. George

Table 2: Comparison of floral nectar and flower nectary traits from five species of Myrtaceae plants sampled from the Kulin Shire of Western Australia. Values are means ± s.e.m

	Species name				
Nectar and nectary traits	*Chamelaucium sp.*	*Ericomyrtus serpyllifolia*	*Kunzea pulchella*	*Verticordia chrysantha*	*Verticordia picta*
DHA(ug flower^{-1})	0.00	0.0837 ± 0.007*a*	0.00	0.6395 ± 0.136*b*	0.00
Tsugar (μg flower^{-1})	81 ± 2.21*a*	11 ± 0.54*b*	870 ± 8.81*c*	30.57 ± 5.13b	18 ± 4.2*b*
DHA : Tsugar (mg kg^{-1})	0.00	7058 ± 308	0.00	20641 ± 2248	0.00
Fructose (μg flower^{-1})	48 ± 1.26*a*	7 ± 0.31*b*	542 ± 4.02*c*	20 ± 3.57*b*	10 ± 2.44*b*
Glucose (μg flower^{-1})	32 ± 0.95*a*	4 ± 0.23*a*	327 ± 9.23*b*	10 ± 1.56*a*	7 ± 1.78*a*
Fructose : Glucose	1.5 ± 0.007*a*	1.6 ± 0.016*ab*	1.7 ± 0.05*bc*	1.997 ± 0.045*d*	1.546,0.043*ac*
Dry flower nectary capsule mass (g)	0.012 ± 0.0004*a*	0.003 ± 0.0003*b*	0.036 ± 0.001*c*	0.004 ± 0.0002*b*	0.004 ± 0.0001*b*
Nectary surface area (cm^{-2})	0.48 ± 0.025*a*	0.3 ± 0.02*b*	0.9 ± 0.02*c*	0.38 ± 0.017*b*	0.38± 0.03*b*
^{N}MA (mg cm^{-2})	0.02 ± 0.002*a*	0.01 ± 0.001*b*	0.04 ± 0.002*c*	0.01 ± 0.0003*b*	0.01 ± 0.0008*b*
Counts (*n*)	4	4	7	3	4

For this work total sugar was the equivalent of glucose + fructose in the nectar per flower. *Kunzea pulchella* produced the highest Tsugar amount per flower and was followed by *Chamelaucium* sp (Table 2). The Tsugar in *Chamelaucium* sp and *Kunzea pulchella* varied between each other and from the other species. The average amount of Tsugar per flower of *V. chrysantha*, *V. picta*, and *E. serpyllifolia* were similar (p = 0.05) (Table 2).

The ratio of DHA : Tsugar is an important index in determining the strength of bioactivity in honey derived from *Leptospermum* spp. that produce DHA. The average DHA : Tsugar ratio in *E. serpyllifolia* and *V. chrysantha* demonstrated that the DHA : Tsugar measured in the two species were above 6000, and 16000 mg kg-1 respectively in all the nectar samples from the two species tested (Table 2).

Like the average Tsugar amount detected in the nectar per flower, the average amount of fructose in the nectar samples per flower was higher in *Chamelaucium* sp. and *Kunzea pulchella* and were significantly different between themselves and the other species (Table 2). *Kunzea pulchella* has the highest fructose amount per flower and was followed by *Chamelaucium* sp. On the other hand, *Kunzea pulchella* produced the highest average amount of glucose in the nectar per flower. This suggested that the Tsugar amount was largely driven by the fructose amount in all the nectar samples. Although *Verticordia chrysantha* did not have the highest Tsugar amount per flower, it had the highest ratio of fructose to glucose per flower and differed from all the nectar samples tested($p < 0.05$) (Table 2). Surprisingly, the two *Verticordia* species had different fructose to glucose ratios. *Kunzea pulchella* which had the highest amount of Tsugar per flower in their nectar, had fructose to glucose ratios that were different to that of *V. chrysantha* values (Table 2).

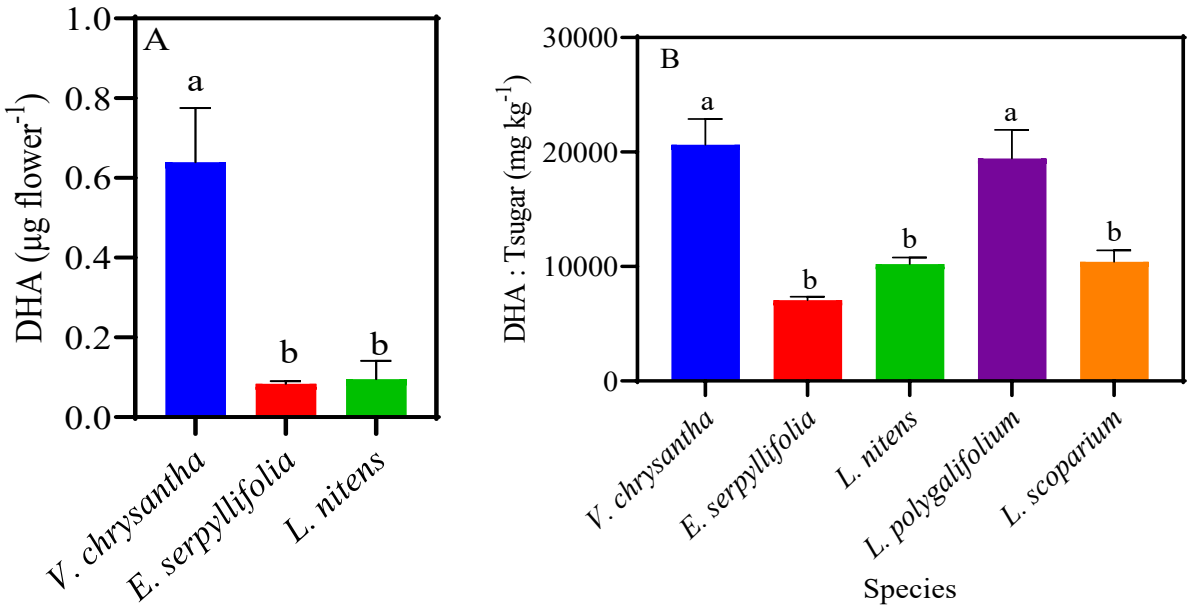

Figure 9: DHA amounts (µg) and DHA :Tsugar ratios (mg kg^{-1}) in *V. chrysantha* and *E. serpyllifolia* compared to some *Leptospermum* species . (A) Comparison of DHA amounts detected in floral nectar of *V. chrysantha*, *E. serpyllifolia* and *L. nitens* collected from the same proximity within the Kulin region of Western Australia. (B) Comparison of DHA/Tsugar concentrations detected in floral nectar of *V. chrysantha*, *E. serpyllifolia*, and *L. nitens, L. scoparium* and *L. polygalifolium.* Nectar of *L. polyglifolium* was collected at Sunshine Coast and *L. scoparium* nectar raw data was sourced from the authors of Williams et al.[1] who used the same detection technique to measure DHA in *Leptospermums* floral nectars they tested. Values are means ± s.e. (*V. chrysantha* n=3, *E. serpyllifolia* n=4, *L. nitens* n=5, *L. polygalifolium* n=6, and *L. scoparium* n=5)

Standing nectar DHA amount per flower in *E. serpyllifolia* and *V. chrysantha* were compared to that of *Leptospermum nitens* growing in the same proximity. *Leptospermum nitens* has high DHA levels and is the preferred species for apiarist involved in the production of bioactive honey from *Leptospermum* in Western Australia. The amount of DHA in *V. chrysantha* was higher than for *E. serpyllifolia* and *L. nitens* ($p < 0.05$) (Figure 9A). However, the DHA amounts for *E. serpyllifolia* and *L. nitens* were similar ($p > 0.05$) (Figure 9A). The DHA : Tsugar ratios from *E. serpyllifolia* and *V. chrysantha* were also compared to that of *L scoparium*, the most widely studied species of Leptospermum targeted by the honey industry and the high DHA accumulators *L. nitens* and *L. polygalifolium* (Figure 8B). The ratios from *V. chrysantha* and *L. polygalifolium* were similar but were significantly different from *L. nitens* and *L. scoparium* ($p < 0.05$). However, the ratio in *E. serpyllifolia* was similar to *L. nitens* and *L. scoparium* ($p < 0.05$) (Figure 9B).

The dry flower nectary capsule mass varied among the species from which floral nectar was sampled ($p < 0.05$) (Table 2). *Kunzea pulchella*, and *Chamelaucium* sp. had the highest nectary dry mass. The flower nectary surface area also varied among the species ($p < 0.05$). *Kunzea pulchella* and *Chamelaucium* sp. were the species with the largest nectary surface area (Table 2). The ratio of the dry flower nectary capsule mass to nectary surface area (NMA) differed among the species ($p < 0.05$) (Table 2). *Kunzea pulchella* had the greatest NMA followed by *Chamelaucium* sp., which differed from each other and the rest of the species. The NMA of *E. serpyllifolia*, *V. chrysantha*, and *V. picta* were similar.

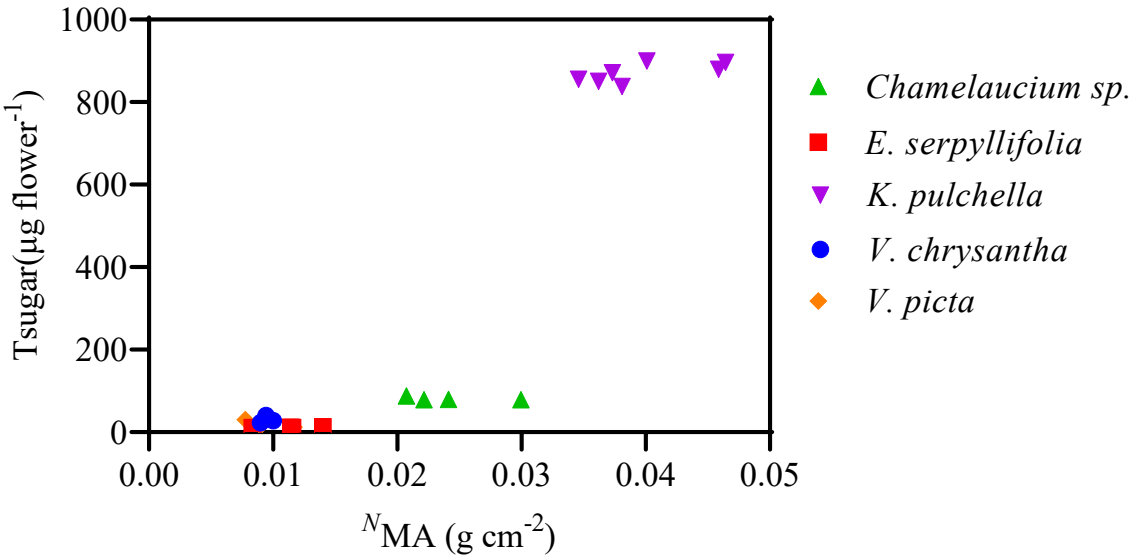

Figure 10: Relationship between Tsugar, and nectary dry mass per nectary surface area, ^{N}MA for the various species of Myrtaceae plants from which nectar was collected.

Finally, NMA and Tsugar content produced per flower were associated in scatter plot (Figure 10). *Kunzea pulchella*, which had the greatest NMA ratio, also had the highest Tsugar. *Ericomyrtus serpyllifolia, V. chrysantha*, and *V. picta*, which had lower NMA ratios, had correspondingly lower Tsugar per flower.

Discussion

This study has identified two new species of Myrtaceae, *Ericomyrtus serpyllifolia* and *Verticordia chrysantha*, that contain DHA in their floral nectar. These genera are phylogenetically related to *Leptospermum*, opening new areas for investigating floral nectar with bioactivity to add to the repertoire of non-peroxide bioactive honeys.

PART III: *Leptospermum nitens* bioactive nectar production

This part of the Project will be reported in the thesis.

Implications

More and more flora capable of producing DHA in their nectar are being identified. Many of these newly identified species flower together with many other flowering species in the spring bloom that occurs in the south-west of Western Australia, and hence why this occurrence may have been overlooked until now when the nectar was analysed.

Generally, honeys produced from the northern regions of the south-west of Western Australia are not assessed for antibacterial bioactivity and this result indicates that this should perhaps now be occurring.

Important is this discovery now offers a wider choice of species, and perhaps a longer flowering period, for the establishment of plantations for non-peroxide bioactivity in honey.

Nectar attractiveness is related to the sugar content and the form of the sugars within the honey. Honey bee preference is for sucrose to fructose, and fructose in preference to glucose. The ideal honey bee sucrose range is between 30-50% sucrose in preference to higher or lower concentrations. The lower the sugar concentration, the more energy is required from the colony to dehydrate the nectar for ripening and final storage. These new species may well have a preferred sugar levels for honey bee attraction.

Recommendations

- Attention needs to be paid to flower age when sampling for DHA in nectar to get an accurate assessment of their production capacity.
- Bees should be put onto *Leptospermum polygalifolium* when the majority of flowers are at their peak production stage. At peak production, nectar harvest will stimulate the production of new nectar with the same properties of the nectar that was harvested.
- Investigate honey quality from apiary sites near *Ericomyrtus serpyllifolia and Verticordia chrysantha* by measuring the conversion of DHA into MGO.
- Investigate other closely related species for DHA in their nectar to broaden the selection of species for planting for non-peroxide bioactive honey production.

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