Companion biota associated with *Leptospermum scoparium* (mānuka; Myrtaceae)

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Abstract

Leptospermum scoparium (mānuka; Myrtaceae) is involved in three crucial ecological interactions that might affect nectar production, and the New Zealand honey industry. First, these plants can be affected by scale insect infestation which have the potential to affect plant health, second, they provide nectar for honey bees (mānuka honey), and third, they are hosts for, and may receive benefits from, dual mycorrhizal fungal associations (both ecto- and endo-). The understanding of these interactions is very important for the honey industry as well as for New Zealand ecosystems. However, there is limited knowledge about the influence of scale insects and mycorrhizal fungi on plant growth and nectar production, and the influence of honey bee visitation on the honey making-process. To better understand the significance of these interactions, a variety of methods, including behavioural observations, histological, molecular, and taxonomic techniques, were used in this thesis.

Findings showed that the eriococcids *Acanthococcus campbelli* and *Acanthococcus leptospermi* are now the main species on *L. scoparium*, rather than *Acanthococcus orariensis*, which was the main causative agent of the mānuka blight in the 1940's and 1960's. Whereas the distribution of *A. leptospermi* was previously reported, the distribution of *A. campbelli* across New Zealand's islands was illustrated for the first time in this thesis. Other scale insect species classified within the families Coelostomidiidae, Diaspididae, and Pseudococcidae were also found, but their incidence and abundance was typically lower in comparison to the family Eriococcidae.

The number of eriococcids was reduced by the application of an Insect Growth Regulator (IGR) on six different cultivars in a split plot designed experiment, but cultivars differed in response to the insecticide treatment. Using the same common garden design, but just the unsprayed plants, honey bees showed a preference for the cultivar with the highest nectar sugar content and nectar DHA content. However, sugar, rather than DHA, was the best predictor of visitation pattern. The number of honey bee visits increased at midday as the day warmed up. The overall number of flowers estimated per plant was included in the model, but did not drive the visit number as, for example, it was found that the cultivar with the highest estimated number of flowers was less visited.

Bioinformatics analysis revealed the association of *L. scoparium* with at least 25 fungal classes, including 16 ectomycorrhizal (EcM) fungal lineages and eight arbuscular mycorrhizal (AM) families. The majority of mycorrhizal fungal lineages were shared among cultivated and wild plants at the three studied sites, which suggests that cultivated plants are naturally colonised by mycorrhizal fungi. The EcM fungal lineages /cortinarius, /laccaria, /tomentella-thelephora, and the AM families Glomeraceae and Claroideoglomeraceae were the most abundant. Among the EcM fungal species, *Laccaria glabripes* and the endemic EcM fungal species *Clavulina subrugosa*, *Cortinarius waiporianus* and *Dermocybe indotata* were revealed as the most abundant. The presence of the exotic EcM fungal species *Amanita muscaria* was limited and mainly found in cultivated plants, that had established on a site previously with *Pinus radiata*. The cosmopolitan AM fungal species *Rhizophagus irregularis* and *Claroideoglomus lamellosum* were the dominant species found in both cultivated and wild plants.

Among cultivated and wild plants, wild plants appeared to be colonised by a more diverse mycorrhizal fungal community. For instance, the lineage /russula-lactarius was more abundant in wild plants than in cultivated plants. The presence of /russula-lactarius and other lineages and species could be improving host performance (seed establishment, drought tolerance, pathogen resistance, and plant growth) on wild plants. However, the absence of some of the mycorrhizal fungal species from cultivated plants, which could be present on wild plants, could limit the potential yield of *L. scoparium* plantation. Finding suitable combinations of mycorrhizal fungal inoculum could help optimise the development of *L. scoparium*, nectar production, and subsequently the New Zealand mānuka honey industry.

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Kilometres do not make the distance, people make the distance.

To:

los pulgos Alessandro, Catalina, Cristina, and Eva

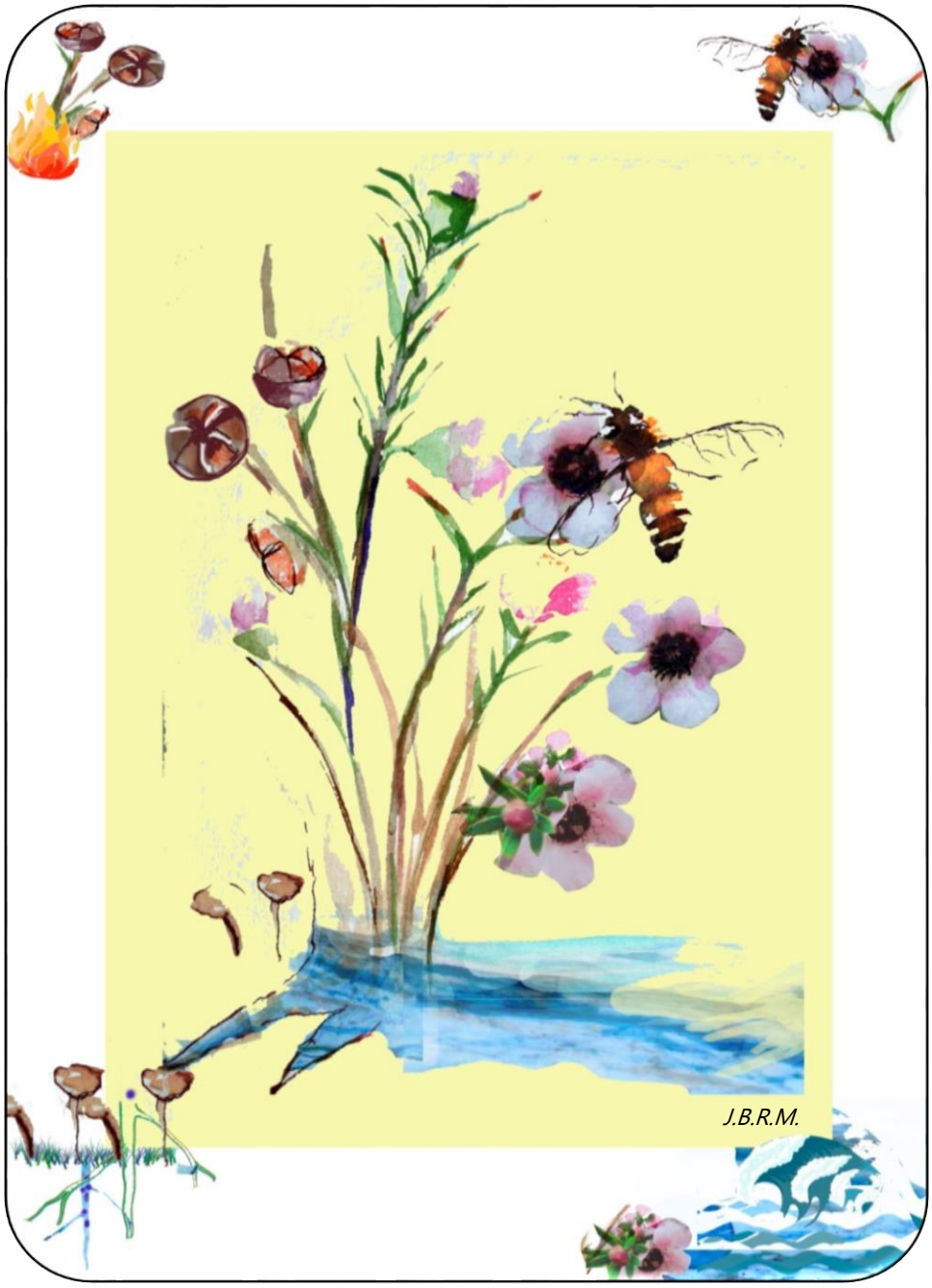

Illustration: Ester Gámez Blánquez

Contents

Glossary of abbreviations

Abbreviation	Full name
AAs	Amino acids
acc. no.	Accession number
ACN	Acetonitrile
AGRF	Australian Genomic Research Facility
AK	Auckland
AM	Arbuscular mycorrhizal
am	Ante meridiem
ANOVA	Analysis of Variance
B	Blue (cultivar)
BA	Basal area
BIOM	Biological Observation Matrix
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
BP	Bay of Plenty
CL	Coromandel
Dev	Deviance
d.f.	Degrees of freedom
DHA	Dihydroxyacetone
DSE	Dark-septate endophyte
E	East
EcM	Ectomycorrhizal
GIS	Geographic Information System
GLM	Generalized Linear Model
GPS	Global Positioning System
H	Height
HA	Hydroxyacetone
HB	Hawkes Bay
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IGR	Insect Growth Regulator
ITS	Internal Transcribed Spacer
KA	Kaikoura
KOH	Potassium hydroxide
LC	Lethal concentration
LG	Lime green (cultivar)
LOD	Limit of detection
LSU	Large Subunit
M	Molar
MG	Mint green (cultivar)
MGO	Methylglyoxal
N	North
NaOH	Sodium hydroxide
NC	North Canterbury
NCBI	National Center for Biotechnology Information
ND	Northland
NGS	Next Generation Sequencing

NIWA	National Institute of Water and Atmospheric
NMDS	Nonmetric Multidimensional Scaling
NN	Nelson
NPA	Nonperoxide activity
NZAC	New Zealand Arthropod Collection
NZGL	New Zealand Genomics Limited
O	Orange (cultivar)
OTU	Operational Taxonomic Unit
P	Pink (cultivar)
PCR	Polymerase Chain Reaction
PCRU	Pasture & Crop Research Unit
PermANOVA	Permutational multivariate analysis of variance
PFBHA	O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine
PGP	Primary Growth Partnership
pH	Potential of hydrogen
Ph. As.	Phylum Ascomycota
Ph. Ba.	Phylum Basidiomycota
Ph. Ch.	Phylum Chytrydiomycota
Ph. Mu.	Phylum Mucoromycota
pm	Post meridiem
QIIME	Quantitative Insights into Microbial Ecology
Ra	Rangitatau
RI	Rangitikei
RI	Refractive Index
Ru	Ruatiti
rRNA	Ribosomal Nuclear A
S	South
SE	Standard error
sec	Seconds
SH	Species hypothesis
SI	Stewart Island
SSU	Small Subunit
Tut	Tutira
UMF	Unique Mānuka Factor
UV	Ultraviolet
VT	Virtual Taxa
W	West
WA	Wairarapa
WD	Westland
WI	Wanganui
Y	Yellow (cultivar)