RESEARCH

TESTING NECTAR TO SELECT MĀNUKA TREES FOR HIGH-GRADE HONEY

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Testing of mānuka nectar is a practical method to identify mānuka *(Leptospermum scoparium)* plants that will contribute to higher-grade mānuka honey. Results can be used to rank the plants according to the dihydroxyacetone (DHA) and Leptosperin content of the nectar.

The benefit of nectar analysis

Methylglyoxal (MG) is the compound in mānuka honey responsible for the non-peroxide activity (NPA). MG is formed from the conversion of dihydroxyacetone (DHA). A hive of bees will visit many flowers from various trees which will dilute the overall DHA and Leptosperin in a batch of honey; hence the more mānuka trees with high DHA and Leptosperin that are in the flight range of the hive, the higher the maximum MG value (and NPA) will be. At present, little information is known about the pathways of expression for both DHA and Leptosperin in the nectar, but the concentration of both compounds is known to vary between Leptospermum species. There is considerable interest from nurseries, landowners and beekeepers to find varieties of mānuka that express high levels of DHA.

Collecting the sample

Analysis of nectar requires representative samples of each tree to be collected; one sample is created by combining the nectar of 10 flowers from one tree. There are three recommended sampling techniques; these are summarised in Table 1. The more care that is taken when collecting a sample, the more reflective the results will be of the tree. Key considerations when sampling are to ensure that there is visible nectar on the flowers (Figure 1) and that it has not recently rained. It is recommended that a fine-mesh bag is placed over the branch a day before sampling to keep insects off the flowers; a plastic bag may cause condensation to form which will dilute the nectar.

Table 1. Overview of three nectar collection techniques.

Sampling Method	Advantages	Disadvantages
Direct Method Collect pure nectar from 10 flowers into one tube	• Preserves flower • 100% nectar	• Impractical in-field • May produce very small sample volume
Wash Method Take 10 flowers from tree and place in a tube with 1.5 mL water	• Quick and easy • Practical	• May dilute sample too much • May introduce extra sugars
10x10 Method Dissolve nectar on flower using 10 µL water; repeat for 9 flowers into same tube	• Sufficient volume for analysis	• Multi-step technique • Time consuming

Figure 1. Visible nectar on a mānuka flower.

Analysis of nectar and normalisation of results

Nectar is primarily made up of glucose (~40%), fructose (~40%), sucrose (~2%) and water (~20%). There are also minor compounds present (e.g., amino acids and phenolic compounds). Samples are analysed using High Performance Liquid Chromatography (HPLC) to detect DHA, Leptosperin, fructose and glucose.

The three methods for nectar collection dilute samples to various volumes (i.e., no dilution, 0.1 mL total volume or 1.5 mL total volume); hence results from different sampling methods cannot be directly compared without normalising the DHA and Leptosperin to the sugar concentration. Honey is made up of approximately 80% sugar (800 g sugar per 1 kg). Therefore the DHA and Leptosperin are reported per 800 g of sugar; this normalised result gives an approximate level of mg/kg that would be expected in a honey that was created entirely from the one sample, allowing samples to be compared. Historically DHA results were been compared to 80° Brix. This is a measure of the sugar concentration of a solution, which is equivalent to 800 g of sugar per 1 kg.

The importance of chilling samples

Once the nectar is collected, it is necessary to chill or freeze it due to the high sugar content which can cause fermentation (depletion of sugars). Alternatively, samples collected using the Wash or 10x10 methods can be preserved using a 10% alcohol solution. To illustrate the effect on the normalised result, a pure nectar sample was divided in two: one half was diluted with water and the other with 10% methanol to preserve the sample and prevent fermentation.

The samples were stored at room temperature and analysed over four days to simulate samples sitting in the field and during postage. The DHA and Leptosperin concentrations did not change over this period; however, the sugar concentration in the sample diluted with water decreased by ~20% during this time due to fermentation.

If we say that the original sample had 100 mg/L DHA and 20 g/L sugar, the normalised DHA would be 4,000 mg DHA/800 g sugar. However, if the sugar concentration dropped by 20%, then the normalised result would be 5,000 mg DHA/800 g sugar, making the tree appear to have a higher DHA content that could cause it to be wrongly selected.

Table 2. Summary of DHA in honey and nectar.

	DHA in honey	Normalised DHA in nectar
	(mg/kg)	(mg/800g sugar)
Average	981	3,887
Median	817	2,940
Maximum	5,330	27,070
Total # samples	3,661	1,309

A tree which produces high levels of DHA, but does not produce many flowers or large volumes of nectar may not be as good as a tree with slightly less DHA, heavy floral density and good nectar flow.

Interpreting results to select good trees

The normalised results for DHA in nectar can be very high (results over 20,000 mg DHA/800 g sugar have been reported). It is important to note that the concentration of honey will not be this high due to the dilution from other flowers. A set of 1,300 nectars and non-related honey samples (3,661 samples with < 4 mg/kg HMF and >100 mg/kg DHA) analysed at Analytica Laboratories showed the nectar results were about four times higher than the honey samples (Table 2). In addition, a paper published in 2009 (Adams, Manley-Harris and Molan) reported the DHA in nectar was more than double the concentration found in honey.

When selecting trees for planting, a number of factors need to be taken into consideration, aside from the DHA concentration. Physical properties of the tree are important, such as floral density, and volume of nectar and resilience of the tree to the environment also need to be taken into consideration. A tree which produces high levels of DHA, but does not produce many flowers or large volumes of nectar may not be as good as a tree with slightly less DHA, heavy floral density and good nectar flow. The flowering period of the species of mānuka tree needs to line up with the latitude of planting—plants that flower early in the season will perform poorly if planted too far south because it will not be warm enough for them to produce nectar at the time of flowering.

Kauri Park (2016) has collated data on a number of mānuka varieties which includes their natural flowering time. For example, trees in the Far North flower in weeks 39 to 44, while Hawke's Bay varieties flower in weeks 49 to 1. Further south, varieties from Westport flower in weeks 51 to 3. Therefore, if the Far North variety was planted too far south, it is unlikely to provide the bees a sufficient nectar source.

Obtaining the most information from a site

For beekeepers and landowners wanting to understand the variability over a hive site, the way a site is sampled may differ depending on whether it is a naturally planted site or if the trees are from a nursery. The higher the number of plants analysed from one site, the greater the amount of information that will be gained. Samples could be collected, then combined, to get an average concentration for a site, but this comes with the risk of masking high-producing plants.

For example, 10 trees were sampled from a naturally planted site. The results were analysed as individual samples and a portion of each were combined to created one sample. Eight of the 10 samples were below detection limit, and the remaining two samples had high DHA (8,243 and 27,070 mg/800 g sugar). When the concentration of individual samples were averaged, the result was 3,531 mg/800 g sugar; however, the composite sample was below the detection limit of the method (see Figure 2 on page 15). In comparison, samples collected from 10 trees on a site that had been planted with nursery-supplied trees had only a 2% difference between the average results and the composite result.

The popularity of nectar testing is growing due to the information that can be learned about a floral variety or a hive site. The normalised results are used to compare sites

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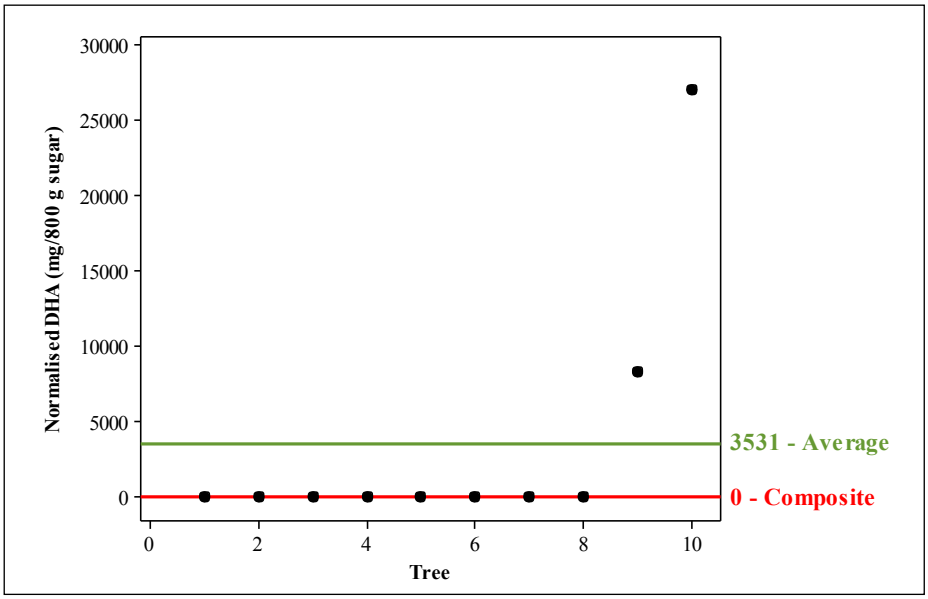
or trees relative to each other and can estimate if the site will produce high-grade mānuka honey.

At right: **Figure 2.** Results for 10 trees analysed from one site. Samples were analysed separately. In addition, equal portions were added together to form one composite sample that was below the detection limit of the method.



References

Adams, C. J., Manley-Harris, M., & Molan, P. C. (2009). The origin of methylglyoxal in New Zealand mānuka *(Leptospermum scoparium)* honey. *Carbohydrate Research, 344*(8), 1050–1053.

Kauri Park. (2016). *Manuka provenances* [Pamphlet]. Northland, New Zealand.

KEY POINTS

- Analysis of the nectar of mānuka trees is a useful tool for beekeepers, landowners and nurseries to identify trees that will contribute to higher-grade mānuka honey.
- Dihydroxyacetone (DHA) and Leptosperin are present in mānuka nectar. The concentration of these compounds differs between varieties of mānuka.
- DHA is converted to methylglyoxal (MG), which is responsible for the non-peroxide activity (NPA) in mānuka honey.
- Samples are collected by combining the nectar from 10 flowers on one tree. Samples may be 100% nectar or a form of dilution (either 0.1 mL or 1.5 mL liquid to dissolve the nectar).
- Samples must be chilled so that fermentation of the sugar does not occur.
- Concentrations of DHA and Leptosperin are normalised to the sugar content, which gives an approximation of the mg/kg that would be found in a honey solely created from the one tree.
- The reported result for the nectar is at least double the concentration that will be found in a honey, due to bees diluting the honey with nectar from many mānuka and non-mānuka flowers.
- Northland varieties of mānuka flower early in the season; flowering occurs later in the season the further south the trees are. If Northland varieties are planted too far south, they will flower before the weather is warm enough for nectar to be produced.
- When choosing good trees, floral density, nectar production and resilience to disease are other factors that should also be considered.

These bees were behaving perfectly, showing off their clever handiwork on their brood frame. Photo: Donna and Jeff Montrose, Warkworth.