Assessment of manuka provenances for production of high 'unique manuka factor' honey

G. Hamilton[1], J Millner[1], A. Robertson[1] and J. Stephens[2]
[1]Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand
[2]Comvita NZ Ltd, 23 Wilson Road South, Paengaroa, Te Puke 3189, New Zealand

Abstract

Manuka (*Leptospermum scoparium*) honey with high methylglyoxal content, commonly expressed as unique manuka factor (UMF®) content, has strong anti-bacterial and anti-fungal properties. Consequently, there is a strong demand for high UMF manuka honey in the health food industry both in New Zealand and overseas. Currently manuka honey is produced from natural stands of manuka, but UMF content varies among regions. The active ingredient methylglyoxal is produced by natural chemical transformation of dihydroxyacetone (DHA) present in the nectar. Production of high UMF honey is insufficient to meet market demand; this is due to variation in UMF amongst stands causing unpredictability in quality as well as inaccessibility of many manuka stands. The feasibility of increasing production by establishing manuka plantations using plants known to produce nectar with high DHA content is being investigated. This study compared establishment, growth and nectar DHA content of four manuka provenances; two from Northland, and one each from Waikato and Wairarapa in a replicated, randomised complete block trial (Site A). In addition a manuka plantation of a single provenance was monitored for nectar DHA content (Site B). Both sites are in the Whanganui area. Survival of seedlings in the Site A trial at 12 months was high in all provenances. Nectar DHA content ranged from 3666 to 6902 mg/kg 80° Brix and there were no significant differences amongst the provenances. These DHA levels were considerably higher than levels measured in the local manuka (2565 mg/kg 80° Brix). At Site B nectar DHA content of the plantation manuka (5770 mg/kg 80° Brix) was significantly (P=0.05) higher than the indigenous manuka (2565 mg/kg 80° Brix). Early results suggest that manuka provenances can be utilised on different sites to produce high DHA nectar and ultimately high UMF manuka honey, providing landowners with an additional income and help prevent erosion of marginal hill country.

Additional keywords: nectar, floral density, dihydroxyacetone (DHA), survival

Introduction

Manuka (*Leptospermum scoparium* J.R.Forst. & G.Forst.) is an indigenous scrub, widespread throughout New Zealand. Manuka has generally been regarded as a weed; large areas are cleared mechanically or through use of herbicides mostly on marginal hill country pastoral farms. However, the value of manuka for producing manuka honey is changing attitudes to the species. This value is based on the presence of the antibacterial compound methylglyoxal (Adams *et al*., 2008). The activity of methylglyoxal in

manuka honey is related to the antibacterial properties of phenol and led to the development of the 'unique manuka factor' (UMF) rating (Allen *et al*., 1991). It is also increasingly recognised as a valuable species for stabilising erosion-prone hill country (Bergin *et al*., 1995).

Methylglyoxal forms in manuka honey overtime by chemical transformation of dihydroxyacetone (DHA), which is present in the nectar of manuka flowers (Adams *et al*., 2009). The level of DHA in the nectar varies significantly between stands, but the reason for this variability is not yet well understood (Adams *et al*., 2009).

There is an increasing world-wide demand for high UMF manuka honey which current production is unable to meet. To increase the production of manuka honey, more stands of manuka economically accessible to beekeepers are needed and the reliability of supply needs to be improved. Because manuka is a good stabiliser of erosion prone land it is proposed that such areas be planted in manuka to increase the supply. There are over 1 million hectares of erosion-prone pastoral land, that would be suitable for plantation, and not only would this help to increase supply for the increasing demand for manuka honey, but it would also protect farmers land and provide them the opportunity to gain a good income from what would otherwise be unproductive land (Ministry for Primary Industries, 2012).

However, if landowners were to retire land from pastoral farming to plantation manuka they need to know that the seedlings they plant will produce good yields of high quality honey.

This study aimed to investigate the viability of high-performance manuka plantations from seedlings grown from seed sourced from manuka stands known to produce high UMF honey. Survival, growth, development and DHA levels in the nectar of four different provenances were assessed in order to compare growth and nectar characteristics outside of their natural environment. Ultimately, farmers will need high nectar yields with good DHA levels to produce high value, high UMF honey.

Methods

Sites and plant material

Trials were conducted at two sites; a trial was established in August 2010 at on a north-west facing hill country site in the Whanganui District (Site A). Seedlings of four provenances, described below, were planted in a randomised complete block design with three replicates. In addition a 50 ha area of hill country which had been retired from pastoral farming was planted with seedlings of a single manuka provenance (Provenance 1) in August 2010, also in the Whanganui District (Site B). At both sites the topography is steep (>25°) and prone to erosion. The soils are a mix of sandstones and limestone at Site A and sandstone and siltstone at Site B. Fertility is low; Olsen P is 12.7 and 9.2 mg/l at Site A and Site B respectively. Both sites had previously been used for pastoral farming.

At Site A container grown seedlings of four different provenances (Table 1) sourced from different regions in the North Island from stands known to produce high UMF honey (J. Stevens, pers. comm. 2013) were planted using standard commercial techniques with a spacing of 1.5m x 1.5m. Each plot consisted of 15 seedlings approximately 45 cm in height. Post-planting weed control consisted of spot spraying with knapsacks (75 cm diameter) using a mix of haloxyfop and clopyralid herbicides at recommended label rates six weeks post-planting to give selective

control of grass and broadleaf weeds. Assessment of survival was carried out in August 2012 and assessment of growth (height, floral density) was carried out in January 2013. Assessment of DHA content (3 plants per plot) was carried out during peak flowering in early December 2012.

The plantation at Site B was established at a population of approximately 1500 plants/ha. This site includes both north and south facing slopes and areas of naturally regenerating manuka. The plantation manuka was assessed by establishing four 0.02 hectare circular plots on each aspect and sampling nectar from 3 plants in each plot during peak flowering (December 2012) for DHA concentration. Additionally survival across both aspects was measured, and growth (height and floral density) was measured using ten random plants for each aspect (January 2013). The DHA concentration of the regenerating indigenous manuka was sampled by randomly selecting 10 plants during peak flowering (January 2013). Separate samples were collected from each aspect.

Table 1: Description of manuka provenances grown.

Provenance	Origin	Description
1	Northern North Island	var. incanum
2	Central North Island	var. scoparium
3	Southern North Island	var. scoparium
4	Central North Island	var. linifolium

Nectar collection

Manuka flowers produce small amounts (<10ul) of nectar so a variation of the wick absorption technique developed by McKenna and Thomson (1988) was used to sample nectar from flowers. A pipette was used to put ~8µl of de-ionised water onto the hypanthium of each manuka flower and a 4mm x 20mm filter paper wick (made of Whatmann Number 1 filter paper) was then used to soak up the water and nectar. Wicks were then placed into a labelled tray and placed on ice and once back in the laboratory, stored in a -80°C freezer until analysis. Nectar was collected from 15 flowers per sample plant which were pooled to form a composite sample. All sampled flowers were enclosed in fine mesh bags for at least 24 hours prior to sampling to prevent access by insects, allowing nectar to accumulate.

Nectar Dihydroxyacetone and sugar analysis

Each composite sample was soaked in 7.5ml of nano-pure water for 30 minutes to wash the nectar into the solution; this solution was analysed.

DHA concentration in the nectar samples was analysed using a reverse phase HPLC method with a diode array detection method adapted from Windsor *et al*. (2012). Previous analysis of DHA using HPLC has resulted in co-eluting peaks. As a consequence this method analysed the O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine derivative of DHA to give a cleaner peak (Windsor *et al*., 2012). An internal standard of hydroxyacetone was used.

Because flowers had different amounts of nectar all samples were normalised to 80° Brix to standardise the DHA values. This is standard practice when noting DHA and MGO values in honey. To do this sucrose, glucose and fructose concentrations in the

samples were quantified by HPLC with a refractive index detector, these were then added to calculate the total sugars for each sample and to normalise the DHA values.

Results

Site A

All provenances flowered from about mid-November through to mid-late December 2012, considerably earlier than the local manuka which started flowering in late December 2012, and finished about a month later. Nectar DHA concentration was lowest in provenance 2 (3666 mg/kg 80° Brix), intermediate in provenance 4 (4385 mg/kg 80° Brix) and highest in provenances 1 and 3 (6902 and 6836 mg/kg 80° Brix respectively) (Table 2). However, an ANOVA showed that differences between provenances were not significant due to a high level of variation among samples (coefficient of variation = 33%). These nectar DHA concentrations were considerably higher than the local manuka (2804 mg/kg 80° Brix). Twelve months after planting survival was uniformly high in all provenances ranging between 14.7 and 13 plants/plot survival. Similarly, 27 months post-planting height growth was almost identical (1.1 to 1.2 m) among provenances, the floral density was similar between provenances 1, 3 and 4 but provenance 4 showed a significantly higher floral density (Table 2).

Table 2: Nectar dihydroxyacetone (DHA) content (mg/kg 80° Brix) at 27 months; survival (plants/plot) at 12 months and height (m) at 27 months in seedlings of four provenances planted August 2010, Site A.

Provenance	DHA	Survival	Height	Floral Density
1	6902	13.7	1.2	0.356a
2	3666	13.7	1.1	1.027b
3	6836	14.7	1.1	0.308a
4	4385	13.0	1.2	0.402a
Significance	NS	NS	NS	P=0.05

Site B

A t-test was used for comparison of the mean (across aspects) DHA concentration of the plantation and indigenous manuka, it showed a significant difference between them; the plantation nectar DHA content (5770 mg/kg 80° Brix) being almost twice the level of the indigenous manuka (2804 mg/kg 80° Brix) (Table 3). Aspect had no effect on DHA concentration for either the plantation or indigenous manuka. However, t-test's showed that there was a significant difference between height and floral density between aspects with the northern face producing taller (Northern = 1.3m, Southern 1.1m) trees with a higher floral density (Northern =2.88 Southern =1.24) than the southern aspect (Table 4). Survival of the manuka seedlings across the northern (1512.5 trees/hectare) and southern (1125 trees/hectare) aspects was good and not significantly different.

Table 3: Nectar DHA content (mg/kg 80° Brix ± SEM) of planation (provenance 1) and local manuka on different aspects at Site B.

	Aspect		
	North	South	Mean
Indigenous	2605 ±392	3046 ±562	2804 ±315
Plantation	5928 ±783	5271 ±874	5770 ±614

Table 4: Height and floral density (number of flowers/basal stem area) of plantation manuka on different aspects at Site B.

	Aspect		
	North	South	Mean
Height (m)	1.33 ±392	1.11 ±562	P=0.05
Floral Density ($cm^{3)}$	2.89 ±0.70	1.24 ±0.42	P =0.10

Discussion

High performance plantation manuka essentially needs to have good survival, grow well, have a high floral density and produce high levels of DHA in the nectar. Manuka predominantly flowers during spring (October through to February) but it can often flower more than once a year. Zieslin and Gottesman (1986) found that manuka flower initiation responded to photoperiod but is also temperature dependent. In New Zealand plants in Northland stands tend to flower 1-2 weeks before those in the central North Island stands. However, it is also noted that there is a genetic influence on flowering period which is apparent in this study. Flowering period is very important for bee keepers as it is the window of opportunity for honey production. Establishing stands of manuka at a site that flower at different times effectively extends the flowering period, allowing increased honey production and providing a buffer in case of unfavourable weather conditions during the time a stand is flowering. In this study the plantation manuka flowered about a month before the indigenous manuka with flowering in the planation manuka finishing at about the time flowering began in the indigenous manuka, indicating that the genetic influence is remaining at other sites and that the flowering period is not entirely environmentally influenced.

Survival of manuka plants was uniformly good in this study. The performance of the provenances from Northland is encouraging because these provenances may suffer from frost damage on cold sites (Greer and Robinson 1995). However, the Whanganui area does not generally have hard frosts and both sites in this study are on sloping ground which reduces the risk of frost (Maunder and Brown, 1972). Frost tolerance in manuka populations has been associated with latitude and altitude of origin of each population (Greer *et al*., 1991). The good growth rates and floral density achieved by seedlings of all provenances suggests that they may potentially produce high honey yields. Floral density and growth were both higher on the northern facing aspect indicating that northern slopes would produce a higher

yield of honey per hectare. The good establishment and early growth of the manuka seedlings is encouraging; a light honey crop was taken from the plantation at Site B two years after planting.

The plantation manuka in this study produced nectar DHA concentrations considerably higher than the indigenous manuka at the study sites, increasing the potential to produce high UMF honey on these sites. In 2011-2012 the value of manuka honey ranged from $14.75-$50.00/kg, depending on UMF value (UMF 5+ to UMF 20+), compared with light/clover honey which ranged from $4.40-$7.30/kg (Ministry for Primary Industries, 2012) indicating the potential for land owners and apiarists to generate significant income by producing high value manuka honey on marginal, erosion prone hill country. This study shows that manuka seedlings from stands known to produce high UMF honey can be established in different environments and produce high nectar DHA concentrations. Further work is needed to assess the viability of a larger range of sites throughout NZ as well as the performance of the seedlings as they reach maturity.

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