Influential Factors in Nectar Composition and Yield in *Leptospermum scoparium*

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science

Institute of Agriculture and the Environment
College of Sciences, Massey University
Palmerston North, New Zealand

Elizabeth Mary Nickless

2015

ABSTRACT

Leptospermum scoparium (Mānuka) is the plant nectar source for medically bioactive honey, commercially marketed in New Zealand as Unique Mānuka Factor honey (UMF-honey). Methylglyoxal (MGO) is the unique bioactive component of UMF honey with Mānuka nectar containing significant amounts of the carbohydrate dihydroxyacetone (DHA), the chemical precursor for MGO. Anecdotal evidence and recently published data from nectar samples collected from various cultivars in natural sites or botanical gardens has indicated that the DHA and overall composition of *L. scoparium* nectar varies according to cultivar. The source of this variation is not clearly understood and although there is considerable literature on climatic and genetic influences on nectar composition and yield within various other plant species, there is little published work available on the influence of genetic and environmental factors on the composition and yield of nectar in *L. scoparium.*

Of value to the commercial UMF honey industry in New Zealand is the ability to assess cultivars from breeding programs for the best potential to increase overall UMF honey yield. Predictive modelling of yields is invaluable to the developing honey industry to allow assessment of environmental influences that may affect overall yield along with seasonal influences on nectar production in Mānuka. The research in this thesis establishes the effect of various parameters on overall DHA yield from Mānuka and the beginnings of modelling influencing environmental factors.

To determine influences on dihydroxyacetone (DHA) concentration and yield in the nectar of *L. scoparium* a number of studies were carried out. Methodologies for the collection and analysis of nectar were established. Ten different cultivars of *L. scoparium*

with a range of genetic parentage were studied in controlled glasshouse conditions to assess phenotypic variability in terms of nectar composition and yield as well as plant growth and flowering amongst these cultivars. Significant differences in plant growth and flowering habits were observed amongst the ten cultivars, significant differences in nectar yield and nectar composition with regard to DHA yield were also observed. DHA yields ranged from 2714-7459 mg of DHA/kilogram normalised to 80 ^{o}BRIX, with total nectar sugar yields ranging between 0.7 and 4.8 mg amongst the ten cultivars studied. Preliminary research into the effect of temperature, radiation and humidity on nectar composition and yield were also undertaken.

Effects of soil composition on these same parameters were researched with a subset of three of the ten cultivars grown on ten different soil types. Plant relative growth rates, dry weights and total plant height were measured throughout a 15 month glasshouse trial. Plant growth, flowering phenology, floral density, nectar yield and DHA composition data was gathered. Soils were analysed for various macronutrient and micronutrient levels and these parameters were modelled against plant data to determine which soil components were influencing plant parameters of interest. Soil type was shown to have no significant effect on DHA concentrations in nectar but results did show that soil type had a significant effect on flowering density amongst the three *L. scoparium* cultivars studied in the trial. Results from regression analysis of soil chemistry against measured plant parameters indicate that a fertiliser regime has the potential to increase nectar yields due to increased flower numbers. Multivariate analysis using partial least squares regression of soil composition data against plant parameters of value showed that soil components; phosphorus, sulphate, ferric and chloride were commonly shown to influence plant parameters measured.

Analytical spectroscopy was investigated as a method to chemotype *L. scoparium* cultivars and also as a method for quantifying nectar components sucrose, glucose, fructose and DHA.

Nectar composition was analysed using high pressure liquid chromatography (HPLC) and compared with fourier transform Raman spectroscopy (FT-Raman) and attenuated total reflectance infrared spectroscopy (ATR-FTIR) analytical spectroscopy methods.

FT-Raman spectroscopy was shown to be useful in chemotyping cultivars and in addition proved to be a useful analytical method to predict DHA yield using leaf material from *L. scoparium* plants from the ten cultivars. FT-Raman and ATR-FTIR proved to be relatively accurate techniques to quantify *L. scoparium* nectar components DHA, fructose, glucose and sucrose, compared with HPLC methods which use extensive preparation techniques. R-squared values were very good for all nectar components measured excepting the sucrose model at $R^2 = 0.77$. The R^2 for the FT-Raman predictions of DHA against HPLC data are very good at 0.85. FTIR prediction data against HPLC data was also good at 0.86 R^2. Overall an accurate model is possible for quantifying DHA concentrations in nectar using both FTIR-ATR and FT-Raman spectroscopy.

Overall results show that various factors need to be considered when assessing plants for commercial use in the (UMF) Mānuka honey industry within New Zealand. Due to their large impact on overall nectar yield; floral density and plant growth rate parameters are the two key factors of value for commercial assessment of Mānuka cultivars. This research also highlights the importance of assessing not just DHA concentration in deducing cultivar value, but overall nectar yield. These key features

must be explored when assessing *L. scoparium* plants within breeding programs, prior to selection for large-scale field production of high UMF Mānuka honey.

Acknowledgements

This work was supported by Primary Growth Partnership (PGP) funding (Ministry of Primary Industries, New Zealand) awarded to Mānuka Research Partnership (NZ) Limited (MRPL), and a scholarship from Callaghan Innovation (NZ) to L.M.N. [Contract: MARP1001].

Supervision and staff support from the Institute of Agriculture & Environment, Massey University; Palmerston North, New Zealand.

The Primary Growth Partnership (PGP) scheme brings the resources of many people together into one research program. And I feel privileged to have worked on my thesis under this collaborative scheme. The PGP scheme brings an extra focus to the work and the connection and interaction with the commercial partners (MRPL) in the scheme has been an invaluable and enjoyable experience.

There are many people, Dr Steve Holroyd, Professor Keith Gordon, Dr Chris Anderson, Dr Jason Wargent, Dr Jonathan Stephens, Professor Michael McManus, Dr James Milner and Associate Professor Alastair Robertson, who my sincere thanks go to for their scientific input and support for the research in this thesis. My special thanks and appreciation go to Georgie Hamilton for the technical support she has given me, along with our many enjoyable discussions on various other topics of interest while we worked on the Mānuka program. Additional thanks and much appreciation go to my supervisor Dr Steve Holroyd whose unfailing support encouraged me to attempt this thesis.

There are many others who I owe a debt of gratitude for the help and support with the experimental work in this thesis in particular the staff at the plant growth unit at Massey

University, Lindsey, Leslie and Steve and in addition Chris Rawlingson for his help with the analytical work on the HPLC and Geoff Smith at Otago University for his help with using the Raman equipment. And my employer: Fonterra Research and Development Centre (FRDC) Palmerston North who have allowed me the time from work to work on my thesis, and especially the support from FRDC statisticians Barbara Kuhn-Sherlock and Maree Luckman.

Last thanks go to my family and the support of my partner Daryl and son Henry who have suffered my lack of attention when my evenings were spent focussed on writing and my thoughts often elsewhere focussed on data analysis. Special thanks to my son Henry who helped me measure plants, collect leaves and chop up plants when I needed his help and entertained me with his wonderful wit, humour and patience along the way.

For myself, I have greatly enjoyed working on this thesis, it has been a very enjoyable experience and I have learnt an immense amount along the way and have really enjoyed getting to know all those involved and have greatly appreciated the input and knowledge and experience shared with me.

Thank you!!

Table of Contents

Contents

List of Tables

List of Figures

List of Abbreviations and Symbols

AAS	atomic absorption spectrophotometry
ACN	Acetonitrile
ANOVA	Analysis of Variance
ATR	Attenuated Total Reflectance
	(Attenuated Total Reflectance Fourier Transform
ATR-FTIR	(Infrared Spectroscopy
B	Boron
BS	Basal Stem
Ca	Calcium
CCD	Charged Couple Device
CCE	Calcium Carbonate Equivalent
CEC	Cation Exchange Capacity
Cl	Chloride
cm^{-1}	Spectral Wavenumbers
Co	Cobalt
Cu	Copper
CV	Cultivar
DHA	Dihydroxyacetone
EDTA	Ethylene-diamine-tetra-acetic acid
Fe	Ferric/Iron
FP	Flowering Period
F	Fructose
FTIR	Fourier Transform Infrared Spectroscopy
FT-Raman	Fourier Transform Raman Spectroscopy
g	Grams
GC	Gas Chromatography
GC-FID	Gas Chromatography with Flame Ionization Detector
GLM	General Linear Model

G	Glucose
HA	Hydroxyacetone
HPLC	High Pressure Liquid Chromatography
HT	Plant Height
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IR	Infrared
K	Potassium
kg	Kilograms
l	Litre
L. scoparium	*Leptospermum scoparium*
LCMS	Liquid Chromatography Mass Spectrometry
me	milli-equivalents of exchangeable base cation
Mg	Magnesium
mg	Milligrams
MGO	Methylglyoxal
Mj/m^2	Micro-Joules of light energy per meter squared
ml	Milli-litre
mM	Milli-Molar concentration
Mn	Manganese
MRPL	Mānuka Research Partnership Limited
MS	Mass Spectrometry
MSC	Multiplicative Scatter Correction
MSI	Mānuka Soil Index
mW	Milli-Watts
N	Nitrogen
Na	Sodium
NA	Not Applicable
NaOH	Sodium Hydroxide
NIPALS	Non-Linear Iterative Partial Least Squares

NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
NP	Nectar Potential
NPA	Non-Peroxide Antibacterial Activity
P	Phosphate
PC	Principal Component
PCA	Principal Component Analysis
PFBHA	O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine
PGP	Primary Growth Partnership
pH	Acidity/Alkalinity level
PLS	Partial Least Squares
PLSR	Partial Least Squares Regression
R	Correlation Value
RGR	Relative Growth Rate
RI	Refractive Index
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSECP	Root Mean Square Error of the Prediction model.
RMSECV	Root Mean Square Error of the Cross Validated model.
S	Sulphur/Sucrose
SC	Seed Capsule numbers
SD	Start Days = Start of Flowering Period in Days
SNV	Standard Normal Variate
SO_4	Sulphate
µg	Micro-grams
µL	Micro-litres
UMF	Unique Mānuka Factor
UV/VIS	Ultra Violet/Visual Absorbance Spectra)
UV	Ultra Violet

Y, MG, G, B, BS, P, O, LG, PU and R	Cultivar Codes
Yd	Yield
Zn	Zinc