BioSS

Ministry for Primary Industries
Manatū Ahu Matua

A definition for the authentication of mānuka honey

Steve Hathaway and Claire McDonald

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Why mānuka honey?

- Honey made by bees collecting nectar from mānuka plants – *Leptospermum scoparium*
- Challenges:
  - No gold standard
  - Claims of health/therapeutic benefits
  - Mānuka plants not isolated
  - Bees forage across large areas
  - Natural product can vary

Selecting candidate markers

Nectar chemicals

- Mānuka markers?
  - 2'-methoxyacetophenone
  - 2-methoxybenzoic acid
  - 3-phenyllactic acid
  - 4-hydroxyphenyllactic acid
  - dihydroxyacetone
  - methylglyoxal
  - leptosperin
  - syringic acid
  - abscisic acid
  - kojic acid
  - linalool oxide
- Kānuka markers?
  - lumichrome
  - methyl syringate
  - 4-methoxyphenyllactic acid

DNA from pollen

- DNA marker from the mānuka plant
- DNA marker from the kānuka plant

Physico-chemical

- Colour
- Conductivity
- Thixotropy

Mānuka

Kānuka

Reference collections

Plant collection

- Over 700 plants collected, 509 tested
  - Collected during 2014/15 and 2015/16
  - 12 regions in New Zealand (29 species of plants)
  - 5 states in Australia (5 *Leptospermum* species)

Honey collection

- Over 800 samples collected, 778 tested
  - 660 samples from New Zealand
    - Primarily single apiary sources
    - 2014/15, 2015/16 and archive samples
  - 118 samples from overseas
    - 16 countries

Marker data analyses

Key questions for assessing markers included:

- Only found in mānuka plants (to date)?
- Separate mānuka from other NZ species?

Nectar data

- Separate mānuka honey from other NZ honey types?
- Separate monofloral from multifloral mānuka?
- Stable over increasing time and temperature?

Honey data

Marker evaluation

- **Factors considered include**:
  - Habitat type for nectar samples
  - Relationships between markers
  - Levels found in different honey types
  - Regional and seasonal variation
  - Honey extraction, storage time and conditions

- **Markers selected for further analysis**:

Mānuka DNA marker

Kānuka DNA marker

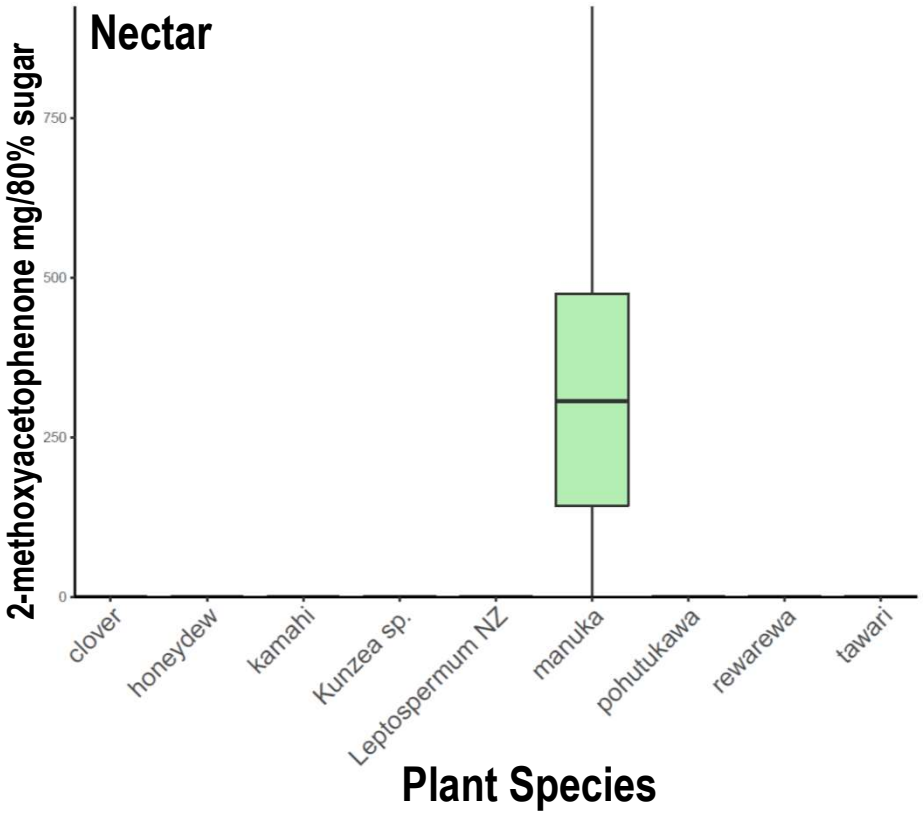
4-hydroxyphenyllactic acid

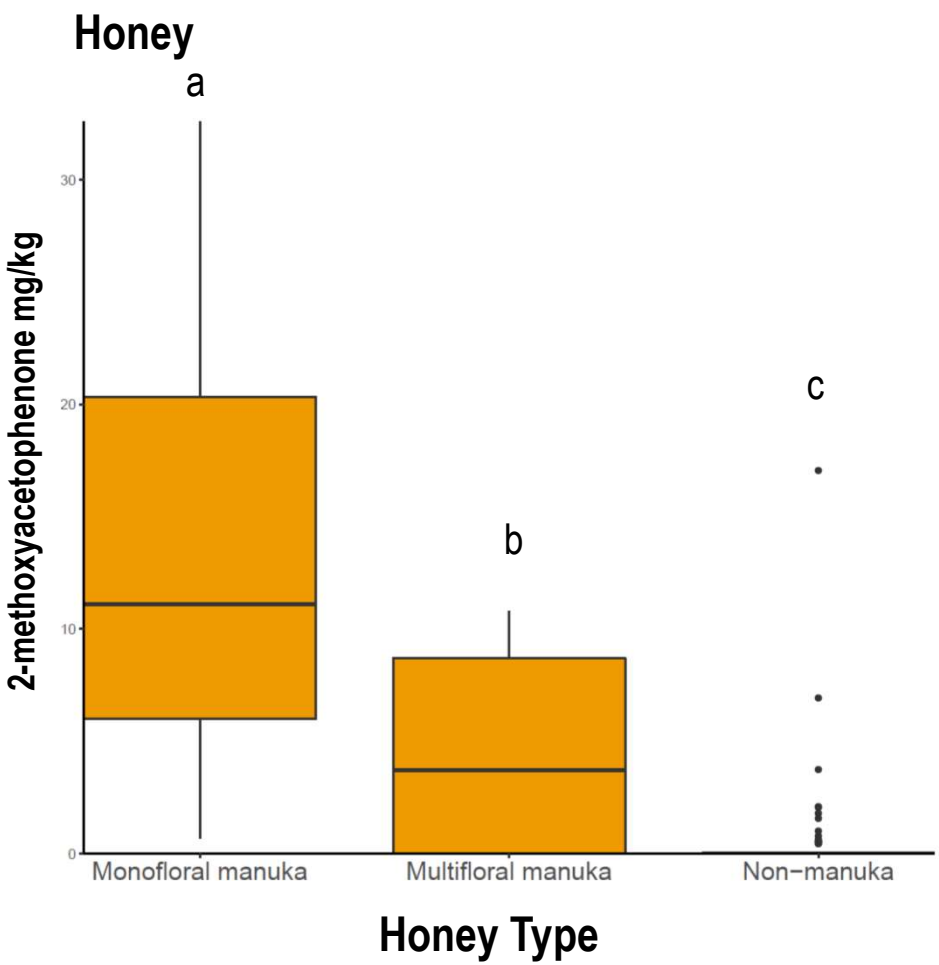
2-methoxybenzoic acid

2'-methoxyacetophenone

3-phenyllactic acid

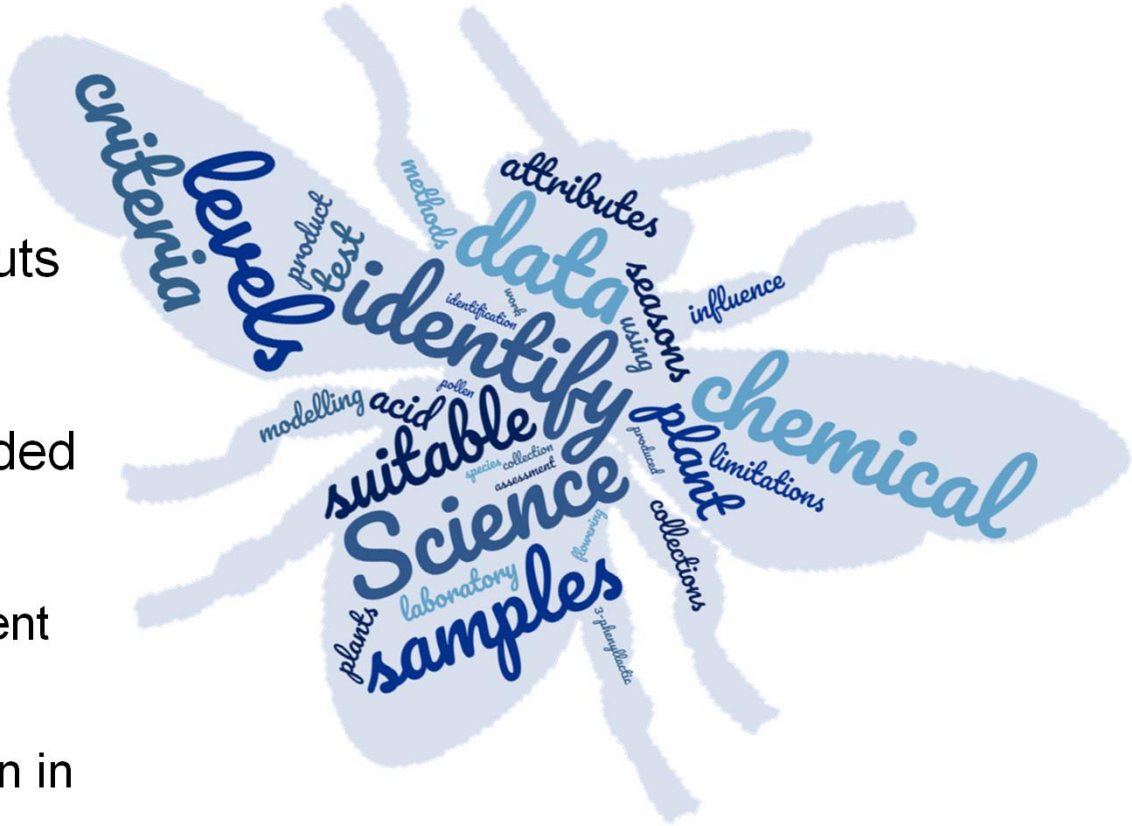
Levels in nectar and honey





Why use classification and regression trees?

- Markers needed to be assessed in combination
- Flexibility to assess outputs with no gold standard
- Identification criteria needed to be:
  - straight forward, transparent and easily interpreted
  - suitable for implementation in regulatory context



Baseline CART model

- Honey type as a 6 level response variable
- Training data: each honey production year plus Australia and non-NZ/Aus samples
- Test data: other honey production year and the archive samples.
- Bootstrap sampling with replacement used to determine:
  - True positives/negatives
  - False positives/negatives
  - Number of times a marker was selected at the first split point
  - Number of times a marker was selected in the CART

Sensitivity of CART outputs

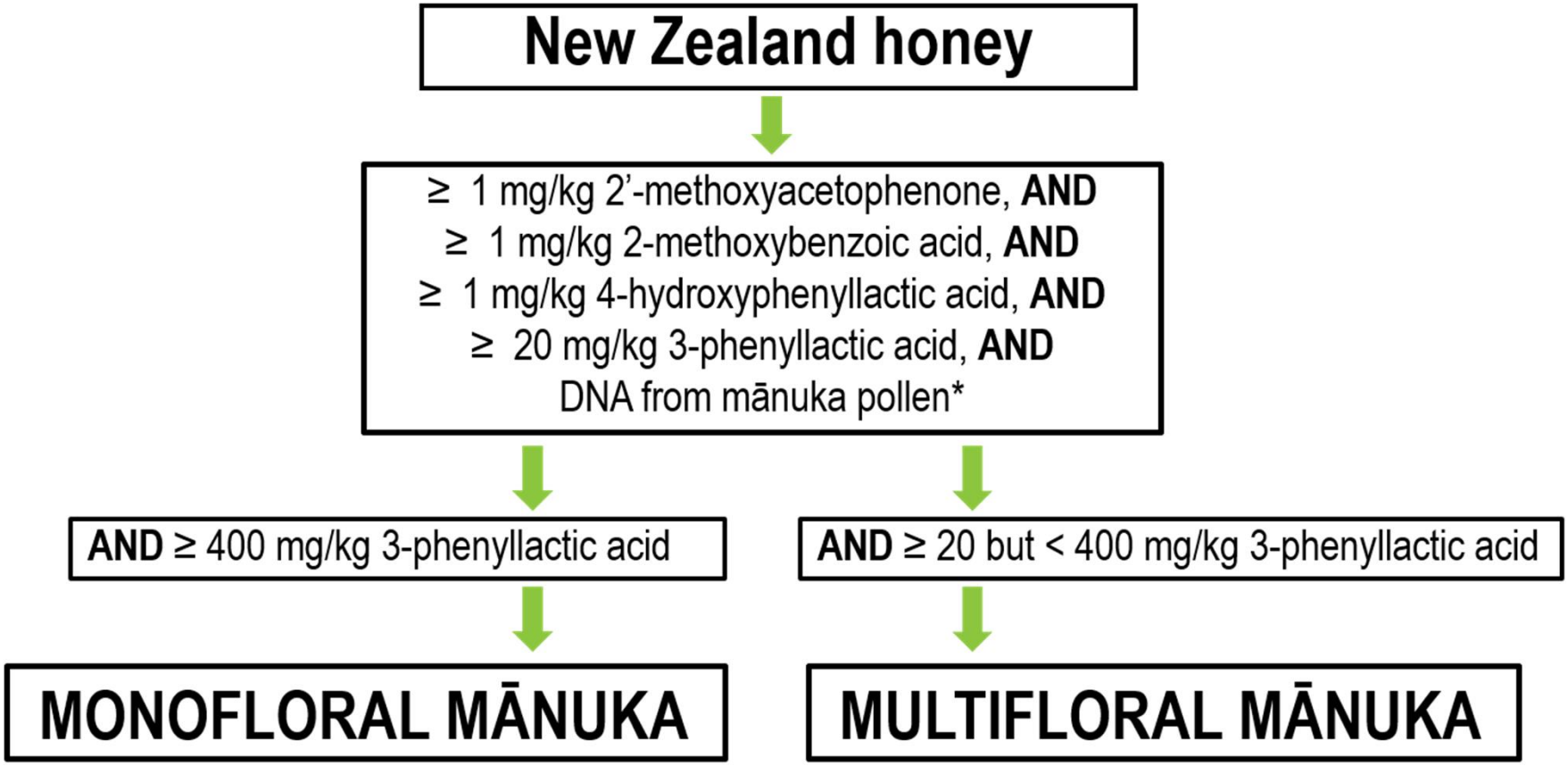
Bootstrap sampling with replacement within each honey type was used to test CART outputs under a range of scenarios:

- different honey production years e.g. 2014/15 vs 2015/16
- different production areas e.g. North Island of NZ vs South Island of NZ
- different numbers of honey types classified e.g. 6 vs 4 classes
- different numbers of markers used to fit the CART
- importance of the test method limit of reporting values in the data

Establishing criteria and testing robustness

- To establish final criteria:
  - 2014/15 data as training set
  - Build CART with both a 4-level and a 6-level response variable
  - Using 3-PA and both DNA markers as other markers had minimal effect
- BUT other markers were selected in the CARTs:
  - add 2-MBA, 4-HPA and 2'-MAP to the criteria and compare classifications
- Robustness:
  - Influence of rounding
  - Systematic bias in laboratory test methods

Final identification criteria



*DNA level required is < Cq 36 which is approximately 3 fg/µL DNA.

Regulatory definition

- A detailed series of blending simulations suggested that in a small number of scenarios, a multifloral manuka honey type could be blended with a non-manuka honey type (kanuka) to form a monofloral manuka honey type

- This scenario was prevented by increasing the level of 2'-methoxyacetophenone from 1 mg/kg to 5 mg/kg in the final regulatory definition

Key findings

- A combination of 5 markers (4 chemical and 1 DNA) can be used to authenticate monofloral and multifloral mānuka honey
- The identification criteria can be used within a regulatory setting as:
  - Based on defendable, robust and transparent science
  - Can easily be used for verification purposes
  - Meet expectations of MPI and overseas authorities
  - Fit for purpose for industry
  - Provide consumer confidence
- Identification criteria can be adapted to accommodate industry practice and potential environmental influences

Acknowledgements

- **Industry**
  - Supply of honey samples
- **Sample collection and plant identification**
  - Scion Research, SPS Biosecurity, University of Sunshine Coast, Peter de Lange (DOC) Peter Wilson (National Herbarium of NSW), Emily Moriarty Lemmon and Alan Lemmon (Florida State University)
- **DNA & pollen**
  - dnature diagnostics & research Ltd, Scion Research, Rachel Chalmers
  - GNS Science, University of Sunshine Coast, Veritaxa
- **Chemical testing**
  - National Measurement Institute, Melbourne, Analytica Ltd, University of Sunshine Coast, Geological Nuclear Sciences
- **Statistical analysis**
  - BioSS (Biomathematics and Statistics Scotland), Scion Research