The factors responsible for the varying levels of UMF® in mānuka (*Leptospermum scoparium*) honey

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Abstract

The variability in the level of the non-peroxide antibacterial component (UMF®) of mānuka honey produced in New Zealand was studied. A field analysis confirmed considerable variability existed in the honeys, and a number of hypotheses to explain this variability were proposed and examined.

Nectar derived from *Leptospermum scoparium* (mānuka), was confirmed to be the source of UMF®.

The dilution of mānuka honey with nectar derived from other floral sources was found to proportionally reduce the UMF® in monofloral mānuka honey. The utilisation of the thixotropic properties of mānuka honey allowed the degree of dilution in the field samples to be established, and an adjustment of the field results to account for the dilution of UMF® by other honey types revealed all monofloral mānuka honey contains UMF®. However, in the monofloral mānuka honey, significantly different levels of UMF® activity were found to come from reasonably well-defined geographic regions.

The cause of the variable levels of UMF® activity in mānuka honey would appear to be the different varieties of *L. scoparium* being harvested by the honeybees, and the environmental parameters influencing nectar production or another species interacting with *L. scoparium* do not appear to influence UMF® activity.

Three methods were used to establish genetic variability within regions of the North Island of New Zealand that gave rise to the various levels of UMF® activity. Analyses of morphological characteristics, chemotaxonomic essential oil profiles, and population genetics of *L. scoparium* populations were conducted, and the conclusions that were drawn from each of these were very similar. Two major divisions were identified, each divided into two varieties. The northern division, which contained the core populations from Northland and Waikato, represented the previously described *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium*. This division yielded mānuka honey with high UMF® activity. The southern division, which contained the core populations from the Central North Island and East Coast, represented the previously described *L. scoparium* var. *myrtifolium* and an unnamed variety. The latter, growing principally on the East Coast, uniquely contains triketones essential oils. The southern division yielded mānuka honey with low UMF® activity. Hybridisation between these varieties will occur, leading to a continuum of UMF® activity in mānuka honey.

The data indicated multiple dispersions of *L. scoparium* to New Zealand from the evolutionary centre of the persistent-capsule *Leptospermum* group in south-east Australia, and later regional dispersal in New Zealand.

From this study two hypotheses were accepted: the variability in the UMF® activity of mānuka honey is due to both the dilution of mānuka honey by other honey types and the variety of *L. scoparium* harvested.

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Chapter 1

Leptospermum scoparium (mānuka) honey

This chapter reviews the traditional and modern use of honey as a medicine, and introduces the four antibacterial components found in honey. Particular emphasis is placed on the non-peroxide antibacterial activity discovered in *Leptospermum scoparium* (mānuka) honey, and the pharmacological development of mānuka honey is discussed. The mānuka and other honey resources of New Zealand are reviewed.

The intention of this study is introduced, namely the determination of the cause of the variability of the non-peroxide antibacterial activity (UMF®) in mānuka honey, and five hypotheses are outlined.

Sections of this chapter have been published in an amended form in the New Zealand Journal of Botany (Stephens, J. M. C.; Molan, P. C.; Clarkson, B D. 2005: A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany 43*: 431–449).

1.1 Pharmacological use of honey

Honey has been recognised as a useful panacea since ancient times, with all of the early historic Eurasian civilisations recording medicinal use (Zumla & Lulat 1989). Honey has continued to be employed as a traditional therapy in many parts of the world, however it fell from favour in Western society with the development of modern antibiotics (Molan 1999a).

A resurgence of interest in honey has led to an explanation of the reported medical properties. Honey has different actions as a treatment, and whilst the principle agent of healing is antibacterial activity; anti-inflammatory action, stimulation of the immune system, stimulation of cell growth, and antioxidant activity all enhance the therapeutic properties (Molan 1999b, 2001a). The literature describing the effect of honey on a wide range of bacterial species and its effectiveness in healing infected wounds has been recently reviewed (Molan 2001b).

The antibacterial activity of honey has been extensively researched and is found to contain four components. The main factor is hydrogen peroxide, termed inhibine until correctly identified (White et al. 1962). Hydrogen peroxide is generated by glucose oxidase, an enzyme added to the nectar during concentration by the honeybee (*Apis mellifera*) from its hypopharayngeal gland (White et al. 1963). The concentration of hydrogen peroxide may be related to the dominant floral source, as ascorbic acid, metal ions and catalase found in varying quantities in nectar all contribute to hydrogen peroxide degradation (Molan 1997). The enzyme is also sensitive to heat and light denaturation, the sensitivity to both dependent to some extent on the floral source (Molan 1997).

Two other antibacterial factors are common to honey, the effect of osmolarity and acidity. The water content of honey varies between 15–21% w/v, however due to the interaction between water molecules and the monosaccaride component only a small and insufficient fraction of the water is available for the growth of bacteria

(Molan 1992). Likewise the relatively high acidity of honey is antibacterial: the acidity varies between pH 3.2–4.5 and is beyond the lower limits for growth of most animal pathogens (Molan 1997).

The fourth component does not appear to be universal in all honey types. Early work found antibacterial activity remained after the removal of hydrogen peroxide by the addition of catalase (Adcock 1962), and the non-peroxide antibacterial activity was reported to be a minor proportion of total antibacterial activity (Dustman 1979).

Antibacterial fractions were recorded in water, alcohol, ether and acetone extracts of honey (Vergé 1951), and Gonnet & Lavie (1960) concluded antibacterial components were derived from phytochemicals present in the nectar source.

The origin of the non-peroxide activity has been examined, and the proposals of lysozyme activity (Mohrig & Messner 1968), pinocembrin (Bogdanov 1984), flavonoids and phenolic acids (Russell et al. 1990; Wahdan 1998; Weston et al. 1999) have been dismissed, as the suggested active components are either too dilute to account for the non-peroxide antibacterial activity or are also found in non-active honeys (Weston 2000).

1.2 Antibacterial activity in mānuka honey

A number of studies analysing the antibacterial activities of New Zealand honeys have been completed. Whilst many honey types contained significant levels of hydrogen peroxide antibacterial activity, only mānuka honey often contained a relatively high level of non-peroxide activity (Molan et al. 1988; Allen et al. 1991a; Allen et al. 1991b), a unique property in the world's honey types other than the recently discovered non-peroxide activity in Australian jelly-bush (*Leptospermum* spp.) honey. The non-peroxide antibacterial activity was considered to be linked to the floral source (Molan & Russell 1988).

However, mānuka honey samples demonstrated a considerable range of non-peroxide antibacterial activity, with a typical agar diffusion assay study reporting a mean antibacterial activity equivalent to 18.6% w/v phenol for 19 *L. scoparium* honey samples, with a significant standard deviation of 8% w/v phenol (Allen et al. 1991a). The variability was initially attributed to sample misidentification or processing differences (Allen et al. 1991a), and later a regional difference in phytochemical composition or concentration (Molan 1995).

For the purpose of indicating the antibacterial activity for the consumer Professor P. Molan named the non-peroxide antibacterial activity of mānuka honey UMF® (Unique Mānuka Factor). The UMF® units of antibacterial activity used commercially are non-peroxide antibacterial activity equivalent to that of w/v% phenol on a standardised antibacterial agar diffusion assay using *Staphlococcus aureus* (NCTC 6571) as the control test organism, established by Allen et al. (1991a). Therefore mānuka honey with a higher UMF® rating has a greater antibacterial effect, and is consequently more desirable for the production of therapeutic goods. UMF® 12+ mānuka honey is marketed for medical usage, however the UMF® 16+ honeys are obviously increasingly sought-after.

Attempts to identify the active component responsible for the non-peroxide antibacterial activity present in mānuka honey have not been successful. Two approaches have been investigated, the seeking of a correlation between the activity of a mānuka honey and the level of any of its components, and the isolation of the fraction responsible for the non-peroxide activity.

Identification of the components of mānuka honey confirmed the constituents are different from those found in the antibacterial *L. scoparium* essential oil (Tan et al. 1988). The antibacterial activity of the essential oils is principally ascribed to the triketones, which are not present in the honey. The identified mānuka honey phytochemical components are similar regionally throughout New Zealand (Tan et al. 1989; Wilkins et al. 1993; Weston et al. 2000). However the New Zealand

mānuka honey differs from the Australian *Leptospermum polygalifolium* jelly-bush honey that also exhibits a non-peroxide antibacterial effect (Yao et al. 2003).

Extraction and identification of some phenolic components with antibacterial activity (Russell et al. 1990) was confirmed by Weston et al. (1999). Oligosaccharides (Weston & Brocklebank 1999) and the antibacterial bee peptides (Weston et al. 2000) have also been identified as possibly contributing to the non-peroxide antibacterial activity. However these components were found to account for little of the non-peroxide activity in mānuka honey. This led Weston (2000) to suggest the non-peroxide antibacterial effect was a residual peroxide effect, and the assay developed to remove peroxide from honey (Molan & Russell 1988) failed to remove the peroxide antibacterial effect in mānuka honey samples.

However the evidence from other studies indicates a non-peroxide antibacterial activity is present in mānuka honey. The residual hydrogen peroxide in mānuka honey has been shown by chemical manipulation not to account for the non-peroxide antibacterial activity (Snow & Manley-Harris 2004). There is a difference in the spectrum of antibacterial action of mānuka honey and other honeys in studies that determined the minimum inhibitory concentrations for species of bacteria. Some wound-infecting bacteria were found to be more sensitive to mānuka honey than a honey with activity due to hydrogen peroxide, and other species vice versa (Willix et al. 1992). Methicillin-resistant *Staphylococcus aureus* responded to the same concentrations of mānuka honey and a honey with activity due to hydrogen peroxide, yet vancomycin-resistant *Enterococcos faecium* required approximately double the concentration of the peroxide honey to be inhibited compared with the mānuka honey (Cooper et al. 2002). Furthermore hydrogen peroxide antibacterial activity of honey is not effective against all bacterial species; *Heliobacter pylori* was inhibited by an application of mānuka honey but was not inhibited by a peroxide honey (Al Somal et al. 1994).

These observations indicate that an agent other than hydrogen peroxide significantly contributes to the antibacterial activity of *L. scoparium* honey.

1.3 Development of mānuka honey as a therapeutic agent

The recognition of the therapeutic value of mānuka honey has led to the development of an extensive range of medical products. Initially mānuka honey was prepared for the retail therapeutic market; however subsequent development for wound management (Molan 1999c) has established mānuka honey as an accepted topical treatment for wounds (Cooper 2004). Particular effectiveness is displayed by wound dressings impregnated with mānuka honey in treating burns, ulcers, skin-grafts, and skin or muscle infections containing antibiotic-resistant strains of bacteria, and several honey items produced for wound care have been approved by the regulatory health authorities in Australia, Canada, and the European Union member states (Molan & Betts 2004).

1.4 New Zealand honeys

Approximately 8000 tonnes of honey is harvested by apiarists annually in New Zealand. This figure represents an average season, and the annual yield may fluctuate from 4000 to 10 000 tonnes dependant on environmental variables (K. Clements pers. comm.). The principal monofloral honey types are derived from nine plant families. Five families represent the indigenous flora; and the honey is marketed under the common names of mānuka, kānuka, rātā, pōhutakawa, (Myrtaceae); rewarewa (Proteaceae); kāmahi (Cunoniaceae); tāwari (Escallioniaceae); and honeydew (Nothofagaceae). The introduced flora is represented by four families; clover (Fabaceae); nodding thistle (Asteraceae); thyme (Lamiaceae); and vipers bugloss/blue borage (Boraginaceae). Many honey blends also exist, and products labelled as bush or pasture honeys are common.

1.5 Mānuka honey resource

Cockayne (1916) recognised at an early date *Leptospermum scoparium* as a major source of honey produced by the introduced honeybee, reflecting both the abundance of the plant and the surplus nectar production. *L. scoparium* is widely distributed throughout New Zealand, and is often described as a dominant member of the indigenous flora (Wardle 1991). Consequently mānuka honey is harvested throughout the country. However geographical differences exist and regions with a greater proportion of indigenous scrub yield a larger harvest of mānuka honey. Regional volumes of mānuka honey are not published for reasons of commercial sensitivity.

The annual mānuka honey harvest in New Zealand is approximately 1500–2000 tonnes, and again this figure fluctuates seasonally (K. Clements pers. comm.). Mānuka honey has a very distinct flavour, colour and consistency, and accordingly a unique set of physical characteristics that are clearly different from other honey types harvested in New Zealand. The colour is described as dark cream to dark brown, the aroma an aromatic damp earth, with a slightly bitter mineral flavour. Until recently mānuka honey was used solely for culinary purposes.

An increasing percentage of the mānuka honey harvested annually is used for the manufacture of therapeutic goods. Being commercially sensitive information, the percentage is not discussed. This use has emerged within the last five years, after the medical effectiveness of mānuka honey first became apparent and was confirmed by medical trials. Growth in the wound care market is expected to increase exponentially, as the product development phases are completed.

Historically the Honey Research Unit, University of Waikato, can link geographical regions of New Zealand with relatively high or low levels of UMF®. However all regions produce a significant range of UMF®, and some areas contain a remarkable variability. The New Zealand honey industry accepts these

observations, and traditionally the north and east of the North Island are considered regions that produce mānuka honeys with UMF® activity, and for the most part other regions do not.

Consequently, high activity UMF® mānuka honey is a desirable resource of limited quantity. The expected demand for this honey is considered to pressure supply in the near future, and the consistency of supply is essential for a therapeutic product to be accepted by the medical profession. Interest in harvesting currently under-utilised *L. scoparium* and in plantation development exists and both programs require considerable capital investment, however such decisions require knowledge of the reasons for variability of UMF® in mānuka honey.

1.6 Intention of thesis

The evidence overwhelmingly indicates mānuka honey carries an unidentified non-peroxide antibacterial component that is not found in other New Zealand honey types. This component, termed UMF®, is highly variable in the harvested mānuka honey. Mānuka honey with a high UMF® rating is becoming increasingly desirable, and supply is neither assured nor predictable.

Allen et al. (1991a) described the range of UMF® found in mānuka honey sourced from many apiarists. The UMF® range provided in this study accurately reflects the variability seen in mānuka honey harvested currently. The reasons for the UMF® variability in mānuka honey are not understood. The published proposals range from sample misidentification, variation in honey-processing, to regional differences of the phytochemical components in *L. scoparium* nectar (Allen et al. 1991a; Molan 1995).

This phenomenon has also received extensive industry analysis, for the most part based on seasonally or geographically limited observations. Climatic influences,

soil types, and interactions with other species are the most common suggestions to explain UMF® variability.

The intention of this study is to explain the variability in levels of UMF® recorded in mānuka honey, and to determine whether management of the resource could allow for a more assured supply in the future. The approach taken was to formulate various hypotheses to explain the variability, and critically investigate the validity of each of these. The final chapter of the thesis summarises the findings of this study, combining and discussing the conclusions of each chapter, and applies the findings to the management of the *L. scoparium* resource in New Zealand.

1.7 Hypotheses

The UMF® variability recorded in mānuka honey may vary for a number of reasons. A single factor may be causative, or a number of factors operating together may bring about an additive or synergistic effect. The hypotheses proposed in this section are explained further and tested in subsequent chapters of this thesis.

The **nectar from another plant species or by-product of an animal species** may be the source of UMF® in mānuka honey. This plant or animal species would be distributed throughout the geographic range of honey containing UMF®, be harvested by the honeybee, and have a geographical frequency that reflects the range of UMF® levels recorded in mānuka honey (see Chapter 4).

The **quantity of *L. scoparium* nectar** incorporated into mānuka honey may influence the UMF® level of that honey. The collection of nectar by honeybees is not controlled, and varying amounts of other floral sources may be included with *L. scoparium* nectar depending on local conditions at the time of harvest. This

dilution of *L. scoparium* nectar may alter the UMF® level recorded in mānuka honey (see Chapter 5 & 6).

The level of UMF® in mānuka honey may be altered by **another species influencing the production of *L. scoparium* nectar constituents**. This species would again be distributed throughout the range of UMF®, and have a geographical frequency that reflects the variability of UMF® recorded in mānuka honey (see Chapter 7).

The **variable impact of environmental factors** may influence the level of UMF® in mānuka honey derived from *L. scoparium* populations. The environmental factors reported to influence plant performance may correlate with the range of UMF® in mānuka honey recorded throughout New Zealand (see Chapter 8).

The **genetic variability between *L. scoparium* populations** may alter the amount of UMF® in mānuka honey derived from *L. scoparium* nectar. The distribution of the varieties throughout New Zealand may account for the variability recorded in UMF® levels (see Chapter 9–11).

Chapter 2

Leptospermum scoparium in New Zealand

This chapter reviews the taxonomy, morphology, anatomy and cytology of *Leptospermum scoparium*. The varieties previously classified in New Zealand are discussed, and the research detailing genetic and phenotypic variability described. The chemotaxonomic analyses arising from the current economic development of essential oils are reviewed.

The distribution and habitats of *L. scoparium* in New Zealand are described, with emphasis placed on the duel roles of *L. scoparium* as either a permanent member of infertile environments or a seral woody colonising species.

The majority of this chapter has been published in an amended form in the New Zealand Journal of Botany (Stephens, J. M. C.; Molan, P. C.; Clarkson, B D. 2005: A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany 43*: 431–449).

2.1 *Leptospermum scoparium*

2.1.1 Taxonomic status

Leptospermum scoparium J. R. et G. Forst. (mānuka, kāhikatoa, tea tree, red tea tree) is a member of the Myrtaceae family. The derivation of the family name is from the Latin *myrtus*, from the Greek *myron*, meaning perfumed, a reference to the scented qualities of the foliage. The genus name is comprised of two words, the Greek *lepto* translates as thin and slender, and Latin and Greek *sperma* as seed, a morphological description of the seed of the genus. The species name is derived from Latin *scoparium*, the noun for a broom, a depiction of the dense twiggy branch structure. Therefore the naming has been precise, and literally translates to English as broom-like slender-seeded plant, in a family of species bearing scented leaves.

The Myrtaceae family contains at least 133 genera and more than 3800 species, with evolutionary centres in Australia, Southeast Asia, and Central and temperate South America. Myrtaceae are characterised by a half-inferior to inferior ovary, usually numerous stamens, entire leaves containing oil glands, internal phloem, and vestured pits on the xylem vessels (Wilson et al. 2001).

Until recently Myrtaceae was divided into two subfamilies, the capsular Leptospermoideae and the fleshy-fruited Myrtoideae. The initial recent extensive review of the Myrtaceae inflorescence structure confirmed this division; the Leptospermoideae contained seven alliances including the *Leptospermum* alliance, which was further subdivided into the *Leptospermum* and *Calothamnus* suballiances (Briggs & Johnson 1979). However, cladistic analysis of morphological and anatomical characters concluded that the subfamilies should be discarded, as the fleshy-fruited *Acmena* alliance did not group within the Myrtoideae subfamily (Johnson & Briggs 1984). A cladistic re-evaluation of non-molecular characters confirmed a high level of homoplasy within Myrtaceae and limited support for any clade (Wilson et al. 1994).

Molecular analysis placed further doubt on the traditional taxonomic groupings. Sequences of the chloroplast matK gene analysed in association with non-molecular data revealed that the *Leptospermum* alliance was polyphyletic and considered an invalid taxonomic concept (Wilson et al. 2001). The sequencing of two chloroplast regions for 31 species within the *Leptospermum* suballiance revealed a monophyletic grouping of eight genera, and the suballiance was considered a valid taxonomic unit (O'Brien et al. 2000). However the same study concluded that the *Leptospermum* genus is polyphyletic, and should be divided into at least four genera; the persistent-fruit group, the East Australian non-persistent-fruit and West Australian non-persistent-fruit groups, and *Leptospermum spinescens* separated as a fourth genus. *L. scoparium* was not included in this analysis but its fruit morphology allies it to the persistent-fruit group.

Analysis of leaf anatomy of 40 *Leptospermum* species showed that *L. scoparium* has the typical xeromorphic structure of the genus (Johnson 1980). The wood anatomy (Johnson 1984; Patel 1994) and the pollen morphology (McIntyre 1963) of *L. scoparium* also support the species generic classification.

A comprehensive taxonomic revision of the genus *Leptospermum* listed 79 species (Thompson 1989), which has been increased to 83 with later additions (Dawson 1997a). *L. scoparium* is one of 13 species included in the *L. myrtifolium* subgroup, the defining characteristics of which are deciduous sepals and persistent strongly wooded fruit-valves (Thompson 1989).

The Australian species within this sub-group are extremely difficult to classify; *Leptospermum continentale* and *L. rotundifolium* were recently elevated by Thompson (1989) from *L. scoparium* varieties to species rank. The species *L. juniperinum* and *L. squarrosum* have both been recorded as varieties of *L. scoparium* (Thompson 1989), and the endemic Tasmanian *L. scoparium* var. *eximium* could be considered to warrant species status, displaying lignotuber

development which is not found in New Zealand's *L. scoparium* (Bond et al. 2004).

2.1.2 *Leptospermum scoparium* in New Zealand

Initially three species of *Leptospermum* were recorded as endemic to New Zealand, the widespread *Leptospermum scoparium* and *L. ericoides*, and *L. sinclairii* which is restricted to Great Barrier Island (Allan 1961). Revision of *Leptospermum* led to the transfer of *L. ericoides* to *Kunzea* as *K. ericoides* (A.Rich.) J.Thompson (Thompson 1983). *L. sinclairii* was included in synonomy to this species, and a new name combination of *K. sinclairii* (Kirk) W.Harris was later published without supporting material (Connor & Edgar 1987). Accordingly, *L. scoparium* is now considered to be the only indigenous member of *Leptospermum* in New Zealand. The species is not endemic to New Zealand as indicated by Allan (1961), as it also occurs naturally in mainland Australia from the southern coast of New South Wales to western Victoria and is widespread in Tasmania (Thompson 1989).

The time of arrival of *L. scoparium* in New Zealand is uncertain. *Leptospermum* pollen has been dated to the Paleocene (Fleming 1975), though the representatives in the upper Cretaceous and older Tertiary beds should be interpreted to represent type pollen and not individual species (Couper 1953, 1960). Thompson (1989) suggested that *Leptospermum* may have originated in the dry Miocene conditions in Australia and that *L. scoparium* dispersal to New Zealand occurred relatively recently, as the species is a not a primitive *Leptospermum* and could not have been present earlier in New Zealand. Wardle (1991) recorded *L. scoparium* as the only New Zealand species to release seed overwhelmingly in concert after fire, a serotinous feature common in Australian flora.

Further evidence for the recent evolution of the genus *Leptospermum* is provided by incomplete sterility barriers and the number of putative hybrids in Australia

(Thompson 1989). Nevertheless only three Australian species are tetraploid, indicating that polyploidy has not been a major influence at the evolutionary centre of the genus (Dawson 1990). One natural intergenic hybrid has been reported in New Zealand, *L. scoparium* × *K. sinclairii* (Harris et al. 1992). Harris (2000) recorded the development of four named inter-specific cultivars having *L. scoparium* as one parent and either one of the Australian species *L. rupestre*, *L. spectabile*, or *L. polygalifolium* as the other, and an earlier hybrid of *L. scoparium* × *L. rotundifolium* has been bred (Bicknell 1995). Intergenic hybrids have also been produced from controlled crosses, however neither the *K. sinclairii* × *L. scoparium* nor the *K.* aff. *ericoides* × *L. scoparium* hybrids had flowered after five years and appeared sterile (de Lange & Murray 2004).

The following species description is drawn from those given by Allan (1961) and Thompson (1989). *Leptospermum scoparium* is a variable shrub or small tree usually about 2 m tall but occasionally reaching 4 m or more, and dwarfed in exposed situations. Bark is close and firm, with young stems bearing a silky pubescence but soon becoming glabrous. Leaves are highly variable both in size and shape, 7–20 × 2–6 mm, ranging from broadly elliptical to lanceolate, coriaceous with incurved margins, rigid, acuminate pungent apex, petiole, young leaves glabrous. Flowers are white or rarely pink or red, axillary or occasionally terminal on branchlets, usually solitary and sessile, 8–12 mm diameter, flowering October–February. Hypanthium is usually glabrous with a distinct pedicel, expanded upper and broadly turbinate. Sepals are deciduous, oblong to broadly deltoid. Petals are 5 × 4–7 mm suborbicular and slightly clawed. Stamens occur in bunches of 5–7(9), 2.5–3.5 mm long. The style is inset with a large stigma, and is often reduced or absent. Ovary is 5-locular, each ovary containing about 100 ovules. Fruits are woody persistent 5-valved capsules 6–9 mm diameter, distinctly exserted beyond receptacle rim. Mature seeds are 2–3.5 mm long, irregularly narrowly linear-cuneiform or sigmoid, curved, striate.

Leptospermum scoparium is an andromonoecious species; however, the variation in percentage of perfect flowers is mostly environmentally produced (Primack &

Lloyd 1980). Overall control of flowering is determined by temperature and day-length. *L. scoparium* flowering is initially activated by a long-day flowering cue. Bud development is restrained by cool temperatures throughout winter leading to spring flowering when the temperature restraint is lifted (Zieslin & Gottesman 1986).

Much of New Zealand's insect-pollinated flora has inconspicuously coloured flowers, which has been historically attributed to the lack of specific insect associations (Godley 1979; Lloyd 1985; Wardle 1991). The small white flowers of *Leptospermum scoparium* are classified as open-access with a dish/bowl shape, and, typical of this type, are visited by a range of insect pollinators (Newstrom & Robertson 2005). Heine (1937) recorded representatives from the orders Coleoptera and Diptera. A detailed study of montane *L. scoparium* visitors revealed a range of insects arriving in a structured pattern (Primack 1978). Open flowers were visited by large tachinid and calliphorid flies at dawn, followed by a great variety of small Diptera with increasing temperature. In fine weather indigenous Hymenoptera visited flowers from mid-morning. The bees and flies ended visits in the late afternoon, and in the early evening in settled weather moths (Pyralidae, Geometridae, Noctuidae) and craneflies (Tipulidae) were recorded. Nocturnal moth visitation has been noted (Newstrom & Robertson 2005). The introduced honey-bee (*Apis mellifera*) also collects both pollen and nectar (Butz Huryn 1995). These observations confirm that non-specific pollinators are associated with *L. scoparium.*

Throughout New Zealand *L. scoparium* is normally diploid with 22 chromosomes (Dawson 1987, 1990), but two triploid and one tetraploid cultivars have been described (Dawson 1990) and wild aneuploids have been recorded (P. de Lange pers. comm.).

2.1.3 Intraspecific variation in New Zealand

Cockayne (1919 p.73) wrote "*Leptospermum scoparium*…presents a diversity of forms which are seemingly impossible to classify. Some, it is true, are distinct races, but most are probably unfixed hybrids between races not yet defined by the plant-classifier". This statement fairly represents the variability displayed by this species. In attempts to classify this variety several wild varieties have been described in New Zealand. Cheeseman (1925) agreed with Cockayne (1919) and listed one species, disputing the earlier classification of four varieties of the species by Hooker (1867). Allan (1961) described two varieties, mentioned a further four, and suggested that the forms may either result from habitat-modification or be genetically determined. The uncertainty regarding the cause of *L. scoparium* variability is reinforced in the genus revision (Thompson 1989). The most recent New Zealand flora discussed two varieties of *L. scoparium*; var. *incanum* and var. *linifolium*, and also listed the naturalised Australian species *L. laevigatum* (Webb et al. 1988).

Leptospermum scoparium var. *scoparium* was listed by Allan (1961) to represent the species description and is considered widespread. *L. scoparium* var. *incanum* (Cheeseman 1925; Allan 1961; Webb et al. 1988) has lanceolate-linear leaves about 8 mm long, rose-tinted petals, and is common especially in the far north of the North Auckland Botanical District. *L. scoparium* var. *prostratum* (Allan 1961) has a prostrate growth form and characteristically appears on mountains. *L. scoparium* var. *myrtifolium* (Allan 1961) has smaller more ovate recurved leaves and is widespread. *L. scoparium* var. *parvum* (Allan 1961) is recorded from the Wellington District, and is a small shrub with very small flowers and hairy leathery foliage. *L. scoparium* var. *linifolium* (Allan 1961) has linear-lanceolate leaves and is again recorded as widespread. Webb et al. (1988) placed *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* together.

The discovery and use of rare wild variants for ornamental plant development reinforces the variability of the wild populations of *L. scoparium*. Outstanding

single white-, pink-, or red-flowered specimens have been identified in the wild, and a number of double white- or pink-flowered plants discovered and propagated (Dawson 1997a). Furthermore the variability of growth habit and foliage colouration and shape has been exploited for ornamental specimens (Dawson 1997b).

2.1.4 Genotypic and phenotypic variability

The variable morphological characteristics exhibited by *Leptospermum scoparium* in New Zealand have been examined. Yin et al. (1984) studied variation of *L. scoparium* using leaf material from 182 herbarium specimens covering most of the natural range of the species, a common garden experiment, and a field analysis of natural populations. The herbarium specimens revealed significant correlations of leaf morphology with altitude, latitude, distance from coast, and annual and winter temperatures. Their common garden experiment established that leaf dimensions and plant morphology had a significant genotypic basis (Yin et al. 1984). Measurement of seven morphological characteristics of the populations grown in common conditions by Yin et al. (1984) also revealed considerable within-population genetic variability (Wilson et al. 1991).

Genotypic variation has also been shown for growth form (Harris 1994), leaf variation (Harris 2002), tolerance of soil acidity (Berninger 1992), soil fertility response (Lyon et al. 1971), root anatomy (Cook et al. 1980), and freezing resistance (Greer et al. 1991; Decourtye & Harris 1992). Genotypic variance within a population of growth habit, leaf size, leaf density, and stem and foliage colour were revealed in a population which was grown under common conditions (Porter et al. 1998).

The flowering times within a population, among adjacent and geographically widely separated populations, and between seasons are highly variable (Primack

1980). This variability also has a genetic component; both age at first flowering and period of flowering differed in a common garden experiment (Yin et al. 1984).

The ability of the species to respond phenotypically to different environments was shown when Burrell (1965) transplanted seedlings of *L. scoparium* from Central Otago to Dunedin, where they immediately produced larger leaves but the proportional dimensions remained typical of the ecotype. Another example of the species' phenotypic plasticity was provided by Gaynor (1979), who showed that branching height in the field was correlated with soil depth.

Burrell (1965) noted that *L. scoparium* in Central Otago retained intact capsules until opening was induced by either drought or fire, and later studies have shown that the rate of capsule splitting differs between populations. Genetic control of capsule splitting was confirmed in a common garden experiment, and it was hypothesised that the difference between New Zealand populations had arisen from rapid selection by regular fire disturbance following human arrival (Harris 2002). However, a South Island field study showed that population differences of capsule splitting related to a much longer history of fire exposure in the regions displaying serotiny (Bond et al. 2004).

2.1.5 Chemotaxonomic analyses of *Leptospermum scoparium*

Essential oils distilled from the leaves of *Leptospermum scoparium* have received considerable commercial attention during the last decade. The New Zealand Phytochemical Register – Part III (Cambie 1976) lists earlier research that identified these oils.

An analysis of 16 commercial samples of *L. scoparium* essential oil revealed 100 components, of which 51 were identified and made up about 95% of the content. The oils fell into three major sections, triketones approximately 20%, sesquiterpene hydrocarbons 60-70%, and monoterpene hydrocarbons about 5%

(Christoph et al. 1999), in contrast to about 75% monoterpene hydrocarbon (α-pinene) present in *Kunzea ericoides* (Perry et al. 1997a).

A review of the essential oils of New Zealand suggested that *L. scoparium* oils would differ between natural populations (Douglas et al. 1994), and this was confirmed by the variation of the component essential oils of natural populations of *L. scoparium* grown in a common garden experiment (Perry et al. 1997b). Two plants from each population were sampled: the East Cape population contained a high triketones level: high levels of α-pinene and β-pinene monoterpene hydrocarbons were found in Northland populations: and the balance of populations contained a complex mix of sesquiterpene and oxygenated sesquiterpene hydrocarbons. Australian *L. scoparium* samples grown in the same common environment had a higher monoterpene level than the New Zealand populations. The *L. scoparium* chemotypes reported matched the morphological types to some degree (Perry et al. 1997b).

Porter and Wilkins (1998) showed a similar pattern to that reported by Perry et al. (1997b), describing four groups of oil profiles found in wild populations; triketone-rich in the East Cape; monoterpene-, linalool- and eudesmol-rich in Nelson; monoterpene- and pinene-rich in Canterbury; and triketone-, linalool-, and eudesmol-deficient in the rest of New Zealand. The average composition of *L. scoparium* essential oil was defined as ≤3% monoterpenes, ≥60% sesquiterpenes, and ≤30% oxygenated sesquiterpenes and triketones. Nevertheless, within-population variation of essential oil content was shown in a study of *L. scoparium* grown in a common garden experiment, with the oil profiles differing by plant age and between seasons (Porter et al. 1998).

A detailed field study of New Zealand *L. scoparium* populations confirmed the presence of *L. scoparium* chemotypes; monoterpenes-enriched areas in Northland and the West Coast, triketones-enriched in East Cape and Marlborough, and sesquiterpenes-rich oils throughout the rest of the country. Eleven chemotypes

were recognised by the division of the major oil types referred to above and subdivision of the sesquiterpenes and oxysesquiterpenes (Douglas et al. 2004).

A chemotaxonomic analysis of *Leptospermum* has been completed. In dealing with species allied to *L. scoparium*, Brophy et al. (1999) showed that Australian *L. scoparium* populations in Victoria and Tasmania had different essential oil profiles from the New Zealand populations; and in particular triketones were not found. The persistent woody-fruited group of *Leptospermum* established by Thompson (1989) was not amended, and in general the *L. scoparium* essential oils did not differ in comparison with this group; however, the authors concluded that *L. scoparium* is a variable taxon that may require division (Brophy et al. 1999).

2.2 New Zealand distribution and habitats of *Leptospermum scoparium*

Although the time of arrival of *Leptospermum scoparium* in New Zealand is uncertain, current opinion suggests a relatively recent dispersal from Australia (Thompson 1989). The distribution within New Zealand would have been restricted until the land clearance associated with human settlement vastly increased the area of low-nutrient environments to which the species was adapted in Australia (Thompson 1989).

Leptospermum scoparium has two principal roles in New Zealand vegetation: permanent dominance of extreme environments or as a seral species (Burrows 1973; Wardle 1991). Permanent dominance occurs on sites that are unfavourable for the development of climax forest due to environmental extremes, whereas the seral role is on disturbed sites (Wardle 1991).

2.2.1 Permanent dominance of *Leptospermum scoparium*

Permanent dominance occurs on sites that are unfavourable for the development of climax forest as they are too wet, dry, cold, exposed, infertile, or unstable (Molloy 1975).

Soils too wet and infertile for the establishment of climax forest are widespread throughout New Zealand, ranging from the gumlands of Northland to mire in Southland, upon all of which *Leptospermum scoparium* dominates (Burrows et al. 1979). The Northland gumlands are typically leached infertile clays with perched water tables or sand podzols sustaining *L. scoparium* heathland (Esler & Rumball 1975; Beever 1988; Enright 1989; Wardle 1991). Whilst much of this land has been cleared and drained for farmland, significant remnants remain. The 16 000 year old Ahipara plateau (Wardle 1991) and the Ngarura swamp in the Waipoua forest (Burns & Leathwick 1996) are examples of self-maintaining *L. scoparium* heathland in this region.

Waikato oligotrophic lowland mires exhibit a range of infertility yet all support permanent *L. scoparium* populations (Burrows et al. 1979; Wardle 1991). A comparison of three of these Waikato environments, the extreme Kopuatai bog (Irving et al. 1984), the intermediary Moanatuatua bog (Burrows et al. 1979; Clarkson 1997), and the relatively more fertile Whangamarino fen (Clarkson 1997), confirmed the presence of *L. scoparium.*

Leptospermum scoparium is prevalent on infertile leached Westland pakihi soils, and a number of widespread communities have been studied in northern Westland (Rigg 1962; Burrows et al. 1979; Norton 1989), the central area (Burrows et al. 1979), and the southern reaches (Mark & Smith 1975). However, *L. scoparium* dominance may be replaced by larger forest species in the pakihi areas provided fire is infrequent and the environment is not exceptionally infertile (Williams et al. 1990).

In south Westland (Wardle 1974) and Fiordland (Wardle et al. 1973) lowland swamps are prime habitat, and montane raised mires in Fiordland (Burrows & Dobson 1972; Mark et al. 1979) and Otago (Johnson et al. 1977) also carry *L. scoparium*. Following fire in a Southland bog, *L. scoparium* dominated the environment rapidly (Johnson 2001). In association with swamp-like environments *L. scoparium* dominates lake shorelines around the southern lakes where it survives temporary submergence (Johnson 1972; Mark et al. 1977; Robertson et al. 1991). In these conditions the species differentiates specialised aeration tissue, aerenchyma, in submerged roots, allowing long-term dominance in waterlogged environments (Cook et al. 1980).

Areas too high and cold for the establishment of climax forest occur in both main islands (Wardle 1991). *L. scoparium* occurs above the tree line (Wardle 1963; Gibbs 1966) and on upland peat and gley soils of both main islands (Burrows et al. 1979), and is frequent on inhospitable sites at low and high altitude in south Westland (Burrows 1964; Wardle 1977) and Southland (Burrows 1964; Burrows et al. 1979), and in montane scrubland on Stewart Island (Wells & Mark 1966), and in the Central North Island (Atkinson 1981; Rogers & Leathwick 1994). However, at high altitude in Otago growth is limited to warmer microclimates (Wilson et al. 1989).

In coastal environments throughout New Zealand *L. scoparium* and *Kunzea ericoides* are found on areas too exposed for forest (Morton & Miller 1968; Molloy 1975). *L. scoparium* occurs on sites as diverse as the edge of mangrove swamps in the Auckland region (Wardle 1991), the Cape Reinga district in the far north (Wheeler 1963), Farewell Spit in Nelson (Burrows 1973), and the coastal cliff zones around southern Wairarapa and Wellington (Burrows 1973).

Areas too infertile for the establishment of forest overlap with the above categories, as the environments are the same. Oligotrophic mires and swamps, extreme coastal and altitudinal sites, and heavily leached soils have all been discussed. *L. scoparium* is also present in other situations: the geothermic heated

environments of the central North Island (Wells & Whitton 1966; Given 1980), edaphically dry pumice in the central North Island (Elder 1962), and as a consistent understorey on poor gleyed soils in forested areas (Burrows 1973). *L. scoparium* is also tolerant of South Island ultramafic soils (Lyon et al. 1971; Lee et al. 1975; Lee et al. 1983; Lee 1992).

Natural unstable environments also lend themselves to colonisation by *L. scoparium*. Landslides in Fiordland forests are rapidly covered by *L. scoparium* in a seral role (Mark et al. 1964). *L. scoparium* establishes on braided river beds (Burrows 1973) and unconsolidated coastal deposits where it is a woody pioneer (Wardle 1991).

Soils too dry for climax forest vegetation present a more complicated picture. Typically these areas occur in the eastern rain shadow of the New Zealand mountain ranges (Wardle 1991). *L. scoparium* occurs as a dominant species in relatively higher rainfall areas of these regions, but becomes uncommon in dryer situations where *Kunzea ericoides* dominates (Wardle 1971, 1991). In Otago where yearly rainfall is less than 650 mm *K. ericoides* is more common, interspersed with *L. scoparium* on boggy land (Burrell 1965). Self perpetuation of *K. ericoides*/*L. scoparium* scrub occurs where broadleaf forest establishment is either prevented (Wardle 2001) or retarded by site conditions (Dobson 1979).

2.2.2 Seral role of *Leptospermum scoparium*

In contrast to the permanent *Leptospermum scoparium* populations, seral communities also exist and form a significant proportion of the species' modern range. *L. scoparium* is an early woody species on disturbed sites, as a succession to forest. This role has been greatly extended by human disturbance (Molloy 1975; Wardle 1991).

Leptospermum scoparium is found in moist forested regions forming similarly aged stands in a nursery role for climax vegetation following fire or other disturbance (Burrows 1973; Payton et al. 1984) where it may persist for more than a century (Mark et al. 1989).

The species also establishes easily in open under-grazed pasture (Grant 1967), and its presence in this situation indicates unsustainable clearance of forest or scrub to establish pasture (Bascand 1973). *L. scoparium* may be the initial woody pioneer on moderately fertile well-drained soil due to prodigious seed set and rapid germination and growth (Mohan et al. 1984a,b). The species has an overriding germination response to full light spectra coupled with an inhibition by far-red wavelengths typical of pioneer species on disturbed sites (Herron et al. 2000; McKay et al. 2002). *L. scoparium* seed does not exhibit dormancy, and the unshed seed in capsules is probably the main reservoir of seed as the soil seed bank is non-persistent (Mohan et al. 1984a).

Accordingly, *L. scoparium* scrub regeneration and re-establishment, which has been a feature of New Zealand hill farming, can be avoided with suitable land management such as the fertilisation and retention of a heavy sward (Levy 1970).

Communities of *L. scoparium* are not permanent in regions where the rainfall is adequate to allow the establishment of climax broadleaved forest (Esler & Astridge 1974; Wardle 1991), and replacement by *Kunzea ericoides* and subsequent establishment of forest has been recorded in Canterbury and Otago (Burrows 1961; Molloy & Ives 1972; Dobson 1979; Allen et al. 1992), Kapiti Island (Esler 1967), and the Hauraki Gulf islands (Atkinson 1954; Bellingham 1955; Esler 1978), and the East Coast (Clarkson et al. 1986).

Where forest on steep slopes has been cleared for pasture establishment the land is often prone to erosion. The value of *L. scoparium* as protective scrub is now recognised, as it provides rapid (Smale et al. 1997) and excellent protection from shallow landslides (Watson & O'Loughlin 1985), and the presence of mature

stands assists erosion control (Bergin et al. 1995). *L. scoparium* foliage can intercept a significant amount of rainfall (Burke 1981), as much as 40-50% in a storm event (Aldridge & Jackson 1968). Together with soil binding by roots this rainfall interruption is effective in erosion control. Carbon accumulation by *L. scoparium* is rapid and similar to plantation forestry (Scott et al. 2000).

Chapter 3

Field study of mānuka honey

This chapter describes the collection of mānuka honey from throughout New Zealand. The commercial apiarist's involvement is discussed, and the sample collection reviewed. The precautions taken to ensure the honey received fitted within the study's guidelines are detailed. The supply of geographical site data is summarised, and the subsequent treatment of data to avoid the disclosure of confidential information is discussed.

The laboratory testing method is described, and alterations to the established industry procedure are outlined.

The results of the non-peroxide antibacterial activity (UMF®) in New Zealand mānuka honey are presented, the data is divided in regions and areas, and statistical comparisons are made within the subdivisions.

The material from this chapter has not been submitted for publication as the thesis is subject to a publication embargo agreed with the commercial sponsors.

3.1 Sampling strategy

The initial stage of the study of non-peroxide antibacterial activity (UMF®) required a sample set representing the geographic range of mānuka honey in New Zealand during one season. This data would provide the first New Zealand-wide comprehensive study of mānuka honey UMF® activity, and allow comparisons with factors outlined in Chapter One that may influence the level of UMF®. The most efficient way to achieve this was to employ the assistance of the commercial apiarists throughout New Zealand.

3.2 Study period

The collection of mānuka honey from throughout New Zealand occurred from early spring September/October 2001 through to summer January/February 2002. *Leptospermum scoparium* flowering occurs later in southern regions of New Zealand due to temperature restraints on the flowering cue.

3.3 Sample collection

Accordingly the beekeepers of New Zealand were personally contacted and this was followed up by mail. The first letter outlined the intentions of the study, describing the requirement for a study to determine the factor responsible for the level of UMF® in mānuka honey.

The second letter elaborated the collection method. Three criteria were considered the most significant and highlighted for the beekeepers. The samples forwarded for the study needed to represent principally monofloral mānuka honey, be collected from a single locality with similar environmental characteristics, and accurate site data was required.

3.3.1 Collection of mānuka honey

When nectar is harvested in New Zealand by the honeybee it is impossible to assure the resultant honey is monofloral. The New Zealand environments which produce honey are comprised of many plant species that are harvested by honeybees. Consequently mānuka honey may contain a high proportion of *Leptospermum scoparium* nectar, but it is unlikely to be pure.

To overcome the difficulty of sample purity precautions were taken with sample collection. Samples needed to be taken from one site or a collection of sites situated together with similar environmental conditions, the dominant floral sources noted, and the period of collection reported. These strategies interconnect to some extent, limiting the likelihood of the inclusion of samples derived principally from other floral sources in the study.

Similar environmental conditions were emphasized to ensure the validity of environmental correlations where more than one site had been incorporated into a sample. When many hives are positioned at a site the foraging honeybees from different hives will collect the nectar from the same sources in the surrounding environment. Therefore honey from one or more hives will provide a representative sample of the honey at the site. Once the flowering period of the desired nectar plant species is complete, hives are often removed and the honey extracted for sale as a monofloral variety.

The information on the dominant floral sources recorded by the apiarists provided a comparison with land use and vegetation maps, establishing a control of the honey samples and an approximation of the honey's monofloral status.

The period and date of collection was also recorded. Usually this documented the period the hives were located at a site and the nectar collected, and in some cases the honey extraction date. The period of collection confirmed the site had been

occupied during the flowering period of *L. scoparium* in each region. The date of collection or extraction also confirmed the year of harvest.

3.3.2 Site data

The accuracy of site data was critical for the success of the study. The beekeepers were advised of this on many occasions, and were well aware of the study requirements. However the sites utilized by beekeepers are commercially sensitive, and a reluctance to disclose the site locations by some beekeepers was noted. To overcome this difficulty it was agreed that the location of sites of production and the production details of the beekeepers would not be disclosed. The results of the study would ultimately be published in the form of a report that detailed the factors which cause the different levels of UMF® activity in mānuka honey, but would not give details of sites. A confidentiality agreement was completed with the suppliers of honey samples for the study.

To ensure that the results would not detail the sites from which the mānuka honey was collected, New Zealand was divided into disclosed regions, and each region sub-divided into areas which are not geographically described. Mean figures are provided, along with appropriate statistical treatment of the data.

3.4 Sample receipt and storage

Upon receipt the samples were listed and all details recorded. The samples were then stored in the dark at 4 ºC until tested. All samples received had the UMF® assay completed and the results forwarded to the supplier. However some samples have not been included in the results presented. The rejected samples were from a previous season, or were improperly labeled and follow-up work with the beekeeper failed to provide the missing details, particularly the site data.

3.5 Testing method

3.5.1 UMF® assay

The UMF® assay was developed by Molan and Russell (1988) to test the non-peroxide antibacterial activity of New Zealand honeys. Alterations to this assay method produced the methodology employed in this study, based on the testing protocol adopted by Allen et al. (1991). This assay is now widely used by the New Zealand honey industry in certified laboratories to establish the level of UMF® in a honey. The UMF® assay employs the agar diffusion method, an appropriate method for topical antibacterial agents (Heggers et al. 1987), however it is of relatively low sensitivity (James et al. 1972) as dilution occurs with diffusion from the well into the surrounding seeded agar (Cooper 1963).

3.5.2 Sample preparation

The samples were prepared aseptically, and were handled away from direct sunlight. All samples were warmed at 37 ºC to liquefy the honey and those containing beeswax and other impurities were strained using a 1 mm mesh to remove unwanted detritus.

Each sample was well mixed, and 5 g of honey was added to 5 ml of sterile distilled water in a universal bottle. The closed bottle was incubated at 37 ºC for 30 minutes, stirring occasionally to facilitate mixing. The 50% w/w honey solutions prepared were diluted to 25% w/w by taking 1 ml of each sample and adding it to 1 ml of a prepared catalase solution. The catalase solution was prepared by the addition of catalase (Sigma EC 1.11.1.6, 3,300 units.mg^{-1} protein) to sterile distilled water at 2 $mg.ml^{-1}$. This provided a final testing concentration of 1 $mg.ml^{-1}$, a concentration shown to remove hydrogen peroxide from honey solutions with the highest level of antibacterial activity due to hydrogen peroxide (Allen et al. 1991). The samples were assayed immediately after the final dilution with catalase solution.

3.5.3 Phenol and internal standards for assay

Phenol standards were prepared for the assay by the dilution of phenol in sterile distilled water. The dilutions used in each assay were 2%, 3%, 4%, 5%, 6% and 7% (w/v) phenol. A 25% dilution in catalase solution of mānuka honey with a known UMF® activity was also prepared as an internal standard. The same honey was used as the internal standard throughout the entire testing program for all samples in the study.

3.5.4 Assay plate preparation

Large square plates (Corning Inc. 431111, 245 × 245 mm) were prepared by seeding nutrient agar with *Staphylococcus aureus*.

The *S. aureus* culture (NCTC 6571) was obtained from the Institute of Environmental and Scientific Research, Porirua. The culture was grown for 24 hours in Bacto™ Tryptic Soy Broth. The *S. aureus* culture concentration was then adjusted to read 0.5 absorbance at 540 nm with a Shimadzu spectrometer using the same broth.

The nutrient agar was prepared by addition of 3.45 g Difco Nutrient Agar to 150 ml sterile distilled water. This solution was autoclaved and stored. Before use the nutrient agar was placed in boiling water for 30 minutes to melt the solidified agar, and then cooled in a 50 ºC waterbath for 30 minutes. The nutrient agar was then seeded with 100 µl of the adjusted *S. aureus* culture, the flask swirled to mix in the culture without producing bubbles, and the plate poured on a level surface immediately after mixing. Once set the plate was stored at 4 ºC for 24 hours before use.

The plate had sixty-four wells cut in the agar with a cooled flamed 8 mm borer, using a quasi-Latin square as a template. The template was prepared on black paper of the same dimensions as the plate. A 25 mm grid was drawn on the

template, 34 mm from the edge, and the wells positioned on the intersections of the grid. The wells were numbered by a quasi-Latin square, allowing random sample placement on the plate (Figure 3.1).

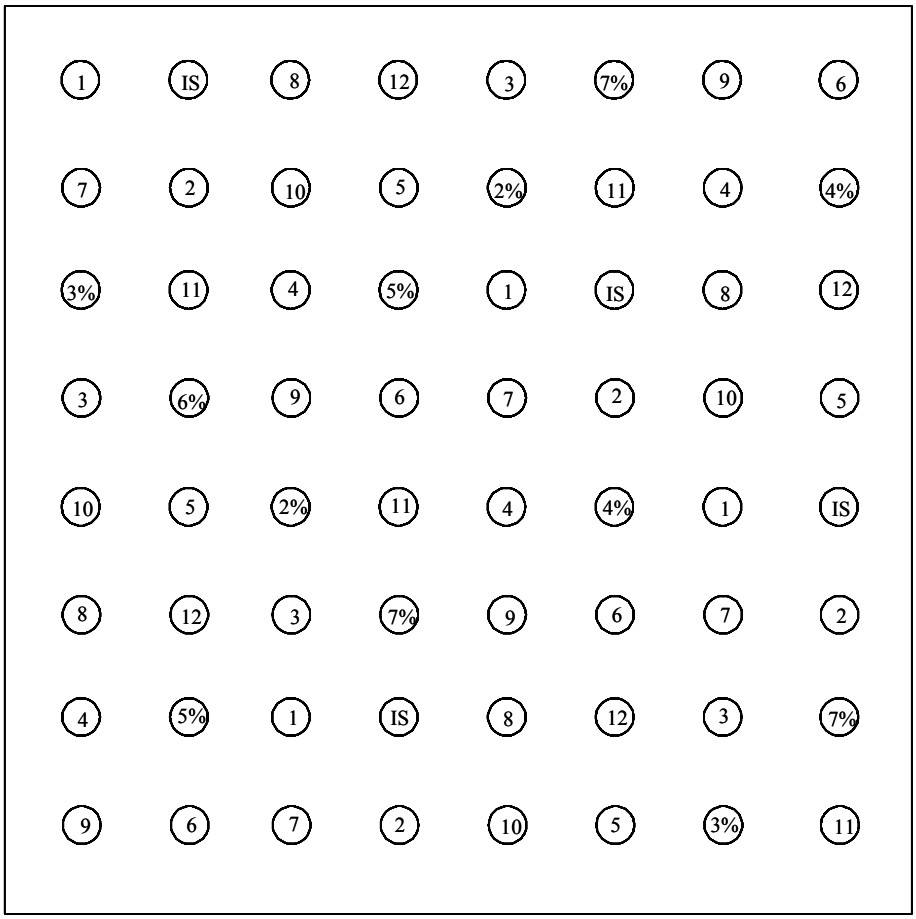

Figure 3.1 An illustration of a prepared plate, showing twelve sets of four randomly positioned wells for samples (1, 2…11, 12), the internal standard (IS), and phenol standards (2%, 3%, 4%, 5%, 6%, 7%).

The samples and internal standard were tested in quadruplicate, 100 µl of the sample being added to each of the four wells with the same number. Each concentration of the phenol standards were tested in duplicate, 100 µl of the standard solution being added to each appropriate well.

The plate was incubated for 24 hours at 37 ºC.

3.5.5 Calculation of antibacterial activity

Following incubation the antibacterial activity of the samples was calculated. The diameter of the clear zone around each well was determined using calipers (Mitutoyo Digimatic 500). The diameter of the clear zone was read along two axes, at right angles to each other, for each well.

The diameter of the clear zone for the phenol standards was measured and the mean value squared. A standard graph was prepared by plotting the % phenol against the squared mean diameter of the clear zone. A best-fit straight line was drawn, and the equation of the line was used to determine the activity of each honey sample from the mean square of the diameter of the clear zones. Antibacterial activity was then expressed as UMF® units, equivalent to phenol concentration (w/v) after multiplying by the dilution factor when preparing the solution of honey from the original sample.

3.6 Re-testing of samples with non-detectable activity

A 2% (w/v) phenol solution is the minimum concentration that provides a readable clear zone in this agar diffusion assay. Therefore the minimum readable non-peroxide antibacterial activity of a honey sample that has been diluted to 25% is equivalent to 2% (w/v) phenol, and this honey would have a rating of 8 UMF® units. Whilst this is acceptable for commercial mānuka honey, lower levels of antibacterial activity needed to be established in this study.

Consequently, honey samples that did not have a clear zone around the well when tested at dilution of honey to 25% were re-tested with the honey diluted to 50% to determine whether an antibacterial activity of less than 8 UMF® units was present. Again 2% phenol (w/v) standard concentration provided the minimum readable clear zone; accordingly a honey sample that has been diluted to 50% and

illustrates an antibacterial activity equivalent to 2% (w/v) phenol has a rating of approximately 4 UMF® units.

Sample preparation for the 50% dilution assay followed a similar course as the standard assay. A 4 $mg.ml^{-1}$catalase (Sigma EC 1.11.1.6) solution was prepared in sterile distilled water. Each warmed sample of honey was well mixed, and 2 g of honey was added to 2 ml of the prepared catalase solution in a universal bottle, giving a 2 $mg.ml^{-1}$ final testing concentration. The closed bottle was incubated at 37 ºC for 30 minutes, stirring occasionally to facilitate mixing. The samples were assayed immediately after dissolving in the catalase solution. The plate preparation, incubation, and reading were the same as the standard method described above.

As the assay relies upon agar diffusion, and as the viscosity of the more concentrated honey solutions may affect the rate of diffusion, an experiment was completed to calculate the relationship between the non-peroxide activity of a honey when tested at 25% and 50% concentration. Twenty samples of active mānuka honey were tested using both of the assay procedures described.

A constant linear relationship was established between honey samples tested at a 25% and 50% concentration (Figure 3.2). This relationship allows prediction of a honey's antibacterial activity at either dilution provided one of the dilutions has been tested. The less dilute sample consistently recorded a relatively lower antibacterial activity after allowing for the dilution factor, most likely as a result of slower diffusion from the well into the surrounding seeded agar.

Therefore as the relationship was constant the UMF® activity of the samples re-tested at 50% concentration were adjusted by the means of the equation shown in Figure 3.2 to equate with 25% concentration UMF® activity, as the 25% dilution is the standard industry test and was initially performed on all the samples in this study.

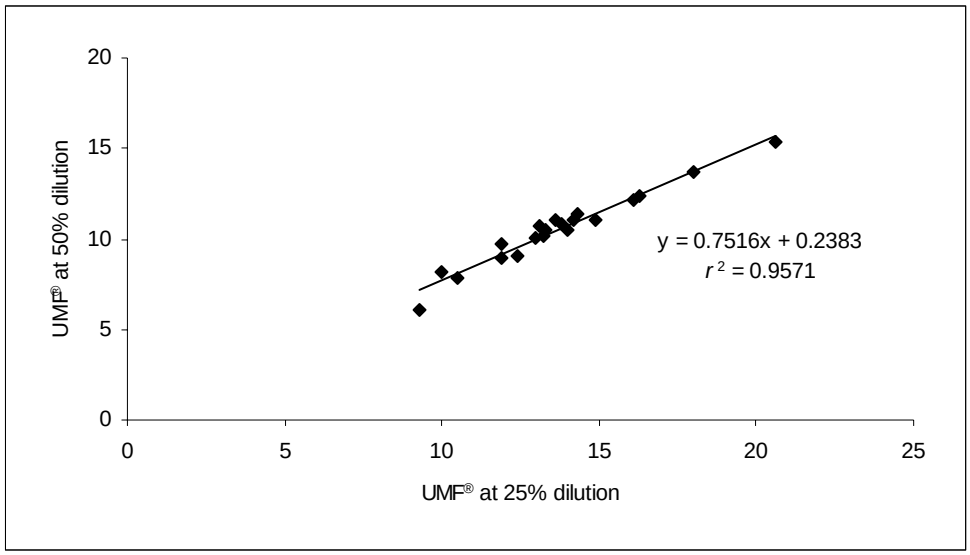

Figure 3.2 The relationship of UMF® values obtained for honeys tested at 25% and 50% concentration, showing the linear correlation.

3.7 Non-detectable samples

The assay methods described above have a minimum sensitivity of 4 UMF® units, the non-peroxide antibacterial activity of a honey. A honey sample that did not contain measurable UMF® was read as a non-detectable sample. A non-detectable honey sample does not necessarily contain 0 UMF® units, but contains 0–4 UMF® units.

A provisional value of 2 UMF® units could be ascribed to these samples, however subsequent transformations and analyses in this thesis makes this unacceptable. Therefore samples with non-detectable UMF® activity are described in this and later chapters, but are not included in the statistical treatments.

3.8 Field study results

New Zealand is divided into regions from which mānuka honey was supplied by the apiarists, and is illustrated in Figure 3.3. The regions followed the boundaries outlined by Crosby et al. (1976), and where a geographical difference exists discussion is made in the relevant section.

Figure 3.3 New Zealand map showing regions from which honey samples were supplied.

Each region was further sub-divided into areas. The areas were geographically distinct within a region, and represent discrete divisions from which similar honey is produced and have relatively constant environmental parameters.

The mean UMF® unit values from each area are detailed, allowing statistical treatment of data within regions, and in combination comparisons between regions. Single factor ANOVA tests (Microsoft® Excel 2000) were performed, equal variance was accepted, and the *alpha* value 0.05 employed.

3.8.1 New Zealand-wide results

A total of 713 samples were received from the apiarists throughout New Zealand. In all cases the apiarists advised the samples were predominantly mānuka honey. Regrettably some samples were not adequately identified, and despite further contact with the apiarist, were finally removed from the study. One supplier, with slightly under two hundred sites spread throughout the North Island later refused to disclose the site details. Thus 463 samples were included in the study.

A summary of the regions, areas, sample numbers, and mean UMF® activity is provided in Table 3.1.

Table 3.1 The mean UMF® activity and standard deviation recorded from the 11 regions throughout New Zealand. The numbers of areas and sample sites from each region are listed. Areas with a non-detectable (ND) UMF® are excluded from the calculations of the mean and standard deviation values in this summary.

Region	Areas	Samples	Mean	SD	Notes
Northland	10	35	14.0	1.4	
Waikato	2	6	14.9	1.2	
Coromandel	17	128	9.1	3.2	ND area excluded
Taranaki	6	51	8.7	2.1	
East Coast	8	39	8.8	2.2	
Gisborne	2	9	10.4	0.6	ND areas excluded
Hawkes Bay	5	48	5.4	0.7	ND areas excluded
Wairarapa	3	20	6.9	1.7	ND areas excluded
Northern South Island	6	50	5.7	1.3	ND areas excluded
Eastern South Island	4	14	6.7	2.0	
West Coast	12	61	10.9	3.0	

The mean UMF values between the regions are significantly different (p <0.0001), and a trend is not immediately obvious. It is apparent why traditionally the north and east of the North Island are thought to yield mānuka honey with comparatively higher levels of UMF®. These regions produce a level of UMF® that is detectable using the standard agar diffusion assay with honey diluted to 25%. Much of the activity recorded south of the East Coast would not be detected unless an assay with honey diluted to 50% was carried out.

3.8.2 Northland region

Thirty-five samples were received from the Northland region, representing a broad swathe from the west to east coasts across the centre of the peninsula. The region was divided into 10 areas, and the mean UMF® activity and standard deviation for the honey samples from each area are described in Table 3.2. This region is generally recognised as a producer of high quality mānuka honey, and the results confirm this opinion. However a significant difference (p< 0.001) was found to exist between the areas.

Table 3.2 A comparison of the mean UMF® activity and standard deviation recorded for areas within the Northland region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	3	14.2	1.2
2	2	15.3	0.2
3	4	15.5	1.6
4	3	13.0	0.4
5	5	14.7	1.5
6	4	11.9	0.8
7	4	13.3	0.2
8	5	14.4	0.8
9	3	14.7	0.3
10	2	12.5	0.2

An illustration of this region (Figure 3.4) demonstrates the relationship between the areas. Honey from the eight central areas recorded a mean non-peroxide antibacterial activity between 13 and 15.5 UMF® units. To the north and south two areas produced a lower activity of 11.9 and 12.5 UMF® units respectively.

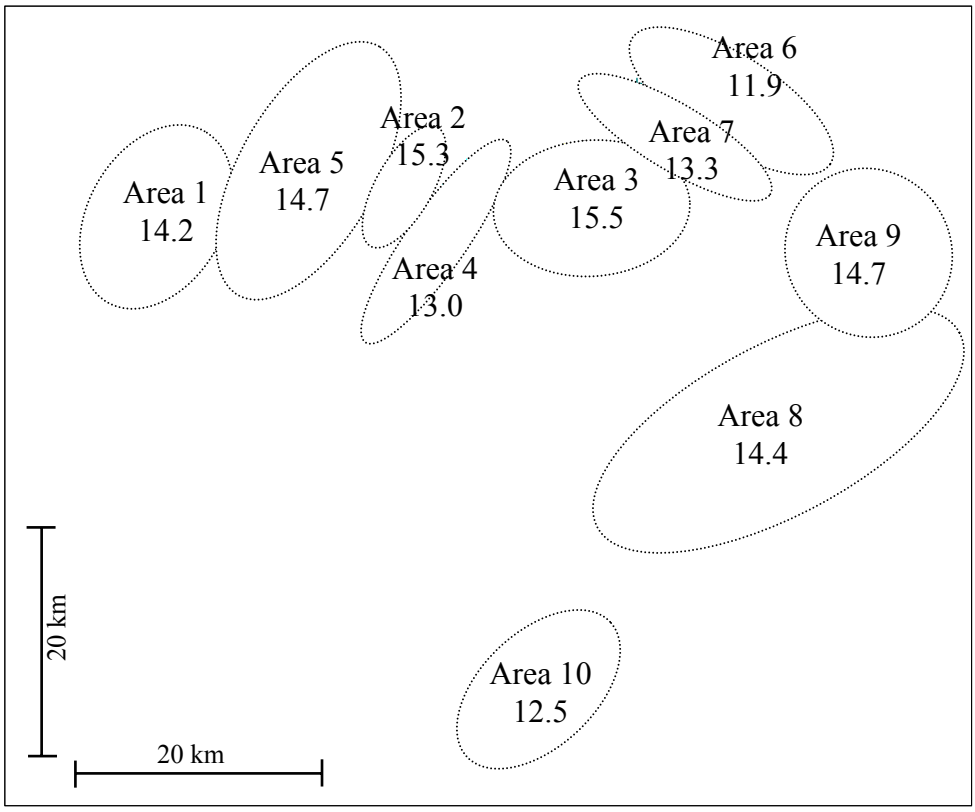

Figure 3.4 The distribution of ten areas producing mānuka honey in the Northland region. The mean activity from each area, expressed as UMF® units, is also shown.

3.8.3 Waikato region

Six samples were received from the Waikato region, representing the northern swamps. The region was divided into two areas, and the mean UMF® activity and standard deviation for the honey samples from each area are provided in Table 3.3. High quality mānuka honey is reported to be produced in this region, and again

the results confirm this. A significant difference ($p < 0.01$) was found to exist between the areas.

Table 3.3 A comparison of the mean UMF® activity and standard deviation recorded for areas within the Waikato region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	4	15.6	0.4
2	2	13.4	0.6

This region is illustrated in Figure 3.5. As only two zones are recorded, no relationship is noted other than the sites within the main swamp produced a honey with a significantly higher level of UMF® than the peripheral area.

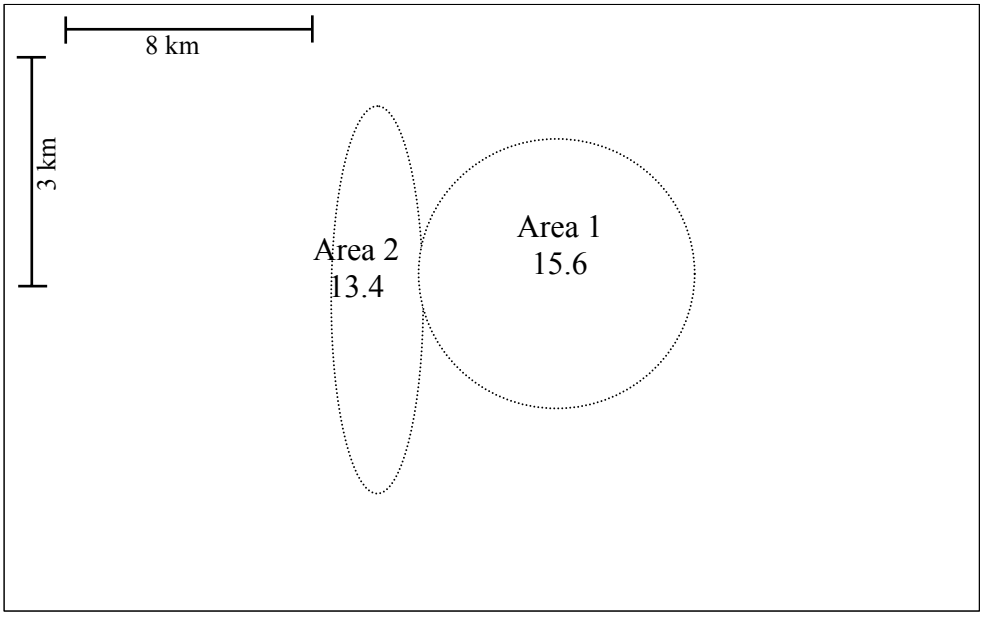

Figure 3.5 The distribution of two areas producing mānuka honey in the Waikato region. The mean activity of honey from each area, expressed as UMF® units, is also shown.

3.8.4 Coromandel region

Apiarists operating in the Coromandel region supplied 128 samples, geographically representing a large proportion of the peninsula. The region was divided into 17 areas, and the mean UMF® activity and standard deviation for the honey samples from each area are provided in Table 3.4. This region historically produces a variable quality of UMF® mānuka honey, and the study results are in accordance with this opinion. After removal of the areas yielding honey with non-detectable UMF® activity, a significant difference ($p < 0.001$) was found to exist between the remaining 15 areas.

Table 3.4 A comparison of the mean UMF® activity and standard deviation recorded for areas within the Coromandel region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	9	6.9	0.9
2	11	11.2	0.6
3	10	10.2	0.6
4	4	14.8	1.5
5	5	8.9	0.6
6	7	11.1	0.5
7	4	13.8	0.1
8	6	15.6	0.8
9	9	7.7	0.6
10	15	ND	
11	6	6.2	1.5
12	3	5.8	0.5
13	2	13.8	0.4
14	15	5.3	0.3
15	4	7.7	0.7
16	11	ND	
17	7	6.8	0.9

The Coromandel region is illustrated in Figure 3.6. Three tentative conclusions can be made from the distribution of UMF® activity. Firstly, the areas from which honey was harvested with non-detectable UMF® are positioned in the centre of the peninsula, in forest-covered hill country. Secondly, six areas yielded a non-peroxide antibacterial activity greater than 11 UMF® units, these areas are located

on an east-west axis through the mid-northern peninsula and south along the coast on the eastern side. The remaining nine areas, the far northern reaches, the balance of the central areas, and the southern areas, exhibited a range of lesser activity, 5.3–10.2 UMF® units.

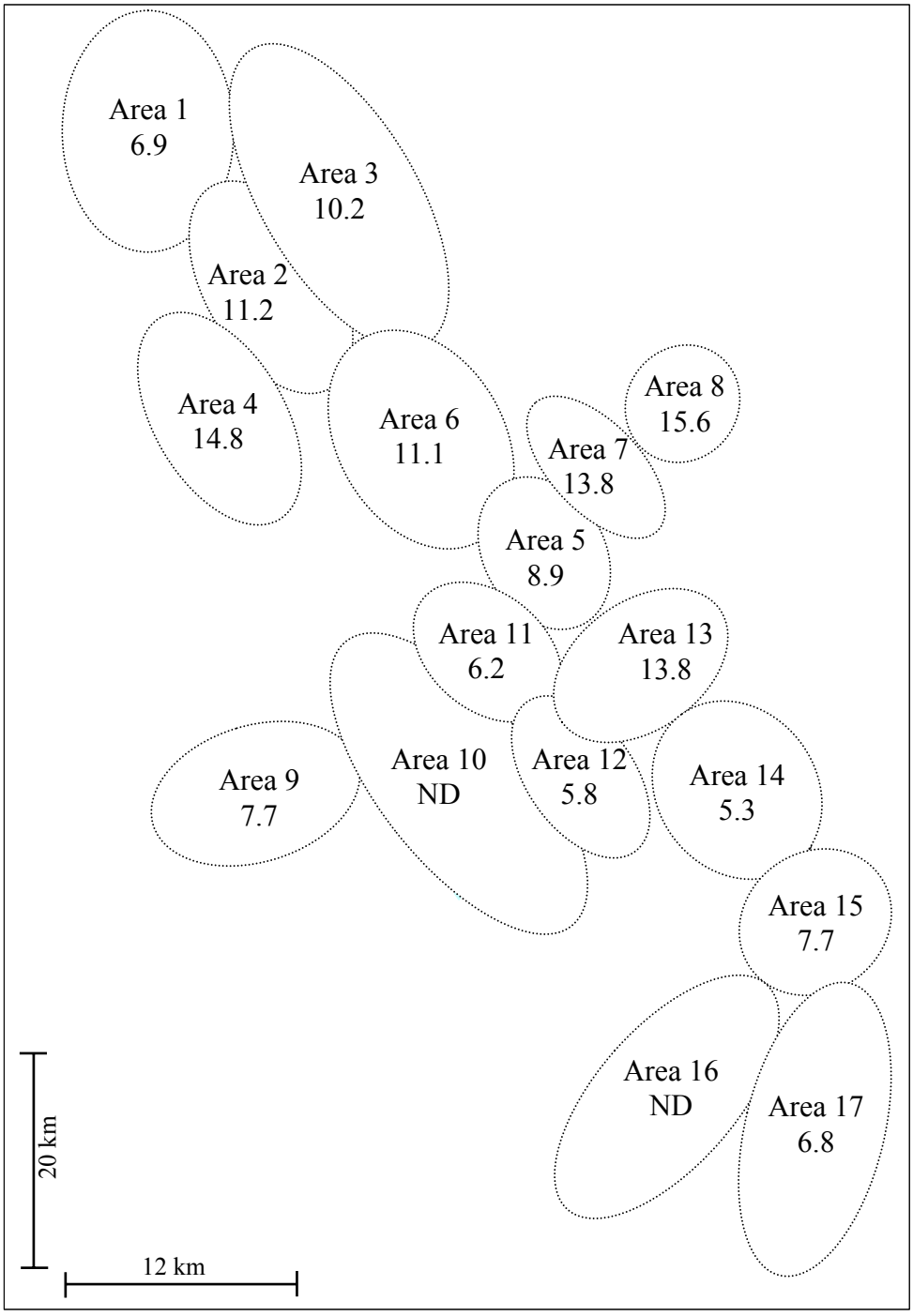

Figure 3.6 The distribution of seventeen areas producing mānuka honey in the Coromandel region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.5 Taranaki region

Fifty-one samples were received from the Taranaki region, representing the less pastoral portions of the province towards the Central Plateau, the western portion of the Central Plateau, and the mid-waters of the Whanganui River. Therefore the western districts of Taupo, Rangitikei and Wanganui (Crosby et al. 1976) are included in this region. The region was divided into six areas, and the mean UMF® activity and standard deviation for the honey samples from each area are detailed in Table 3.5.

This region is not considered a producer of high UMF® mānuka honey, and the UMF® activity of the honey agrees with this general belief. The areas yielded a significantly different ($p < 0.001$) level of UMF® activity.

Table 3.5 A comparison of the mean UMF® activity and standard deviation recorded from the areas within the Taranaki region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	6	10.1	0.8
2	13	9.9	1.1
3	10	10.1	1.0
4	4	4.6	0.4
5	15	7.1	1.1
6	3	10.9	1.5

The Taranaki region is illustrated in Figure 3.7. Whilst taken together the areas within the Taranaki region were significantly different, yet the four western areas recorded remarkably similar ($p < 0.6$) levels of mean activity between 9.9 and 10.9 UMF® units, however the eastern and north-eastern areas were lower.

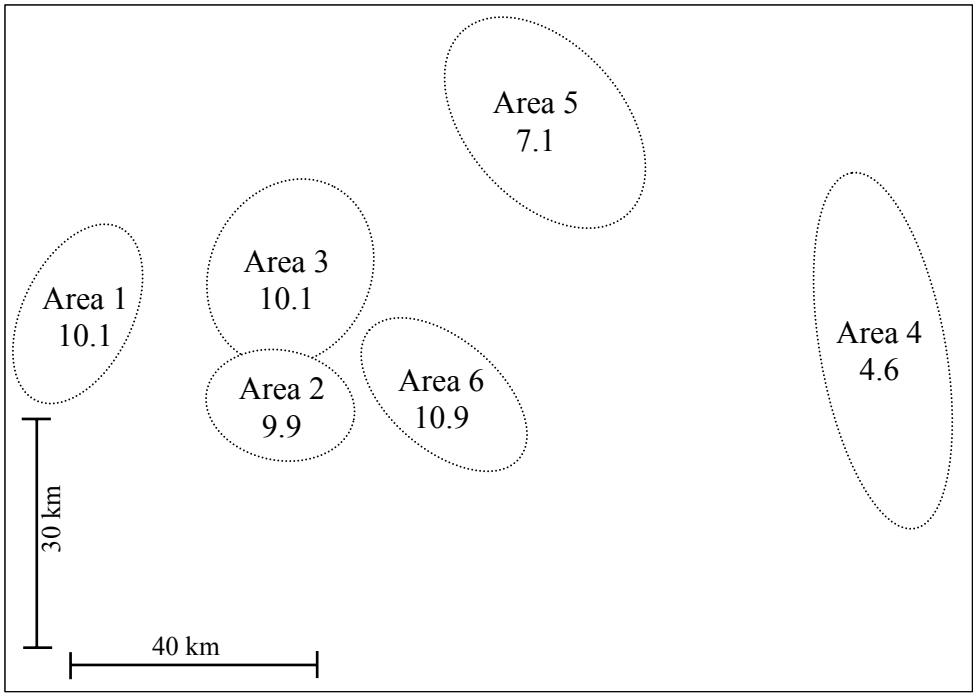

Figure 3.7 The distribution of six areas producing mānuka honey in the Taranaki region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.6 East Coast region

Thirty-nine samples were received from the East Coast region, collected from the coastal reaches and inland through the river valleys. This region represents the eastern district of the Bay of Plenty and north-east Gisborne defined by Crosby *et al*. (1976). The region was divided into eight areas, and the mean UMF® activity and standard deviation for the honey samples from each area are described in Table 3.6. This region is thought to produce a range of levels of UMF® in mānuka honey, however the UMF® activity of the honey reported in this study suggests the East Coast is not a high activity region. A significant difference ($p < 0.001$) existed between the UMF® activity recorded in these areas.

Table 3.6 A comparison of the mean UMF® activity and standard deviation recorded for areas within the East Coast region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	4	11.3	1.5
2	2	10.1	0.4
3	2	7.5	0.3
4	5	5.1	0.3
5	6	7.5	0.4
6	7	8.1	0.6
7	7	9.7	0.4
8	6	11.4	0.4

Figure 3.8 illustrates the distribution of areas in the East Coast region. A pattern of distribution is not clearly discernable, the coastal areas fluctuating over a range of mean values of 7.5–11.4 UMF® units, and the most inland area yielding 11.3 UMF® units.

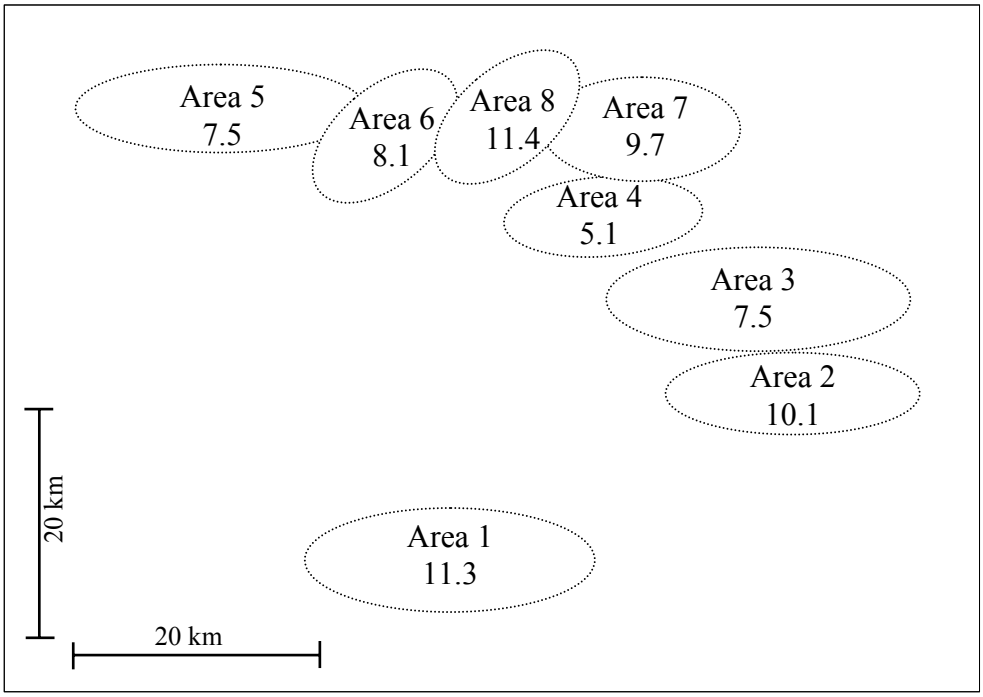

Figure 3.8 The distribution of eight areas producing mānuka honey in the East Coast region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.7 Gisborne region

The apiarists supplied nine samples from inland sites in the Gisborne region. The region was divided into two areas, and the mean UMF® activity and standard deviation for the honey samples from each area are detailed in Table 3.7. This region is generally thought to produce low activity UMF® mānuka honey. However one area did not exhibit activity, therefore a statistical test was not possible.

Table 3.7 A comparison of the mean UMF® activity and standard deviation recorded from areas within the Gisborne region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	9	ND	
2	2	10.4	0.6

Figure 3.9 illustrates the distribution of areas in the Gisborne region. The sites in Area 1 were well spread and none registered detectable UMF® activity. However further west in similar country a mean value of 10.4 UMF® units was recorded.

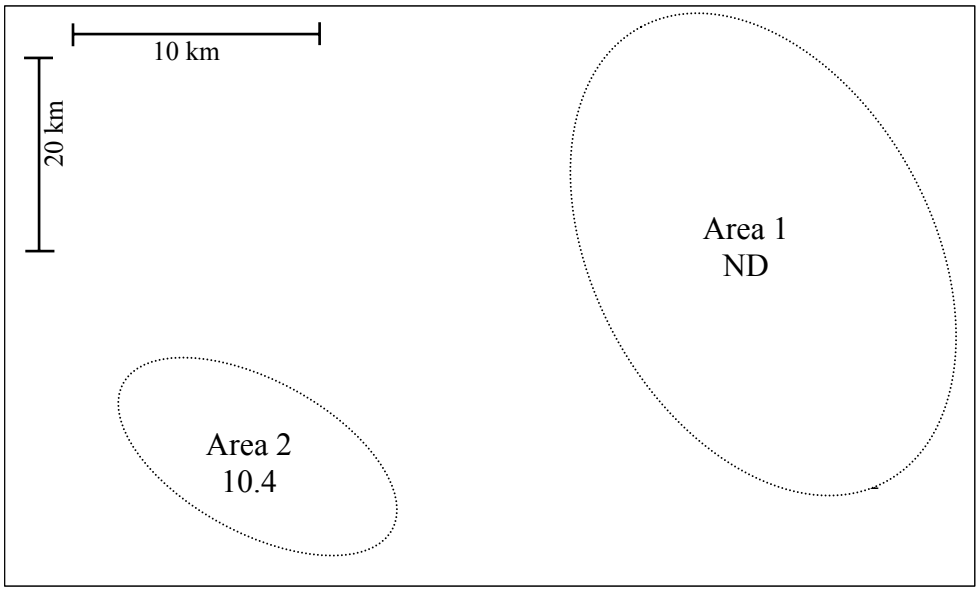

Figure 3.9 The distribution of two areas producing mānuka honey in the Gisborne region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.8 Hawkes Bay region

Forty-eight samples were supplied from inland sites in the Hawkes Bay region, collected in the hill country beyond the intensive horticultural centre of the province.

The region was divided into five areas, and the mean UMF® activity and standard deviation for the honey samples from each area are described in Table 3.8. This region is considered to yield mānuka honey with low levels of UMF® activity, and the study result confirms this opinion.

One area did not yield detectable UMF® activity. After the removal of this area the UMF® activity recorded in the remaining four areas was not significantly different ($p < 0.5$).

Table 3.8 A comparison of the mean UMF® activity and standard deviation recorded for areas within the Hawkes Bay region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	10	ND	
2	4	5.9	0.5
3	6	5.1	0.5
4	10	5.5	0.7
5	18	5.4	0.8

Figure 3.10 illustrates the distribution of areas in the Hawkes Bay region. Apart from the most northerly area the region yielded a very similar level of mean non-peroxide activity in the honey, ranging between 5.1 and 5.9 UMF® units.

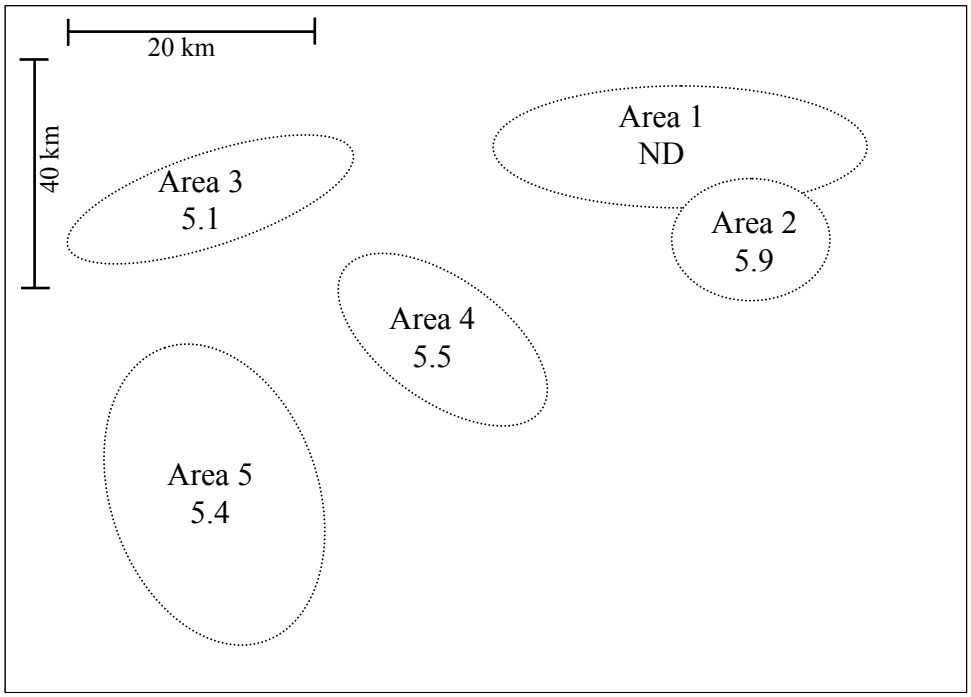

Figure 3.10 The distribution of five areas producing mānuka honey in the Hawkes Bay region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.9 Wairarapa region

The apiarists supplied 20 samples from Wararapa from inland sites. The region was divided into three areas, and the mean UMF® activity and standard deviation for the honey samples from each area are provided in Table 3.9.

This region is considered to produce low activity UMF®, and the study supports this. Honey from one region did not contain detectable UMF®. The UMF® activity in the honey harvested in the remaining two areas was found to be significantly different ($p < 0.001$).

Figure 3.11 illustrates the distribution of areas in the Wairarapa region. The mean activity ranged between 5.7 and 8.6 UMF® units in the northern areas whereas UMF® was not detected in the southern area.

Table 3.9 A comparison of the mean UMF® activity and standard deviation recorded for areas within the Wairarapa region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	8	ND	
2	7	5.7	0.8
3	5	8.6	0.9

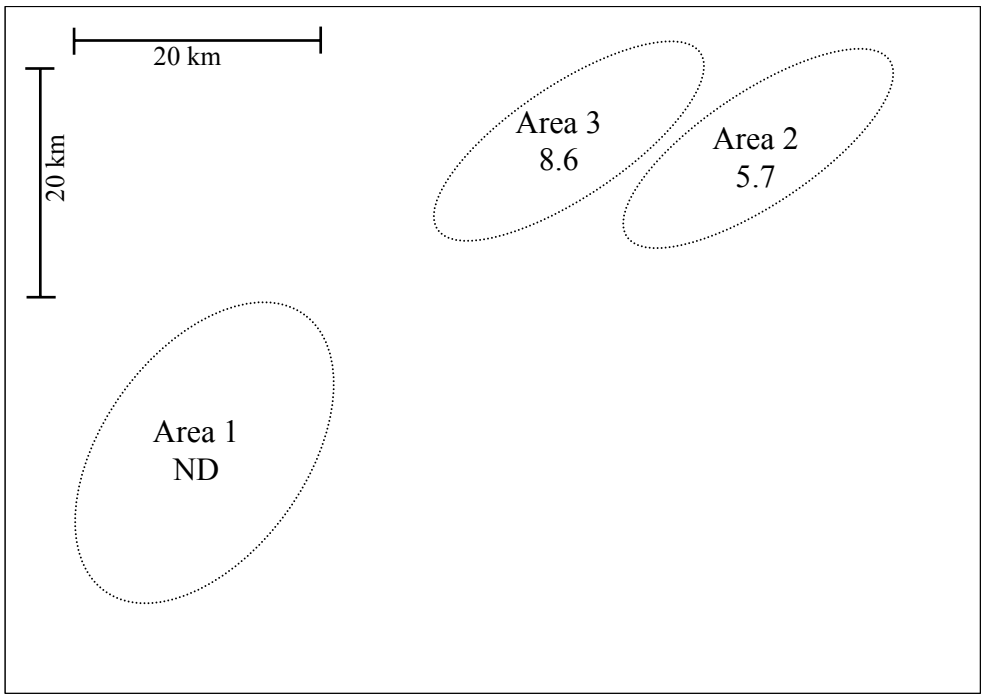

Figure 3.11 The distribution of three areas producing mānuka honey in the Wairarapa region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.10 Northern South Island region

Fifty samples were supplied from the northern South Island. This region includes the Marlborough Sounds and the northern section of Marlborough, Kaikoura, and Nelson defined by Crosby et al. (1976). The region was divided into eight areas,

and the mean UMF® activity and standard deviation for the honey samples from each area are described in Table 3.10.

This region is considered to produce mānuka honey with low activity UMF®, and the study confirms this general opinion. One area did not exhibit activity, and with this area removed from analysis the remaining seven areas yielded significantly different ($p < 0.001$) UMF® activity.

Table 3.10 A comparison of the mean UMF® activity and standard deviation for areas recorded within the northern South Island region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	9	ND	
2	10	5.0	0.4
3	2	7.2	0.1
4	2	9.9	0.3
5	15	5.1	0.4
6	7	6.6	0.4
7	2	10.0	1.0
8	3	5.2	0.9

Figure 3.12 illustrates the distribution of areas in the northern South Island region. A cluster in the central area of the Marlborough Sounds and a valley running southwest produced higher UMF® activity than the surrounding areas, however the nearby Area 1 recorded no activity. The west and southeast areas exhibited low UMF® activity.

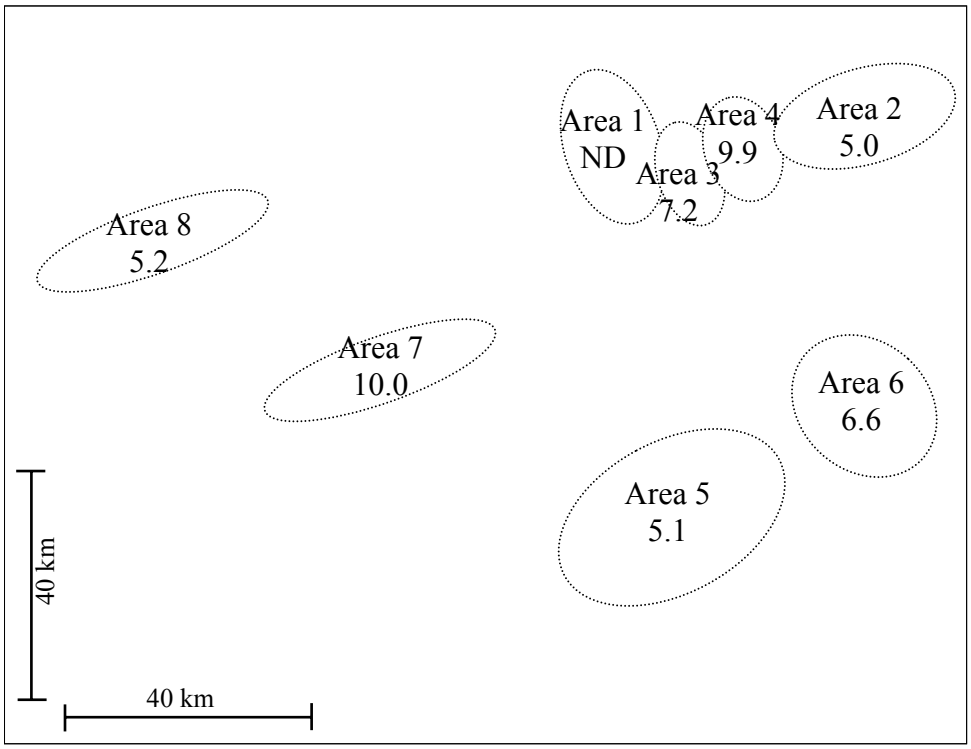

Figure 3.12 The distribution of eight areas producing mānuka honey in the northern South island region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.8.11 Eastern South Island region

Fourteen samples were supplied from the eastern South Island region. This region includes the North Canterbury and Dunedin districts defined by Crosby et al. (1976). The region was divided into four areas, and the mean UMF® activity and standard deviation for the honey samples from each area are provided in Table 3.11. This region does not produce large quantities of mānuka honey. The mean UMF® activity of the honeys yielded in each area were significantly different ($p < 0.001$). This region is not illustrated as the districts are approximately 420 km apart, and in those districts the areas from which the honey was collected were less than 25 km apart.

Table 3.11 A comparison of the mean UMF® activity and standard deviation recorded for areas within the eastern South Island region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	2	5.2	0.4
2	3	10.1	0.2
3	4	5.3	0.4
4	5	6.5	0.6

3.8.12 West Coast region

The apiarists supplied 61 samples from the West Coast, from Westport to Greymouth. This region covers Buller, southern Nelson and northern West Coast as described by Crosby et al. (1976). The region was divided into twelve areas, and the mean UMF® activity and standard deviation for the honey samples from each area are detailed in Table 3.12. This region is considered to produce a range of UMF® activities in honeys, and the study confirms this. The areas yielded significantly different ($p < 0.001$) levels of UMF® activity in the mānuka honey tested.

Table 3.12 A comparison of the mean UMF® activity and standard deviation recorded for areas within the West Coast region. The number of sites (*n*) within each area is listed.

Area number	*n*	Mean UMF®	SD
1	5	9.2	0.6
2	2	11.9	1.1
3	14	12.6	0.7
4	10	13.3	0.4
5	5	15.2	0.8
6	8	10.8	0.7
7	5	6.8	1.1
8	3	5.9	0.8
9	3	8.2	0.9
10	2	4.1	0.1
11	2	6.6	1.3
12	2	12.2	1.0

Figure 3.13 illustrates the distribution of areas in the West Coast region. In the southern areas mean UMF® activity is greater in the coastal area. The same situation applies in the northern areas: in the more coastal areas mean activity was in the range of 10.8–15.2 UMF® units, whereas the four inland areas yielded a range of 5.9–9.2 UMF® units. Therefore a tentative conclusion could be made; the coastal locations in the West Coast region produce mānuka honey with higher UMF® activity.

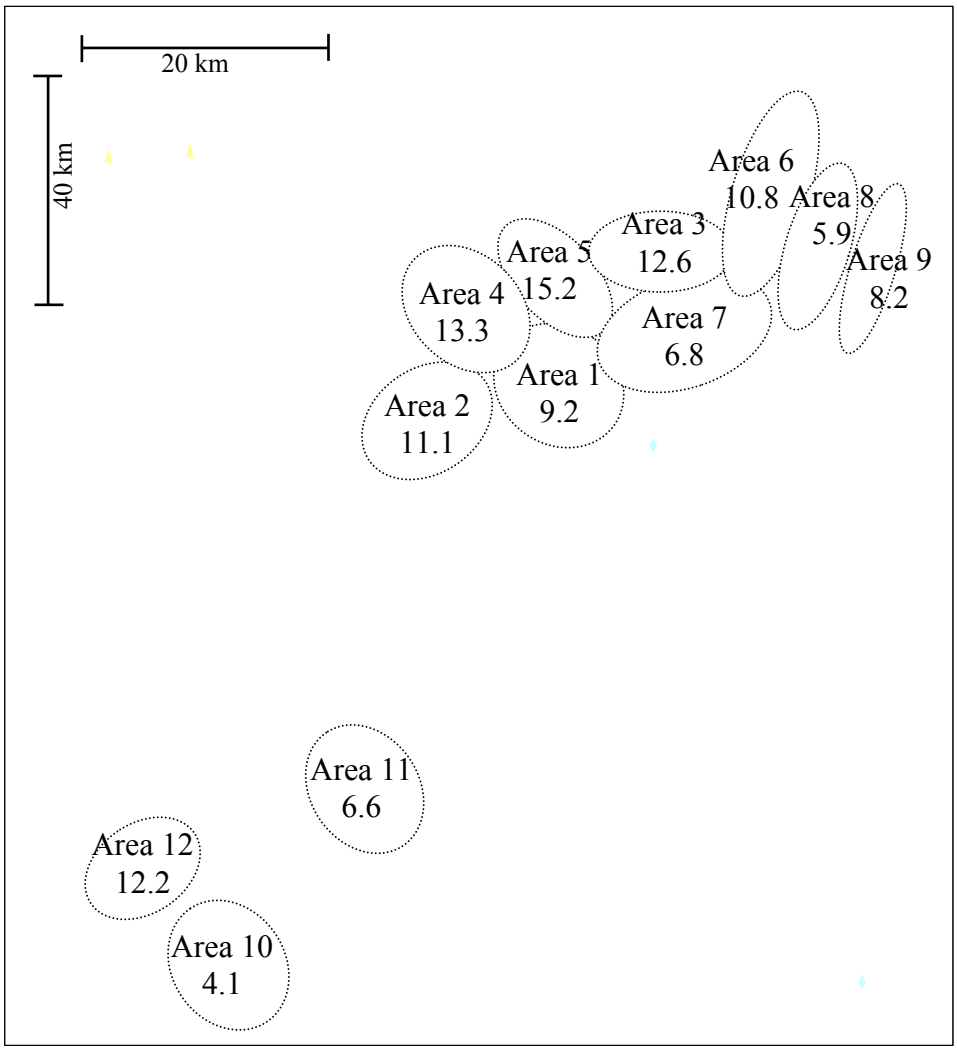

Figure 3.13 The distribution of twelve areas producing mānuka honey in the West Coast region. The mean activity of the honey from each area, expressed as UMF® units, is also shown.

3.9 Conclusion

The general opinion that the UMF® activity present in mānuka honey harvested throughout New Zealand varies substantially is confirmed by this study.

The mean regional UMF® activities recorded range from 14.9 to 5.4 units in the Waikato and Hawkes Bay respectively. Two of the most northern regions, Northland and Waikato, yielded high activity UMF® honey, yet the third northern region, Coromandel, only yielded high activity UMF® honey in a relatively limited area. Further south regional mean UMF® activities are generally less than 10 units, but a geographically distinct area of the West Coast in the South Island also yielded high UMF® activity honey. The Hawkes Bay, Wairarapa, and north and east South Island would appear to yield a fairly uniform low UMF® activity honey.

Likewise for the most part variability within regions is also pronounced yet differs between regions. Northland and Waikato regions yielded similar mean UMF® values and levels of variability. In contrast the Coromandel and West Coast regions were highly variable, brought about by the pockets of high UMF® activity yielding areas. The Taranaki, East Coast, Wairarapa and east South Island regions were moderately variable, whilst the Gisborne, Hawkes Bay and north South Island were relatively constant.

A unifying trend determined by an environmental parameter is not readily apparent, though several generalised statements may be made. Firstly, the high UMF® activity is more prevalent in the north of the North Island, and secondly activity appears to decrease in a relatively linear manner in a southerly direction. However the UMF® activity reported in the West Coast region disagrees with both of these assumptions.

The field data reported in this chapter is used in the following chapters to develop and explore the hypotheses outlined in Chapter One.

Chapter 4

Nectar or by-product from another species is the source of UMF®

This chapter develops the hypothesis that the UMF® in mānuka honey arises from the nectar of another plant or the by-product of another species. For the hypothesis to be acceptable this species would need to meet a number of prerequisite conditions.

The candidate plant species are considered. The plant species' nectar production, the collection of their nectar by honeybees, flowering periods, and distribution are summarised. The plant communities of five regions are described, from which mānuka honey containing UMF® is harvested. The other candidate species are considered. These species' by-products production, period of production, and distribution are summarised.

The necessary requirements are not met by any species other than *Leptospermum scoparium*. *L. scoparium* is indigenous, a surplus nectar producer, and has a range that encompasses all UMF® producing regions.

Sections of this chapter have been published in an amended form in the New Zealand Journal of Botany (Stephens, J. M. C.; Molan, P. C.; Clarkson, B D. 2005: A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany 43*: 431–449).

4.1 Hypothesis

The hypothesis that UMF® in mānuka honey arises from the nectar of another plant species, or from the by-product from another species, is explored.

The proposed species must fulfil a number of requirements –

(i) Be an indigenous New Zealand species.
(ii) Produce nectar/by-product collected by honeybee.
(iii) Be present in all regions where UMF® is reported in honey.
(iv) Be present in varying densities to explain the variability of UMF® reported in honey.

4.2 Nectar from another plant species

4.2.1 Indigenous New Zealand species

The premise is that UMF® is restricted in New Zealand to the indigenous flora. Non-peroxide antibacterial activity in honey shows a relationship with honey harvested from the indigenous flora of New Zealand, and has not been reported, other than in barely detectable levels, in honeys elsewhere in the world (Molan 1992).

The introduced plant species in New Zealand that yield surplus nectar for honey production are harvested in other geographic regions of the globe where UMF® is not recorded. Therefore UMF® is not associated with any introduced plant species.

However non-peroxide activity has also been recorded in honey harvested in southeast Australia (Blair 2004). The physical and anti-microbial characteristics of this honey type, known as jelly bush honey, are remarkably similar to mānuka honey harvested in New Zealand (Blair 2004). A comparison of the New Zealand and Australian *Leptospermum* honey types revealed different levels of phytochemical components (Yao et al. 2003). Australian jelly bush honey is derived from *Leptospermum* species, probably *L. polygalifolium*, an endemic Australian species present in south-eastern Australia and not found in New Zealand. The most recent taxonomic analysis of the genus separates *L. scoparium* and *L. polygalifolium* into related sub-groups, however both species share a number of characteristics (Thompson 1989).

4.2.2 Surplus nectar collected by honeybees

Whilst nectar is collected from 55 indigenous plant families in New Zealand, only 14 families contain species known to provide surplus nectar for the creation of honey stores (Butz Huryn 1995). Only 11 species from these families are found throughout New Zealand with a range that is equivalent to that reported to yield UMF® in honey.

These species are listed in Table 4.1, and the flowering periods are described. *Weinmannia racemosa* (kāmahi) and *W. sylvicola* are included as a generic entry; together these closely related species are found throughout New Zealand. Likewise the *Metrosideros robusta* (northern rātā) and *M. umbellata* (southern rātā) have been combined.

Therefore nine indigenous plant taxa are present throughout New Zealand, with a distribution and flowering period that may account for the UMF® reported in mānuka honey.

Table 4.1 Indigenous and endemic New Zealand plant species that provide nectar for surplus honey production

Family[1]	Species[1]	Distribution[1,2,3]	Bloom[1]	Habitat[4]	Commercial honey[1]
Araliaceae	*Pseudopanax arboreus*	NZ	Jul-Sep	Lowland & montane forest, lowland scrub	
Asphodelaceae	*Cordyline australis*	NZ	Oct-Dec	Damp forest margins	
Cunoniaceae	*Weinmannia* spp.	NZ	Nov-Feb	Lowland & montane forest	Kāmahi
Elaeocarpaceae	*Elaeocarpus dentatus*	NZ	Oct-Dec	Lowland forest	
Myrtaceae	*Kunzea ericoides*	NZ & Aus	Jan-Feb	Widespread	Kānuka
	Leptospermum scoparium	NZ & SE Aus	Sep-Feb	Widespread	Mānuka
	Metrosideros spp.	NZ	Oct-Apr	Lowland & sub-alpine forest	Rātā
Phormiaceae	*Phormium tenax*	NZ	Oct-Jan	Lowland swamps	
Rhamnaceae	*Discaria toumatou*	NZ	Oct-Jan	Dry coastal to sub-alpine	

[1]Butz Huryn 1995; [2]Webb et al. 1998; [3]Allan 1961; [4]Salmon 1991.
Distribution abbreviations. AUS–Australia; NZ – New Zealand; SE Aus – Southeast Australia

4.2.3 Plant communities in regions producing honey with UMF®

Five different vegetation communities are considered in detail to establish whether the nine surplus nectar-producing plant taxa are always present in areas that produce UMF® mānuka honey. The Northland gumlands, Waikato wetlands, East Coast regenerative seral scrub, North Island Volcanic Plateau heathlands, and Westland pākihi swamps are discussed. The prevalent woody dicotyledonous members of these communities are listed in Table 4.2. Honeybees visit the listed species for either nectar or pollen, even though the majority of these species do not provide surplus nectar for honey stores (Butz Huryn 1995).

Honey with high activity UMF® is harvested in the Northland gumlands. *Leptospermum scoparium* is common in large swathes of the undeveloped areas (Burrows et al. 1979), and is reported as a principal species in the far north (Enright 1989), the Waipoua Forest heathlands (Burns & Leathwick 1996), and represented 89% of the total dicotyledonous shrub dry mass near Kaikohe (Esler & Rumball 1975). Eight species were considered common and recorded in two or more studies.

Honey with high activity UMF® is also harvested in the Waikato wetlands. *L. scoparium* dominates large areas, and is found on a gradient of environments, ranging from the more extreme Kopuatai bog (Irving et al. 1984), the intermediary Moanatuatua bog (Burrows et al. 1979; Clarkson 1997), to the Whangamarino fen (Clarkson 1997) that is relatively more fertile and well covered by *L. scoparium* (Clarkson 2002). There are few other dicotyledons present, and only two species are noted in each report.

A range of UMF® activities is found in honey harvested from the lowland East Coast. A number of different communities exist; in particular large areas of coastal and lowland hillsides are covered with dense seral scrub that has become established since forest clearance for pasture establishment.

Table 4.2 Principal plant species in five environments producing honey with UMF® activity

Northland gumland	Waikato wetland	East Coast seral scrub	Volcanic Plateau heathland	Westland pākihi
Dracophyllum lessonianium[1,2,3,4]	*Epacris pauciflora* [5,6,7]	*Coprosma rhamnoides* [8,10]	*Dracophyllum longifolium* [12,13]	**Dracophyllum* spp. [17,18,19,20]
Epacris pauciflora [1,2,3,4]	*Leptospermum scoparium* [5,6,7]	*Coprosma robusta* [8,9,11]	*Dracophyllum subulatum* [12,13,14,16]	*Epacris pauciflora* [17,18]
Leptospermum scoparium [1,2,3,4]		*Coriaria arborea* [8,9,11]	*Kunzea ericoides* [12,13,16]	*Leptospermum scoparium* [17,18,19,20]
Leucopogon fasiculatus [1,2,3,4]		*Cyathodes juniperina* [10,11]	*Leptospermum scoparium* [12,13,14,15,16]	*Metrosideros umbellata* [18,19]
Pimelea prostrata [1,3]		*Hebe stricta* [8,11]	*Leucopogon fasciculatus* [13,15,16]	*Weinmannia racemosa* [18,19,20]
Pomaderris kumeraho [1,3,4]		*Kunzea ericoides* [8,9,10,11]		
Pomaderris phylicifolia [1,3]		*Leptospermum scoparium* [8,9,10,11]		
Weinmannia silvicola [2,3]		*Pittosporum ralphii* [9,11]		
		Pittosporum tenufolium [8,9,10]		
		Pseudopanax arboreus [8,9]		
		Weinmannia racemosa [8,11]		

Northland gumland, [1]Enright 1989; [2]Burns & Leathwick 1996; [3]Esler & Rumball 1975; [4]Burrows et al. 1979;

Waikato wetland, [5]Irving et al. 1984; [6]Burrows et al. 1979; [7]Clarkson 1997;

East Coast seral shrub, [8]Clarkson et al. 1986; [9]Regnier et al. 1988; [10]Clarkson & Clarkson 1991; [11]Whaley et al. 2001;

Volcanic Plateau heathland, [12]Rogers & Leathwick 1994; [13]Atkinson 1981; [14]McQueen 1961; [15]Leathwick 1987; [16]Clarkson 1984;

Westland pākihi, [17]Rigg 1962; [18]Burrows et al. 1979; [19]Mark & Smith 1975; [20]Norton 1989.

**Dracophyllum* spp. represents *D. longifolium* and *D. palustre*, possibly the same taxon (Allan 1961 p.533).

Leptospermum scoparium is a dominant member of the seral communities, interspersed with pockets of broadleaved trees (Molloy 1975). In the Motu Ecological District three types of *L. scoparium* scrub were described by Clarkson et al. (1986); *L. scoparium*, *L. scoparium*/*Coprosma* sp./*Hebe* spp., and *L. scoparium*/*Kunzea ericoides* scrub. Generally the same situation exists around the East Cape (Clarkson & Clarkson 1991; Regnier et al. 1988; Whaley et al. 2001). Eleven dicotyledons were recorded in two or more studies. Most of these species are associated with regenerating forest, expected in a seral *L. scoparium* environment. The coastal and lowland areas of the Coromandel Peninsula are similar to the East Coast, where forest clearance has led to the formation of significant areas of *L. scoparium* seral scrub.

Honey with low activity UMF® is harvested from the Central Plateau. *L. scoparium* dominates heathlands on the immature soils of volcanic debris above the tree line (Burrows et al. 1979). The vegetation at two high altitude areas, the Rangipo Depression (Rogers & Leathwick 1994) and the volcanic slopes of Mt Tongariro (Atkinson 1981), is a mixture of tussock grassland and scrubland. The three lower altitude studies, a widespread ignimbrite pumice plain near Tokoroa (McQueen 1961), the Waipapa Ecological Area (Leathwick 1987) and Pureora mountain mires (Clarkson 1984), illustrate more diversity as forest species return to more hospitable environments. Five species are noted as common throughout the region and recorded in two or more studies.

Honey with a moderate to high UMF® activity is harvested in coastal Westland. *L. scoparium* is prevalent on the low fertility pākihi soils. A number of widespread communities have been studied in northern Westland (Rig 1962; Burrows et al. 1979; Norton 1989), the central area (Burrows et al. 1979), and the southern reaches (Mark & Smith 1975). The vegetative communities differ according to latitude yet a common theme is found. Five dicotyledons are noted as common in this environment and recorded in two or more reports.

Only one indigenous species, *Leptospermum scoparium*, is found throughout the variety of environments in which honey with UMF® is harvested in New Zealand. *L. scoparium* exhibits the widest environmental adaptability of the indigenous flora, and no other plant species inhabits the range of *L. scoparium* throughout New Zealand.

Leptospermum scoparium is a surplus nectar producer. Two other surplus nectar producing species are reported as common in some UMF® producing environments. *Weinmannia* spp. (yielding kāmahi honey) are common in the regions of the Northland gumlands, East Coast seral shrub and Westland pākihi, however not in the Waikato wetlands or the Volcanic Plateau. Likewise *Kunzea ericoides* (yielding kānuka honey) is common in the East Coast and the Central North Island, but is not frequently found in two other areas and is absent in the Waikato wetlands. Accordingly these species cannot be responsible for UMF® in mānuka honey.

An alternative proposition may be suggested. The UMF® in mānuka honey may be derived from a plant that provides only pollen or very little nectar to the honeybee. The honeybee is reported to visit 224 native plant taxa in New Zealand (Butz Huryn 1995).

However, only *L. scoparium* is found throughout the entire range of environments that yield honey containing UMF®. *Epacris pauciflora* is common on the infertile lowlands, but is not present in the seral scrub of the East Coast or the Volcanic Plateau heathland. Likewise *Dracophyllum* spp are found in the lowland and montane heathland, but not in the Waikato wetlands or the East Coast seral scrub. Common members of the seral shrub communities are *Weinmannia* spp. and *K. ericoides*. These taxa are only recorded in two or three of the five environments respectively, but are not present in the Waikato wetlands. Therefore the collection of minor amounts of nectar or pollen by the honeybee from another plant species cannot give rise to UMF® in mānuka honey.

4.2.4 Other plant species conclusion

Leptospermum scoparium is the only species that meets the prerequisite conditions; it is indigenous, a surplus nectar producer visited by honeybees, and has a range that encompasses all UMF®-producing regions. No other indigenous species fulfils these requirements, and consequently the hypothesis that the UMF® in mānuka honey arises from the nectar or pollen of another plant species is rejected.

4.3 By-product of another species

Alternatively a by-product produced by another species may be incorporated into the honey by the honeybee and be responsible for the UMF® in mānuka honey. The prerequisite conditions remain identical; this species must be indigenous, the by-product harvested by the honeybee, be present throughout New Zealand where UMF® honey is harvested, and the duration of production of the by-product correlate with the harvest of UMF® honey. The candidate species are limited to the scale insects in New Zealand.

In New Zealand the scale insects (order Hemiptera) secrete quantities of honeydew that is attractive to other insect species. The honeydew honey produced in New Zealand is derived from the excretion of scale insects (*Ultracoelostoma* spp.) feeding on Nothofagaceae sap. The composition of the scale insect's honeydew is determined by both the insect and plant species (Hendrix et al. 1992).

4.3.1 Scale insect distribution

Eighteen species of scale insects have been recorded as associated with *Leptospermum scoparium*, however only two species (family Coccidae) are distributed throughout New Zealand and are commonly often found on *L. scoparium*. Both *Eriococcus orariensis* and *E. leptospermi* were involuntarily introduced from Australia in the mid-20th century (Hoy 1961). Though not indigenous, these species inhabit a range through south-eastern Australia where the non-peroxide jelly-bush honey is harvested, and whilst a different *Leptospermum* species is thought to be associated with that honey type these insects may generally inhabit *Leptospermum* on that continent and accordingly cannot be dismissed on an endemic status and geographical range basis.

The principal host species of *Eriococcus orariensis* in Australia are *Leptospermum juniperinum* in the southern and eastern areas of that continent and *L. scoparium* in Tasmania (Hoy 1961). Once introduced into New Zealand it was deliberately spread and brought about a rapid eradication of large areas of *L. scoparium*, as the removal of plant nutrients by the scale insect weakens the plants so that they are unable to survive environmental stress (Hoy 1961). The condition is commonly described as mānuka blight and is associated with infestation by the insect species and the development of a visually diagnostic covering of *Capnodium* spp. (sooty mould) growing on the resultant honeydew (Hoy 1961).

Whilst this scale insect was originally widespread, though absent in wetter regions and the sub-alpine belt (Wardle 1991), the virulence of *E. orariensis* has been significantly reduced by the subsequent spread of the entomogenous fungus *Myriangium thwaitesii*, and the revival of *L. scoparium* has been as spectacular as the initial decline (Hoy 1961). The range of *M. thwaitesii* is restricted to comparatively wetter and warmer environments (Hoy 1961). Consequently *E. orariensis* is no longer common throughout much of the North Island and northern South Island. The current distribution of *E. orariensis* does not correlate with the production of honey with UMF®. The numbers of this scale insect have been

significantly depleted in areas known to produce honey with high UMF® activity, whereas honey with low UMF® activity is harvested from the cooler drier environments still carrying a considerable population of *E. orariensis*.

Eriococcus leptospermi is found throughout New Zealand, also having been involuntarily introduced in the mid 20th century from Australia. *E. leptospermi* is usually found at low population levels, and infestation does not lead to plant death. It appears to be immune to *M. thwaitessi* (Hoy 1961). The widespread low-density distribution of *E. leptospermi* does not correlate with the variation of UMF® reported in honey.

In conclusion, two widespread scale insect species are commonly found in association with *L. scoparium*. These species do produce honeydew which may be harvested by the honeybee, but the distribution of the scale insects does not correlate with UMF® in honey.

4.3.2 Duration of production of honeydew

In the North Island all stages of the *Eriococcus orariensis* life cycle can be found on infested plants throughout the year (Hoy 1961). Sap feeding begins after hatching, and the insect continues to feed and secrete honeydew during the 16-week life cycle (Hoy 1961).

Consequently honeydew is produced throughout the year from successive populations of the scale insects. A rapid increase in insect population would be expected through the spring and summer seasons, however the harvest duration of honey containing UMF® is restricted to the period when *Leptospermum scoparium* is in bloom.

Honey harvested from the flora of an area containing a significant proportion of *L. scoparium* and populations of scale insects, hosted by *L. scoparium* or other plant

species, does not contain UMF® after the flowering period of *L. scoparium*. Therefore the duration of honeydew production by scale insects that inhabit the same range as *L. scoparium*, but are not necessarily hosted by *L. scoparium*, does not correlate with the production of honey containing UMF®.

4.3.3 The composition of honeydew honeys

Scale insects draw nourishment from the plant phloem and secrete surplus phloem as a waste by-product known as honeydew. Whilst the honeydew is predominately sucrose, glucose, and fructose; higher sugars are also present. The higher sugars are produced by the action of glycosyl transferases or glycosidases in the insect gut (Astwood et al. 1998). Therefore the presence of these modified sugars is a diagnostic test for honeydew honey. An analysis of the oligosaccaride composition of New Zealand honeys revealed mānuka honey with UMF® activity did not exhibit the complex oligosaccaride composition characteristic of a honeydew honey (Weston & Brocklebank 1999). The mānuka and clover honeys analysed contained a remarkably similar composition of oligosaccarides. These observations confirmed mānuka honey is derived from plant nectar, and not the honeydew by-product excreted by the scale insects found on *Leptospermum scoparium*.

4.3.4 Other species conclusion

Scale insects do utilise *L. scoparium* as a host. Consequently honeydew that is unique to the plant/insect combination is produced. However the distribution of the scale insects in New Zealand does not account for the variability of UMF® in mānuka honey. This is confirmed by the different duration of nectar and honeydew production in a season, and the absence of complex oligosaccarides in mānuka honey containing UMF®. Therefore the hypothesis that UMF® in mānuka honey is derived from the by-product of another species is rejected.

Chapter 5

Purity of mānuka honey and its effect on the level of UMF®

This chapter describes the development of a method to quantify the proportion of mānuka honey in the samples of honey collected, and the impact of purity of floral sources on the level of UMF® in mānuka honey.

The honey types frequently harvested with mānuka honey are described. The likelihood of harvesting monofloral honey in the New Zealand environment is considered, and the melissopalynological and physical methods of certifying a honey as monofloral are discussed.

Measurement of the viscosity of mānuka honey was developed as a method for determining purity. The viscosity properties of six New Zealand honeys commonly harvested in conjunction with mānuka honey were analysed. The dilution of pure mānuka honey with the other honey types resulted in an exponential reduction of the viscosity of mānuka honey, allowing a means of determining the purity of a mānuka honey to be established. The UMF® activity was found to be proportional to the amount of mānuka honey in the samples of honey collected.

An amended form of this chapter has been prepared for submission to Lebensmittel-Wissenschaft und Technologie – Food Science and Technology.

5.1 Hypothesis

The hypothesis that the variability encountered in UMF® activity in mānuka honey is a result of dilution of mānuka honey by the collection of nectar derived from other plant species is explored.

Two requirements must be fulfilled –

(i) Surplus nectar from other plant species is collected by the honeybee with nectar derived from *Leptospermum scoparium*.

(ii) Dilution of mānuka honey by other honey types alters the level of UMF® in mānuka honey.

5.2 New Zealand monofloral honeys

From spring to summer New Zealand monofloral honey is derived from seven main sources; the indigenous species *Leptospermum scoparium* (mānuka), *Kunzea ericoides* (kānuka), *Weinmannia* spp. (kāmahi), *Ixerba brexioides* (tāwari), *Knightia excelsa* (rewarewa), *Metrosideros* spp. (rātā/pōhutukawa) (Butz Huryn 1995), and the pasture-type honeys which are derived principally from introduced *Trifolium* spp. (clover). Therefore these honey types are most likely to form mixtures when honeybees harvest multiple sources of nectar during the mānuka season in New Zealand.

Thymus spp. (thyme), *Echium* spp. (vipers bugloss), *Carduus nutans* (nodding thistle), and honeydew (*Nothofagus* spp. and Homoptera association) honeys are also marketed as monofloral varieties, however these types are harvested from distinct regions where *L. scoparium* is not a significant member of the floral assemblage. Thyme, vipers bugloss and nodding thistle honeys are harvested in the summer-dry areas of the eastern South Island, and honeydew is produced from *Nothofagus* spp. forests of the northern South Island, particularly the mountainous regions of north-west Canterbury and Nelson.

5.2.1 Purity of monofloral honey types

Free-flying bees may collect nectar from any floral source available at the time of the honey production. Therefore there can be no certainty about the floral source of a honey, and those honeys marketed as monofloral would be better described as predominately produced from the nectar of one floral source. Whilst purity is initially controlled by floral source, hive management by the apiarists and subsequent techniques of honey extraction from the hives also have a considerable impact on the final honey purity.

5.2.2 Floral source

The foraging worker honeybee exploits the closest fruitful source, maintaining a delicate balance of transit time, energy expenditure in travel, and yield to the hive. Accordingly the honeybee will harvest nectar from any plant species in proximity to the hive, provided the collection proves worthwhile. Whilst an environment may be dominated by one plant species, other plant species may be visited. An example of this is seen in the north Waikato wetlands. The environment is dominated by *Leptospermum scoparium* and high quality mānuka honey is harvested from this region, yet other plant species, particularly the introduced pasture species, are flowering in conjunction with *L. scoparium* and are visited by honeybees.

5.2.3 Hive management

Modern beekeeping is not a passive occupation. Hives are moved into areas depending on the principal flowering plant species, the commercial demand for a honey type, and the need for pollination services in horticulture and agriculture. Many hives will be positioned at a site to fully exploit the floral resource, and the honeybees from each hive harvest the same plant species concurrently. Therefore the combined honeys from these hives represent the dominant floral source at the time of harvest. Many apiarists reposition hives at another site for a second

harvest of a different floral source after removing the initial honey gathered at the first site.

The honeybee hive is a dynamic system (Figure 5.1). The hive is made up of a number of stacked layers, termed supers. Each super is a frame containing combs. The hive initially contains a brood super at the base, and a few empty supers above. The hive is fed on sugar syrup until positioned in readiness for the nectar flow in spring. Once positioned, the honeybees collect nectar and concentrate it to produce honey, filling the lower super first, then the next, and so on. The apiarist regularly checks the hives at a site, and adds supers to the top of the hives depending on the honey flow.

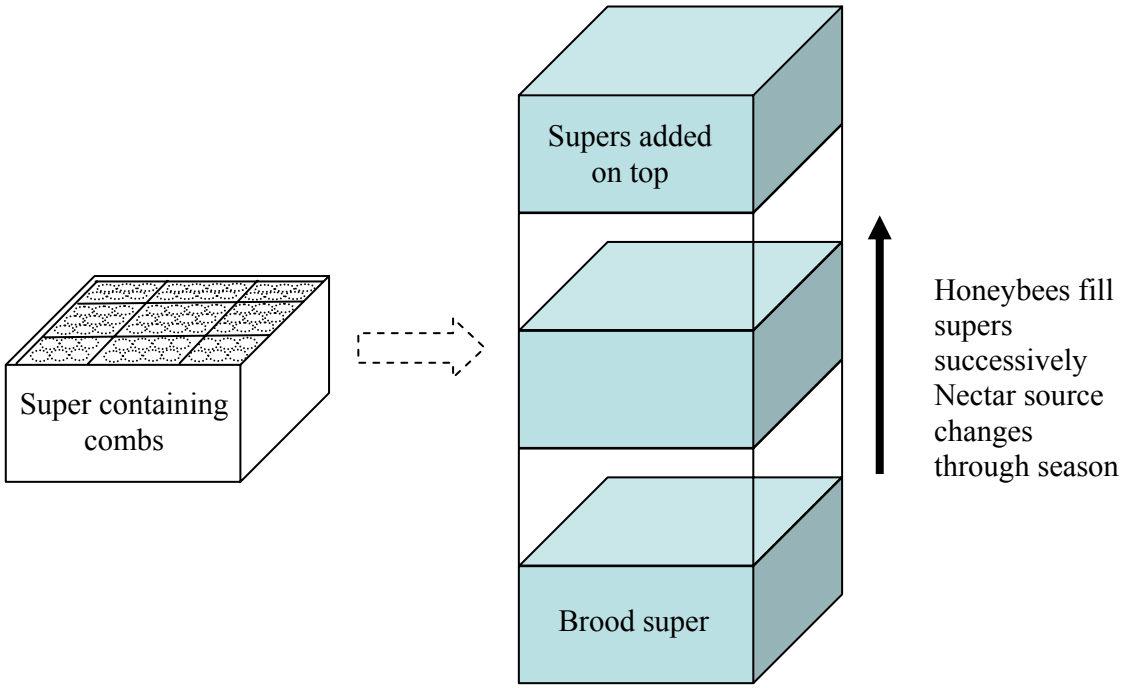

Figure 5.1 An illustration of a commercial honeybee hive and super, showing the upward filling of the supers with honey from different nectar sources.

Consequently hives left at one site for the duration of a season will contain a sequence of honey types representing the plant species which flowered throughout spring and summer within the collection range of the hive. Current practice in the industry encourages active hive management and removal to other sites for better

exploitation of new nectar sources. A site well-covered with *Leptospermum scoparium* scrub, for example, will produce surplus nectar through spring, but once the main flowering period is over will produce small amounts of honey derived from nectar of other species. The honeybees are forced to spend more time and distance foraging leading to reduced yield, and in extreme circumstances the honeybee population of the hive will begin to significantly deplete the honey stores even during summer. Therefore the hives are removed to another site where the nectar flow is sufficient to allow continued accrual of honey stores.

The extraction technique adopted by an apiarist significantly affects the monofloral nature of the honey. Experienced beekeepers are familiar with the characteristic colour, scent, flavour, and viscosity of the honey types. Therefore the division of supers within hives and sites before extraction of honey from combs allows the grouping of periods when the harvest represented a plant species and the honey was principally monofloral. An example is a site in 100 hectares of *L. scoparium* scrub surrounded by *Lolium perenne* / *Trifolium repens* pasture. In spring the *L. scoparium* and *T. repens* flower, but the honeybees collect nectar from the nearest viable source, the *L. scoparium*. Two months later the *L. scoparium* has finished flowering and the honeybees are collecting nectar from *T. repens* in the surrounding pasture. Therefore to extract monofloral honey the apiarist divides the supers. The lower supers will be predominately *L. scoparium* honey with a little *T. repens* (clover honey), and the upper supers will contain a pasture-derived clover honey.

The extraction of honey from the supers of a hive needs to be appropriately managed to avoid grouping different floral types together. The extraction of all the supers from hives that have been left at a single site for the duration of a season cannot yield monofloral honey, unless the plant species flowering has remained constant. The plant species from which honey is harvested in commercial quantities do not flower for an entire honey collection season. However hives that have been restricted to pasture species throughout a season will produce a clover-dominated pasture honey.

5.2.4 Identifying the floral source of New Zealand honeys

The New Zealand honey industry provides tentative standards for honey, having recently produced a draft 'New Zealand honey profiles monofloral varieties' (Anon. 2004) for discussion. Standards are common in most honey-exporting nations; however a system has not been established in New Zealand. Monofloral varieties, particularly mānuka honey, command a premium and the difficulties associated with mislabelling are prevalent.

The 'New Zealand honey profiles monofloral varieties' discussion paper divides the characteristics of monofloral honey into three components, appearance, pollen content, and the organoletic qualities of smell and flavour. These tests should be completed together, and the subjective sensory methods carried out by an experienced practitioner. The most recent report on pollen analysis in New Zealand (Mildenhall & Tremain 2005) concludes melissopalynology should not be carried out in isolation, while remaining an accurate indicator of a honey's purity, quality and source in association with the other tests. Moar (1985) published a set of standards for identification of New Zealand honey by pollen analysis.

However melissopalynology may give misleading results for a variety of reasons (Molan 1998). Floral morphology, variable pollen contribution from the flowers, the division of worker honeybees exploiting different floral sources for nectar and pollen collection, and pollen contamination introduced by both the honeybees and apiarists all contribute to the inaccuracies inherent in this method (Molan 1998). Trace organic constituents have the potential to be used to identify the floral sources, but these methods are not practical as databases of normal levels of constituents in each floral honey type are yet to be established (Molan 1998).

An amalgamation of the current standards for the seven honey types studied is provided in Table 5.1. The criteria for each monofloral honey type are outlined.

Table 5.1 The characteristics of seven New Zealand honey types adapted from current industry standards. Additions resulting from this study are italicised.

Monofloral honey	*Minimum % of nominated pollen[1]	Representation in honey[1]	Pollen frequency (%)[3]	Pollen count range grains/10g (mean ± SD) [3]	Colour[3]	Aroma[3]	Flavour[3]
Mānuka	70[1]	Over-represented[1]	70[3]	517,000 ± 280,000[3]	Dark cream to dark brown[3]	Damp earth, heather, aromatic[3]	Mineral, slightly bitter[3]
Kānuka	Same as mānuka[2]	Same as mānuka[2]	Same as mānuka[2]	Same as mānuka[2]	*Pale yellow, clear*	*Delicate, sweet, slightly aromatic*	*Sweet, slightly aromatic*
Kāmahi	60-70[1]	Over-represented[1]	45[3]	185,000 ± 67,000[3]	Light-pale yellow[3]	Intense, musky, quite complex[3]	Rich, sweet, aftertaste, buttery texture[3]
Tāwari	Not given[1]	Not given[1]	Low pollen count[3]	Not given[3]	Light cream[3]	Rich, musk, incense, sandalwood[3]	Rosehip, very sweet golden syrup[3]
Rewarewa	10[1]	Under-represented[1]	Not given[3]	113,000 ± 102,000[3]	Amber-red[3]	Light, mild mixed fruit[3]	Clean, sweet, smoky, malty[3]
Rātā/ Pōhutukawa	45[1] Not given[1]	Not given[1]	45[3] 10-15[3]	123,000 ± 34,000[3] Not given[3]	White-cream[3] Off-white[3]	Heady, aromatic[3] Musky, salty[3]	Sweet, salty[3] Clean earthy, butterscotch[3]
Clover	45[1]	Mixed representation[1]	45[3]	100,000 ± 90,000[3]	Light pale gold[3]	Herbal, dry grass[3]	Clean, mild, sweet, delicate[3]

[1]Moar, 1985; [2]Mildenhall & Tremain, 2005: [3]New Zealand honey profiles monofloral varieties, unpublished industry source.
*Pollen count should reveal a minimum % of pollen derived from nominated monofloral nectar source.

Mānuka and kānuka honey do not have individual standards, and pollen differentiation between *Leptospermum scoparium* and *Kunzea ericoides* is extremely difficult (Mildenhall & Tremain 2005). At least 70% of the pollen in the honey should be derived from these Myrtaceae species, and the honey should have between approximately 240 000–800 000 Myrtaceae pollen grains in 10 g. Mānuka honey is the more valuable resource and unadulterated material commands a premium, whereas kānuka is a palatable culinary honey. However these plant species often inhabit the same environments throughout New Zealand, being woody scrub pioneers that flower at similar times, though *K. ericoides* flowers a little later through early summer. Therefore it is likely that honeys will be produced that are a mixture of mānuka and kānuka nectar.

It has been noted that many apiarists have difficulty in correctly identifying these plant species. Observations from this study highlight the honey differences. Kānuka honey is pale brown, clear, with a delicate sweet smell and slightly aromatic taste, and is not thixotropic, whereas mānuka honey is a darker brown, strongly aromatic with a distinctive bitter flavour, and markedly thixotropic. The flower morphology *Kunzea ericoides* and *Leptospermum scoparium* are similar, and accordingly honeys derived from these sources would be expected to provide the same pollen count, which is suggested by Mildenhall and Tremain (2005). The characteristic strong smell and taste of mānuka honey is more distinctive and easily identified. Therefore the common mixture of these honey types is often described as mānuka honey, and pollen analysis is ineffective differentiating these types.

Kāmahi honey has a distinct flavour, smell and appearance. *Weinmannia* spp. are forest trees inhabiting lowland regions. The pollen is distinct and well represented in the honey, the kāmahi standard being 60–70% of the pollen grains and numbering between approximately 112 000–250 000 grains in 10 g of honey.

Tāwari honey does not have a set standard, but smell and taste are distinct. *Ixerba brexioides* is also a forest tree, present in the northern half of the North Island.

The pollen is large and distinctive and expected to be under-represented in the honey, and 13% *I. brexioides* grains was considered adequate to label the honey as monofloral (Mildenhall & Tremain 2005). The number of grains of pollen has not been recorded.

Likewise *Knightia excelsa* pollen is under-represented in rewarewa honey, and 10% of the total pollen is the minimum standard in a monofloral honey, with a range between 10 000–215 000 *K. excelsa* grains in 10 g of honey. Rewarewa honey is visually distinct, clear and darkly coloured with a fruity smell and flavour. *K. excelsa* is a lowland forest tree species found from Marlborough northwards.

The *Metrosideros* spp. range widely throughout the North Island and northern-central South Island forests. Monofloral rātā honey should contain at least 45% *Metrosideros* pollen with a total count between approximately 90 000–160 000 grains in 10 g of honey.

Pōhutukawa (*Metrosideros excelsa*) honey is harvested from the coastal northern North Island regions. Mildenhall and Tremain (2005) note *M. excelsa* pollen is difficult to distinguish from the rātā species. The flavour and scent of these honeys are fairly similar, and colour may also be the same. A standard of 10–15% *Metrosideros* pollen has been proposed for pōhutukawa honey, which appears low compared to the rātā honey standard. The narrow coastal distribution of *M. excelsa* may be a simpler method for differentiating between these honeys.

The pasture honey is often treated as a monofloral clover type. This honey is harvested throughout New Zealand. Pollen grains from *Trifolium* spp. are the most common constituent, being at least 45% of the total pollen grains and having between 10 000–190 000 grains in 10 g of honey. The sweet clean taste and smell are reasonably distinctive.

5.2.5 Pollen content of monofloral honeys

Mildenhall & Tremain (2005) analysed the pollen content of 45 honey samples supplied by the Honey Research Unit, University of Waikato.

Table 5.2 details the pollen types recorded in the monofloral samples with the highest percentage of type pollen for each of the seven honey types discussed above. Three mānuka, rewarewa, rātā/pōhutukawa, and clover honeys, and one kānuka, kāmahi, and tāwari honey are described.

All the monofloral honeys contain at least 13% pollen from other plant species, and in many cases considerably more, supporting the opinion monofloral honey is not harvested naturally, but because pollen counts are an unreliable indicator the floral source cannot be confirmed.

Trifolium and *Taraxacum* spp. pollen is represented in all the samples, and *Weinmannia* and *Lotus* pollen in all but one sample, and these plant species produce nectar for collection. Poaceae pollen is also very common, but this family does not produce nectar and the pollen is wind dispersed.

This data reinforces the shortcomings of the melissopalynological method. Whether the pollen grains were gathered with nectar, were representative of independent pollen collection by the honeybees, or have been introduced by some other form of contamination is unknown.

Table 5.2 Pollen analysis of seven monofloral honey types: % of pollen grains in honey sample. Adapted from Mildenhall & Tremain (2005).

	Study number	Asteraceae	*Borago*	*Coprosma*	*Echium*	*Geniostoma*	*Griselinia*	*Ixerba brexioides*	*Knightia excelsa*	*Leptospermum scoparium*	*Kunzea ericoides*	*Lotus*	*Mentha*	*Metrosideros*	Poaceae	*Quintinia*	Ranunculaceae	*Rosa*	*Salix*	*Taraxacum*	*Trifolium*	*Weinmannia*	*Other*
Mānuka	M24	<1								**85**		2		1	1		1		<1	1	4	1	2
Mānuka		<1								**87**		<1			2		<1			<1	10		<1
Mānuka	M11			<1		<1		<1	<1	**73**		10	<1	1	1		<1	<1	1	1	6	1	3
Kānuka	K6	<1			<1						**87**	4		<1	1		<1		<1	<1	7	<1	<1
Kāmahi	Ka3											<1		11			<1		<1	<1	<1	**79**	8
Tāwari	T3	1						**13**	1	5		14	1	<1	2	<1	2	1	1	2	41	11	4
Rewarewa	R1	2		<1				8	**22**	<1		10	<1	3	<1		1		4	2	41	1	6
Rewarewa	R2					1			**37**	37		1		<1	1		1	<1		2	7	5	9
Rewarewa	R4	2				<1		1	**27**	<1		10	3		1	<1	8	2	3	5	26	10	2
Rātā		<1										2	<1	**84**	<1			1		<1	1	6	6
Rātā	Ra1											1		**94**		1				<1	1	2	1
Pōhutukawa	Ra2	<1		<1	1	<1	<1	<1	<1	1		10		**48**	<1	9	<1		<1	<1	8	18	4
Clover	C9	1				<1			<1			5		<1	<1				<1	1	**89**	2	1
Clover		<1	1										1	<1	<1				<1	<1	**88**	<1	10
Clover	C8	<1	1						<1	<1		1	<1	<1	1		<1	<1	1	1	**84**	1	8

5.3 Purity of mānuka honey

The identification of a honey by colour, aroma, and flavour, coupled with melissopalynology, does not provide an exact measure of the purity of mānuka honey. The strong aroma and flavour of mānuka honey override those of the more subtle honey types, and colour varies immensely. Pollen analysis will only indicate *Leptospermum scoparium* was the main floral source.

Therefore another method had to be developed to determine the percentage of mānuka honey in the samples of honey collected. A method dependent on the thixotropic nature of mānuka honey was developed, and the six other honey types harvested with mānuka were investigated in comparison with mānuka honey.

5.2 1 Viscous nature of honey

Honey is a viscous liquid prepared by honeybees from plant nectar. The viscous properties of a honey are principally influenced by the solids composition, water content, and temperature (White 1975a; 1975b), and the physical properties of honey differ depending on the nectar sources collected. Considerable variability exists in the solids composition of honey; the main factors influencing the physical characteristics are the monosaccaride components (glucose and fructose) which make up approximately 84% of the solids and accordingly are the dominant cause, the disaccarides and higher sugars, the protein fraction and other organic molecules, and the mineral fraction (White 1975a, 1975b).

Consequently the viscosity of different honeys varies significantly, typical of a sugar solution containing different constituents and proportions (White 1975b; Bourne 1982). Furthermore, water content and temperature also influence physical properties; an increase of either independently reducing the viscosity (Monro 1943; Mossel et al. 2000, 2003).

Most honey is reported to be a Newtonian fluid (White 1975b), following an Arrhenius type relationship correlated with solid content (Junzheng & Changying 1998; Mossel et al. 2003), water content (Mossel et al. 2003), or temperature (Bhandari et al. 1999; Mossel et al. 2000). However some honeys have been noted to exhibit thixotropic behaviour attributed to the presence of colloids (Monro 1943; Pryce-Jones 1953; White 1975b).

5.3.2 Viscosity of New Zealand honeys

The viscosity of New Zealand honey types has not received attention. Mānuka honey has previously been recorded as distinctly thixotropic (Pryce-Jones 1953), this property being attributed to the presence of certain proteins (White 1975b). *Calluna vulgaris* (heather) honey was also reviewed (Pryce-Jones 1953), and whilst this honey is also thixotropic, the late summer flowering period and montane distribution of this introduced species is not equivalent with *Leptospermum scoparium*, and accordingly these honeys are unlikely to be harvested together. Honey derived from the introduced *Trifolium* spp. (clover) has been shown to exhibit only Newtonian characteristics (White 1975b).

5.4 Viscosity method and materials

This section details the method used for determining the viscosity of honey samples, to establish whether mānuka honey was more viscous than the other honeys, and whether the mānuka content of a honey could be determined in a honey mixture.

5.4.1 Sample collection

Honeys were collected from commercial apiarists throughout New Zealand. The samples were unprocessed other than having been extracted by centrifuge from the combs. Each honey was considered monofloral by the apiarists based upon colour, aroma, flavour, and knowledge of what plant species were in flower around the location of hives at the time of production.

5.4.2 Sample preparation

To remove crystals or air bubbles in the honey, which may interfere with viscosity measurements, the honey samples were placed in a sealed 35 ml container, heated at 55 °C for 2 h, gently stirred, and then stored at 25 °C for 48 h. The samples did not contain detritus such as comb wax and insect body parts. To prevent incorporation of air bubbles during transfer, the viscosity of samples was measured in the storage containers (Mossel et al. 2000).

For the latter part of the study the mānuka honey was blended with the other honeys and also prepared in the same way, however bulk samples were heated at 55 °C first, and three mixtures made, 75%, 50% and 25% mānuka honey with each of the other six honey types. The various blends were then stored in 35 ml containers as above

5.4.3 Total soluble solids

The moisture content of a honey significantly alters viscosity. The moisture content of each honey was determined by measurement of total soluble solids (°Brix) at 20 °C using a refractometer (ATAGO ACT-1). For these measurements 1 ml of honey was diluted with 4 ml of water and the °Brix reading multiplied by the dilution factor (Bhandari et al. 1999).

5.4.4 Removal of proteins

To determine whether proteins were responsible for the thixotropic behaviour of mānuka honey, the proteins were precipitated and removed. A diluted solution of mānuka honey (70 ml, 50% w/v) had a small volume (5 ml) of phosphotungstic acid (2% w/v) added to precipitate the proteins (Blair 2004). Following mixing, the solution was centrifuged (10 min, 3000 rpm) and the honey solution decanted from the precipitate. The moisture content of the honey solution was returned to the initial level by rotary evaporation under vacuum at 37 ºC, and then the honey was allowed to rest for 48 h at 25 °C to allow re-gelling following the disturbance, and the viscosity tested.

5.4.5 Viscosity measurement

Measurements were taken with an Anton Paar DV-1 P digital viscometer, spindles L3 and L5 immersed to the same depth, and variable rpm. The samples were heated at 35 °C for 1 hr prior to testing to ensure a homogeneous temperature, which was maintained throughout the analysis by the use of a temperature-controlled jacket with circulation from a water-bath.

Three experiments were conducted. Firstly, the shear rate was increased stepwise, 5, 10, 20 and 50 rpm, after a reading taken immediately (within 3 seconds) for the time-independent variable shear rate analysis. The second experiment, investigating thixotropy, utilised a time-dependent constant 50 rpm shear rate, with readings taken every 15 s for 2 min and an initial reading at 5 s. Thirdly, the mānuka honey blends with the other honey types were measured by time-independent variable 5 rpm shear rate to determine whether any changes in viscosity would be apparent. All the tests, apart from the initial sample sorting process selecting the honeys to bulk together to represent the standard monofloral honeys, were conducted in duplicate and mean values and standard error of the mean are presented.

5.5 Results and Discussion

5.5.1 Monofloral honey standards

The first stage of this method development required the selection of standard monofloral honeys. Therefore a number of honey samples from the Honey Research Unit collection were analysed to determine the viscous characteristics of mānuka, kānuka, kāmahi, tāwari, rewarewa, rātā/pōhukakawa and clover honeys. These samples were examined by the time-independent variable shear rate method.

5.5.1.1 Mānuka honey

Table 5.3 provides the results of the analysis performed on 32 samples of mānuka honey. Many of the samples, despite being supplied as mānuka honey, did not have the colour, odour, taste, or viscosity of a pure mānuka honey, and were a mixture of other floral types. Complete analysis was not always carried out when the sample was not a monofloral mānuka honey. Five of these samples had a pollen analysis completed (Mildenhall & Tremain 2005). M3, M8, M11 and M24 were considered monofloral mānuka honey, but M5 contained a significant amount of *Trifolium* spp. pollen.

The 5 rpm viscosity of the four samples shown to be monofloral by pollen analysis did correlate positively with the percentage of *Leptospermum scoparium* pollen content (r^2 0.6693), indicating a higher percentage of *L. scoparium* pollen shows a greater concentration of *L. scoparium* nectar has been incorporated into the mānuka honey. However, the sample numbers are small and the correlation weak, so pollen and viscosity analysis of a greater number of samples of mānuka honeys would be required before this conclusion could be validated.

Table 5.3 Viscosity analysis of monofloral mānuka honeys supplied to the Honey Research Unit by New Zealand apiarists; presented in order of decreasing viscosity at 5 rpm.

Sample	Viscosity (Pa.s)				Observations on physical characteristics
	Spindle speed (rpm)				
	5	10	20	50	
M28	29789	20630	15198	10593	Mid-dark brown, strong gel, aromatic
M24*	29711	19908	14193	10471	Mid-brown, gel, aromatic
M22	29333	19598	14289	11001	Mid-brown, gel, aromatic
M15	28643	17035	12413	8520	Mid-brown, aromatic, gel
M18	28084	17893	13177	10016	Mid-brown, strong gel
M21	27998	18989	13463	9640	Mid-brown, gel very aromatic
M8*	27325	16125	11210	8821	Mid-brown, aromatic, bitter, gel
M3*	26735	16303	11857	8724	Mid-brown, aromatic, bitter, gel
M19	25491	16297	11525	8612	Mid-dark brown, gel, aromatic
M11*	25324	15218	10994	7364	Mid-brown, aromatic, bitter, gel
M27	25311	17949	13732	9143	Mid-brown, gel, aromatic
M30	25030	15580	11254	8370	Mid-brown, aromatic, gel
M26	24073	15055	11593	8547	Mid-brown, gel, aromatic
M12	23144	15046	10871	7908	Mid-brown, aromatic, gel
M13	23506	15694	11137	8272	Mid-brown, aromatic, gel
M31	20694	13369	9427	7821	Light-mid brown, sweet, mix
M32	19266	12332	8753	6533	Dark brown, aromatic, mix
M23	17603	12797	9637	5729	Mid-brown, sweet, semi-gel, mix
M10	15218	10047	8095	5933	Pale brown, mix
M7	11029	9077	7342	5882	Mix
M2	9590	7517	5863	5214	Dark, bitter, mix
M5*	9383	8287	7453	7010	Mid-brown, bitter, mix
M6	7300	6056	5189	4268	Light brown, mix
M1	6932	5293	4091	3648	Very dark, bitter, mix
M20	11269	10997	8612		Mid-brown aromatic, mix
M9	8926	8631			Grey-brown, unpalatable, mix
M25	5801				Mix
M17	5615	5048			Light brown, no gel, slightly aromatic
M4	5221				Very dark brown, mix
M29	4299	4307			Mid-brown, sweetish, mix
M16	4125	3342	3190		Light brown, no gel, mix
M14	3298	3004	2816		Very dark, no gel, mix

*Pollen analysis completed by Mildenhall & Tremain (2005).

The mānuka samples displayed shear-thinning non-Newtonian viscosity (Figure 5.2).

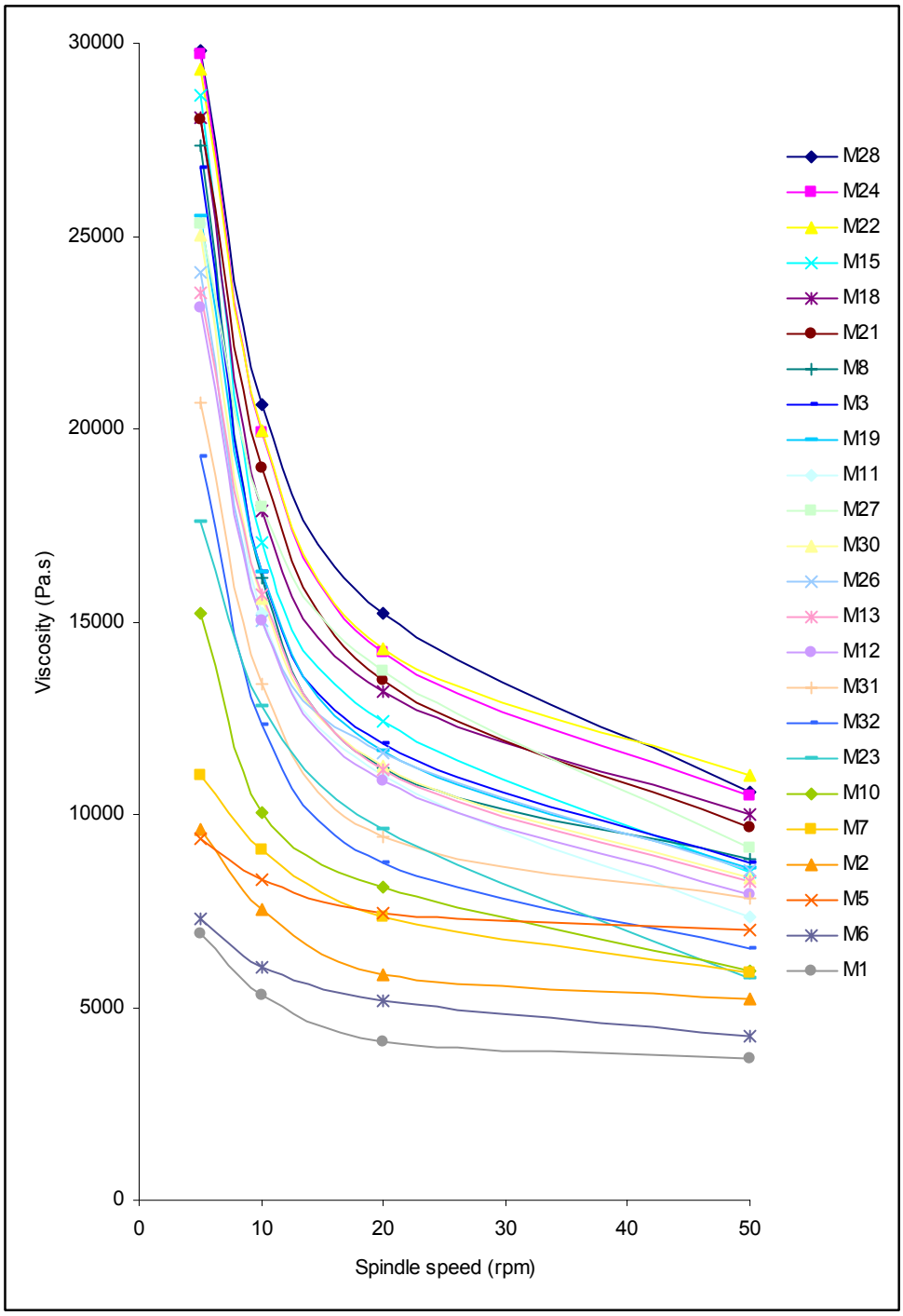

Figure 5.2 The viscosity of the mānuka honey samples, illustrating non-Newtonian shear-thinning behaviour.

The range of shear-thinning behaviour differed and the samples may be divided into three groups entirely on the basis of the recorded 5 rpm viscosity. It can be seen that a continuum exists however the data has been made discrete to enable the main features to be described (Figure 5.3).

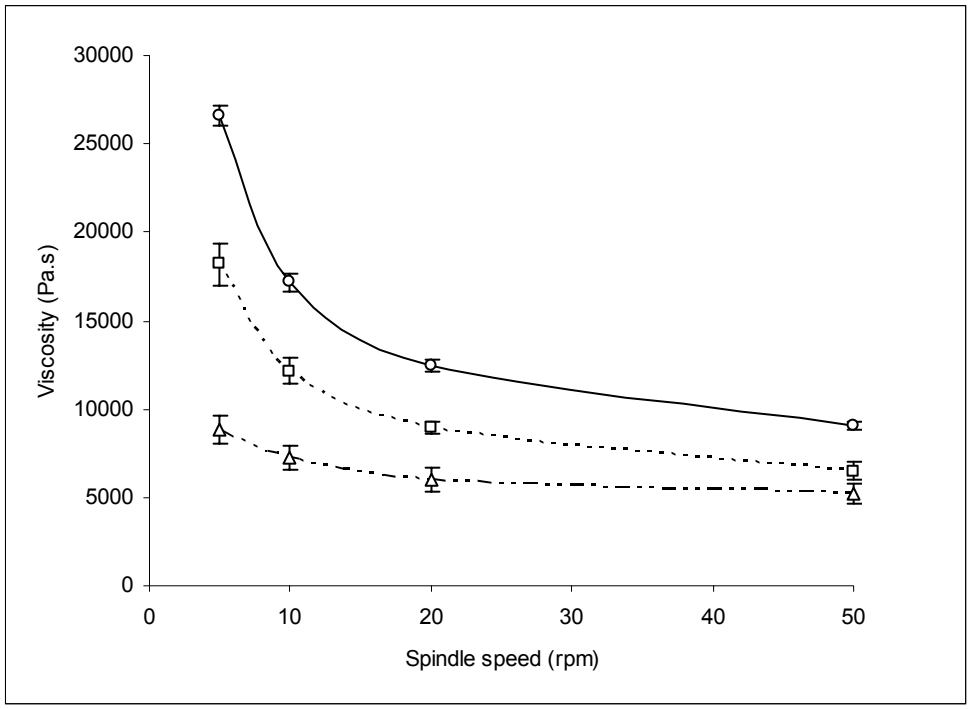

Figure 5.3 The viscosity, at various spindle speeds, of three groups of mānuka honey samples, high viscosity, (–○–), mid viscosity (–□–), and low viscosity (–Δ–). Mean value shown and error bars represent SEM.

Fifteen samples with higher viscosity samples were noted. These samples were extremely viscous at 5 rpm, with a reading greater than 23 000 Pa.s. When subjected to shear stress of 50 rpm the viscosity of this group decreased by an average of 66% from the viscosity recorded at 5 rpm. The samples had very similar physical characteristics, and four of these samples had been shown, by pollen analysis, to be principally derived from *L. scoparium*.

The remaining samples recorded a viscosity of less than 21 000 Pa.s, and the physical characteristics of the samples indicated a blend of floral sources had been incorporated into the honey. The viscosity of four samples was 15 000–20 700 Pa.s at a shear stress of 5 rpm, and in this group again the viscosity decreased markedly by an average of 65% at 50 rpm. The five samples with the lowest viscosity, less than approximately 11 000 Pa.s at shear stress 5 rpm, showed an average decrease of 41% at the final 50 rpm spindle speed. One of these samples had been shown to contain a large amount of *Trifolium* spp. pollen, indicating clover was a major nectar source.

Therefore the material supplied as monofloral mānuka high exhibited a range of behaviour. The high viscosity group was accepted to represent monofloral mānuka honey, confirmed by the high *Leptospermum scoparium* pollen counts in four of these samples and the balance of the samples shared the same physical characteristics. These mānuka honey samples had the greatest viscosity, in agreement with the reported thixotropic nature of this honey type. Equal volumes of the fifteen honeys in the high viscosity group were bulked together to be representative of mānuka honey, well mixed, and stored for use in the later experiments.

5.5.1.2 Kānuka, kāmahi, tāwari honeys

Table 5.4 provides the results of the analysis performed on 11 kānuka, 6 kāmahi, and 4 tāwari honey samples. Again the physical characteristics of these samples indicated some of the samples were not monofloral. Complete analysis was not always carried out when the sample was physically different to the named monofloral type. The samples that have had a pollen analysis completed are noted (Mildenhall & Tremain 2005).

Seven of the eleven kānuka samples had similar viscous characteristics. The samples were not shear-thinning and had a relatively low constant viscosity. One

of these samples, K6, had a pollen analysis completed (Mildenhall & Tremain 2005), categorising 87% *Kunzea*, 4% *Lotus*, and 7% *Trifolium* pollen, and the other six accepted samples had similar physical characteristics. Two of the rejected kānuka samples contained small impurities which may influence the viscosity test, and the other two rejected samples appeared to contain mānuka honey. The physical properties of this honey are very different from the mānuka monofloral honey type. Accordingly equal volumes of the seven samples were bulked together and well mixed to represent kānuka honey.

Four of the six kāmahi honeys had similar characteristics. The samples were not shear-thinning, and were approximately a quarter more viscous than kānuka honey. Three of these samples had pollen analysis completed (Mildenhall & Tremain 2005). Sample Ka3 was considered monofloral containing 79% *Weinmannia* and 11% *Metrosideros* pollen; but Samples Ka1 and Ka2 contained approximately 20% *Leptospermum scoparium* pollen, accounting for the shear-thinning and viscous nature of these honey samples. Therefore the four samples with similar properties were bulked together in equal volumes and well mixed to represent kāmahi honey.

The four tāwari honeys had similar characteristics. The samples were not shear-thinning, exhibiting approximately half the viscosity found in kānuka honey. A pollen analysis has been completed for one sample (Mildenhall & Tremain 2005). Sample T3 was considered monofloral, containing 13% *Ixerba*, 14% *Lotus* and 41% *Trifolium* pollen. The low percentage is considered acceptable as the pollen grain of *Ixerba brexioides* is large (Mildenhall & Tremain 2005). Tāwari honey does not fully granulate on storage and has a relatively high moisture content (Anon. 2004), which probably accounts for the low viscosity. Equal volumes of the four samples were bulked together to represent tāwari honey.

Table 5.4 Viscosity analysis of monofloral kānuka, kāmahi, and tāwari honeys supplied to the Honey Research Unit by New Zealand apiarists; presented in order of decreasing viscosity at 5 rpm.

Sample	Viscosity (Pa.s) Spindle speed (rpm)				Observations on physical characteristics
	5	10	20	50	
Kānuka					
K4	2136	2098	2107	2132	Yellow, clear, sweet
K1	1955	1972	1902	1883	Fairly dark yellow, not thinning
K6*	1882	1834	1846	1852	Mid-yellow, clear sweet
K9	1818	1834	1869	1849	Yellow, clear, kānuka
K8	1767	1826	1793	1821	Yellow, sweet
K5	1742	1735	1714	1708	Yellow, clear, sweet
K7	1732	1726	1705	1724	Yellow, clear, sweet
K11	27152	19259	14781		Pale-brown, creamed, aromatic, mix
K10	4650	4445	4291		Crystals present, discarded
K2	4321	4216	4210	4038	Very dark, thinning, mix
K3					Faintly waxy, discarded
Kāmahi					
Ka5	2355	2392	2363	2349	Pale brown, clear, slightly aromatic
Ka6	2398	2410	2436	2412	Pale brown, clear
Ka4	2470	2414	2385	2412	Pale brown, clear, slightly aromatic
Ka3*	2529	2555	2477	2463	Pale brown, clear, slightly aromatic
Ka2*	6971	6828	6673	6538	Dark yellow, aromatic, mix
Ka1*	3541	3698	3378	3255	Mid-brown, aromatic, mix
Tāwari					
T2	916	914	906	902	Light brown, sweet, clear
T1	1028	961	983	974	Light brown, sweet, clear
T3*	1057	1018	996	969	Light brown, sweet, clear
T4	1136	1121	1128	1109	Light-mid brown, sweet, clear

*Pollen analysis completed by Mildenhall & Tremain (2005).

5.5.1.3 Rewarewa, rātā/pōhutukawa, clover honeys

Table 5.5 provides the results of the analysis performed on 8 rewarewa, 7 rātā/pōhutukawa and 11 clover honey samples. The physical characteristics of some of the samples indicated a blended composition, and complete analysis was not always carried out when the sample was evidently not monofloral. The samples that have had a pollen analysis completed are noted (Mildenhall & Tremain 2005).

Six of the eight rewarewa samples had similar viscosity characteristics. The samples were not shear-thinning and had a low constant viscosity similar to kāmahi honey. Five samples had a pollen analysis completed (Mildenhall & Tremain 2005), and R1, R3 and R4 were categorised as monofloral rewarewa honey and contained 22%, 27% and 11% *Knightia excelsa* pollen respectively, an acceptable quantity for this floral type. Sample R4 also contained 52% *Trifolium* pollen. However Sample R2 contained 37% *K. excelsa* and 37% *Leptospermum scoparium* pollen, the presence of the latter explaining its shear-thinning viscosity. Sample R6 contained only 6% *K. excelsa* and 71% *Trifolium* pollen, allowing certification as a monofloral clover honey and indicating the dark colour of rewarewa blend honeys may be misleading. Equal volumes of the six samples with similar viscosities were bulked together and well mixed to represent rewarewa honey.

Five of the seven rātā/pōhutukawa honeys had similar characteristics. The samples were not shear-thinning, and were approximately half as viscous as rewarewa honey. Three of these samples had pollen analysis completed (Mildenhall & Tremain 2005). Samples Ra1 and Ra2 contained 94% and 48% *Metrosideros* pollen respectively, an adequate quantity to certify both honeys as monofloral rātā/pōhutukawa. Sample Ra7 contained a variety of pollens, 7% *Metrosideros*, 28% *L. scoparium*, 32% *Lotus*, 16% *Weinmannia* and *Trifolium* 7%, and exhibited the shear thinning characteristics of a mānuka honey blend. The other rejected sample appeared dilute. Equal volumes of the five samples with

similar viscosity properties were bulked together and well mixed to represent rātā/pōhutukawa honey.

Table 5.5 Viscosity analysis of monofloral rewarewa, rātā/pōhutukawa and clover honeys supplied to the Honey Research Unit by New Zealand apiarists; presented in order of decreasing viscosity at 5 rpm.

Sample	Viscosity (Pa.s) Spindle speed (rpm)				Observations on physical characteristics
	5	10	20	50	
Rewarewa					
R1*	2452	2412	2444	2438	Dark, clear, slightly aromatic
R7	2352	2329	2346	2331	Dark, clear, slightly aromatic
R3*	2218	2177	2169	2192	Dark, clear, slightly aromatic
R5	2115	2125	2110	2103	Dark, clear, slightly aromatic
R8	2094	2138	2115	2091	Dark, clear, slightly aromatic
R4*	2049	2038	2012	2027	Dark, amber, clear, slightly aromatic
R2*	5512	4836	4514	4450	Dark, clear, thinning, mix
R6*	1657	1592	1604		Paler brown, sweet, mix
Rātā/pōhutukawa					
Ra4	1224	1210	1194	1188	Light brown, sweet
Ra6	1181	1130	1133	1138	Pale brown, sweet
Ra1*	1172	1193	1215	1194	Pale cream, sweet
Ra5	1138	1146	1129	1112	Pale cream, sweet
Ra2*	1033	990	1042	1018	Pale cream, sweet
Ra7*	4280	3895	3707	3612	Light brown, aromatic, mix
Ra3	832				Very pale, dilute, dilution
Clover					
C7	1847	1852	1863	1835	Pale yellow, sweet
C5	1834	1816	1819	1803	Pale yellow, sweet
C2*	1721	1738	1720	1708	Pale yellow, sweet
C11	1609	1655	1633	1644	Pale yellow, sweet
C9*	1538	1555	1583	1520	Pale yellow, sweet
C10	1437	1426	1434	1429	Pale yellow, very sweet
C8*	1407	1483	1420	1461	Pale yellow, sweet
C3	4662	4591	4469	4452	Light brown, thinning, mix
C4	3896	3796	3701	3642	Light brown, thinning, mix
C1	3456	3321	3210	3104	Light brown, thinning, mix
C6	880	843			Very pale yellow, diluted

*Pollen analysis completed by Mildenhall & Tremain (2005).

The viscosity of seven of the eleven clover honeys was similar. The samples were not shear-thinning, exhibiting a viscosity that was approximately in the centre of the range of the other five Newtonian monofloral honeys studied. This honey type is common throughout New Zealand and a ubiquitous contaminant of other floral types to a greater or lesser degree. Three samples had a pollen analysis completed (Mildenhall & Tremain 2005). Samples C2, C8 and C9 held 76.5%, 84% and 89% *Trifolium* pollen respectively. Equal volumes of the seven samples with similar viscosity were bulked together and well mixed to represent clover honey.

5.5.1.4 Moisture content of standard honeys

Whilst some countries have set maximum water content standards for honeys, and therefore New Zealand honey exporters are required to meet these regulations, detailed industry guidelines have not been prepared for moisture content in New Zealand honeys prepared for the domestic market. However a number of generalised observations can be reported.

Whilst the moisture content of honey types differs, the moisture content of samples of a honey type also differs. Therefore the range of moisture contents of a honey type will not be clearly separate from another honey type.

These ranges are principally determined by three factors, the region of harvest, climatic conditions, and hive management and honey extraction procedures of the apiarists.

Because of these difficulties the moisture content is used as a general guideline, and whilst 21% moisture is set by the industry as the maximum water content,

many honey processors prefer to adopt 20% as the upper moisture limit to minimise the possibility of fermentation (V. O'Kane, pers. comm.).

The moisture content of the honey samples used for the standard samples is described in Table 5.6. For the most part the ranges are similar. Mānuka honeys are expected to have greater moisture content than 15.9% (V. O'Kane, pers. comm.). Tāwari monofloral honey contains a relatively higher percentage of moisture than the other honey types. None of the samples contained moisture content beyond acceptable limits, and therefore inclusion in the composite monofloral sample was warranted.

Table 5.6 Moisture content of honey samples included in the composite monofloral honey types.

Monofloral honey type	% moisture range	Mean % moisture
Mānuka	16.4 – 17.4	16.98
Kānuka	16.4 – 17.0	16.57
Kāmahi	17.0 – 17.2	17.10
Tāwari	20.0 – 21.0	20.63
Rewarewa	16.4 – 16.8	16.68
Rātā/pōhutukawa	16.3 – 17.0	16.64
Clover	16.4 – 17.2	16.84

5.5.2 Viscosity of monofloral honey standards

The composite samples of monofloral honey types prepared in the above section were subjected to a number of viscosity tests in duplicate.

5.5.2.1 Characteristics with variable shear rate

Analysis at a constant temperature utilising a range of shear rates from 5 to 50 rpm illustrated monofloral mānuka honey was non-Newtonian and shear-thinning (Figure 5.4). The very high viscosity of approximately 25 000 Pa.s recorded at the lowest shear rate of 5 rpm decreased to 8500 Pa.s at the highest shear rate, 50 rpm.

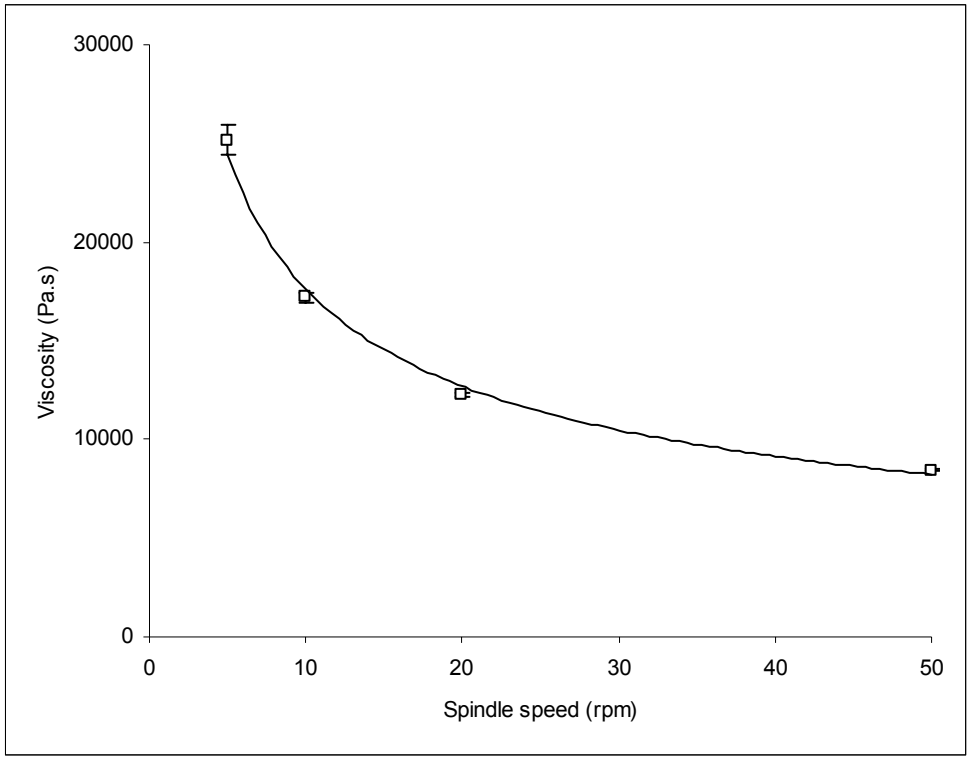

Figure 5.4 Time-independent viscosity of monofloral mānuka (–□–) honey subject to various shear rates. Mean values from duplicate measurements are shown, error bars represent SEM.

The analysis at a constant temperature utilising a range of shear rates from 5 to 50 rpm revealed the other six honey types behaved in a Newtonian manner (Figure 5.5).

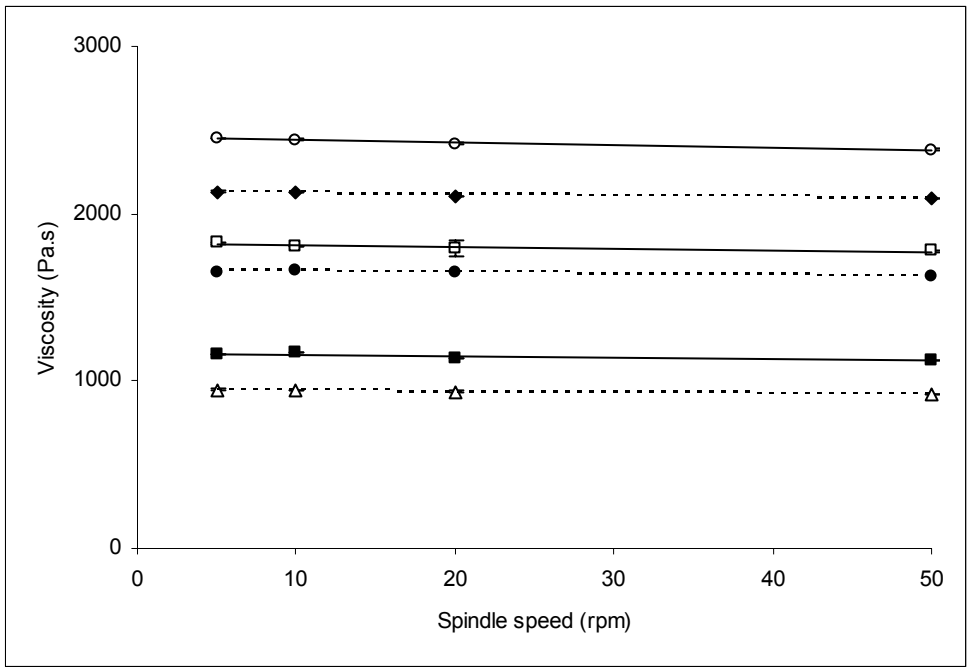

Figure 5.5 Time-independent viscosity of monofloral kāmahi (− ○−), rewarewa (− ▲−), kānuka (–□–), clover (−●−), rātā/pōhutukawa (− ■−) and tāwari (−Δ−) varieties of honey subject to various shear rates. Mean values from duplicate measurements are shown, error bars represent SEM.

The viscosity remained very constant despite the increase in shear rate, typical of most previous analyses of other honey types (White 1975b; Junzheng & Changying 1998; Mossel et al. 2003). The mean viscosity for each honey type revealed a range from approximately 950 Pa.s (tāwari) to 2400 Pa.s (kāmahi). The mean viscosities of these honey types did not linearly correlate with the total soluble solid measurements (r^2 0.33, data not shown), indicating the narrow range of moisture contents did not significantly alter viscosity.

Thus other varying factors, for example the ratio of soluble sugar types and colloid or protein content, must have been responsible for the range of viscosity in the honeys. There is also a possibility of the inclusion of small amounts of

mānuka honey in these samples that may explain a degree of the different reported viscosities. Future work should utilise samples where *Leptospermum scoparium* is not present, therefore eliminating the possibility of contamination with mānuka honey.

Mānuka honey exhibited a different set of properties from the other six monofloral honeys examined. Mānuka honey was shear-thinning, with a very high viscosity compared to the other Newtonian honeys. The results the analysis at 5 rpm revealed an order of magnitude differentiates mānuka honey, and when tested at 50 rpm mānuka honey remained more than three times as viscous as the other honey types.

5.5.2.2 Characteristics of thixotropy

To further investigate the viscosity of the monofloral honeys a time-dependent analysis at a constant temperature and 50 rpm shear-rate over a period of two minutes was conducted. It was found that the viscosity of the six honey types that had remained practically unchanged by a variable shear rate was also unaltered by continual exposure to a constant shear rate (Figure 5.6). From these results and those of the first experiment it was concluded that these six honeys are time-independent Newtonian fluids.

In contrast, the same test confirmed the reported thixotropic behaviour of mānuka honey (Pryce-Jones 1953). Viscosity reduced by approximately 20% in 2 min, a reduction from approximately 8500 to 6900 Pa.s in the test period (Figure 5.7).

A thixotropic fluid should re-set following disturbance, illustrating the gel-sol-gel transformation outlined by White (1975b). The mānuka honey samples, left to re-set for a period of 48 h at 25 °C following shear disturbance from an initial test, exhibited almost the same behaviour in viscosity as in the first measurement (Figure 5.7).

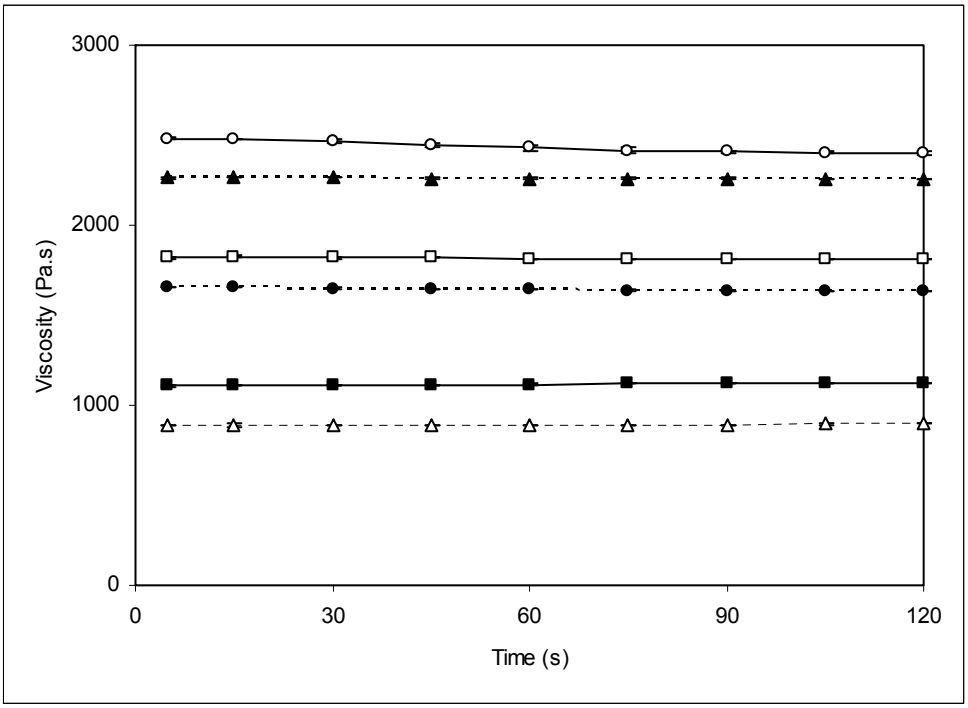

Figure 5.6 Time-dependent viscosity of monofloral kāmahi (− ○−), rewarewa (− ▲−), kānuka (–□–), clover (−●–), rātā/pōhutukawa (− ■−) and tāwari (–Δ–)varieties of honey subject to a constant shear rate of 50 rpm. Mean values from duplicate measurements are shown, error bars represent SEM.

Following the removal of proteins and colloids by precipitation, the reconstituted mānuka honey behaved in a time-independent Newtonian manner (Figure 5.7). The mean recorded viscosity (1500 Pa.s) was similar to that of the other Newtonian honey types (Figure 5.6) and was approximately five times less than the mean viscosity of intact mānuka honey in this experiment. Despite the difficulty in replicating the original moisture content of the manuka honey, in all probability the thixotropic nature of mānuka honey can be ascribed to the protein or colloid fraction, in accordance with the opinion of White (1975b).

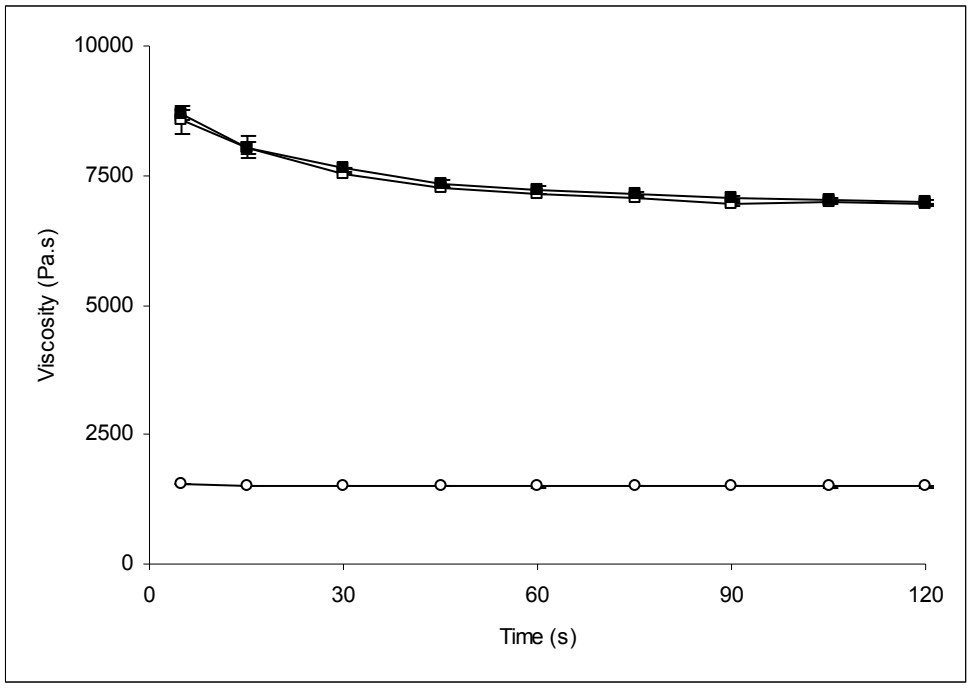

Figure 5.7 – Time-dependent viscosity of monofloral mānuka honey (–□–), mānuka honey retested after resetting for 48 hours (–■–), and mānuka honey following precipitation of proteins and colloids (–○–), subject to a constant shear rate of 50 rpm. Mean values from duplicate measurements are shown, error bars represent SEM.

5.6 Mānuka honey mixed with other types of honey

The greatest difference between mānuka and the other honeys was seen during the time-independent variable shear rate analysis at 5 rpm. Therefore these conditions were used to investigate the viscosity of mānuka honey mixed with the other honeys. Samples were prepared in duplicate by mixing 25%, 50%, and 75% of the composite standard mānuka honey with each of the other composite monofloral honey types, and then stored for 48 hr to allow the honey to re-gel.

Monofloral mānuka honey mixed with the other honey types exhibited an exponential increase of viscosity with an increasing percentage of mānuka honey in the mixture (Figure 5.8).

The results were similar for mānuka honey mixed with each of the other six honey types. The variation reflects the six Newtonian honey types exhibiting slightly different behaviour as monofloral honeys (Figure 5.5)

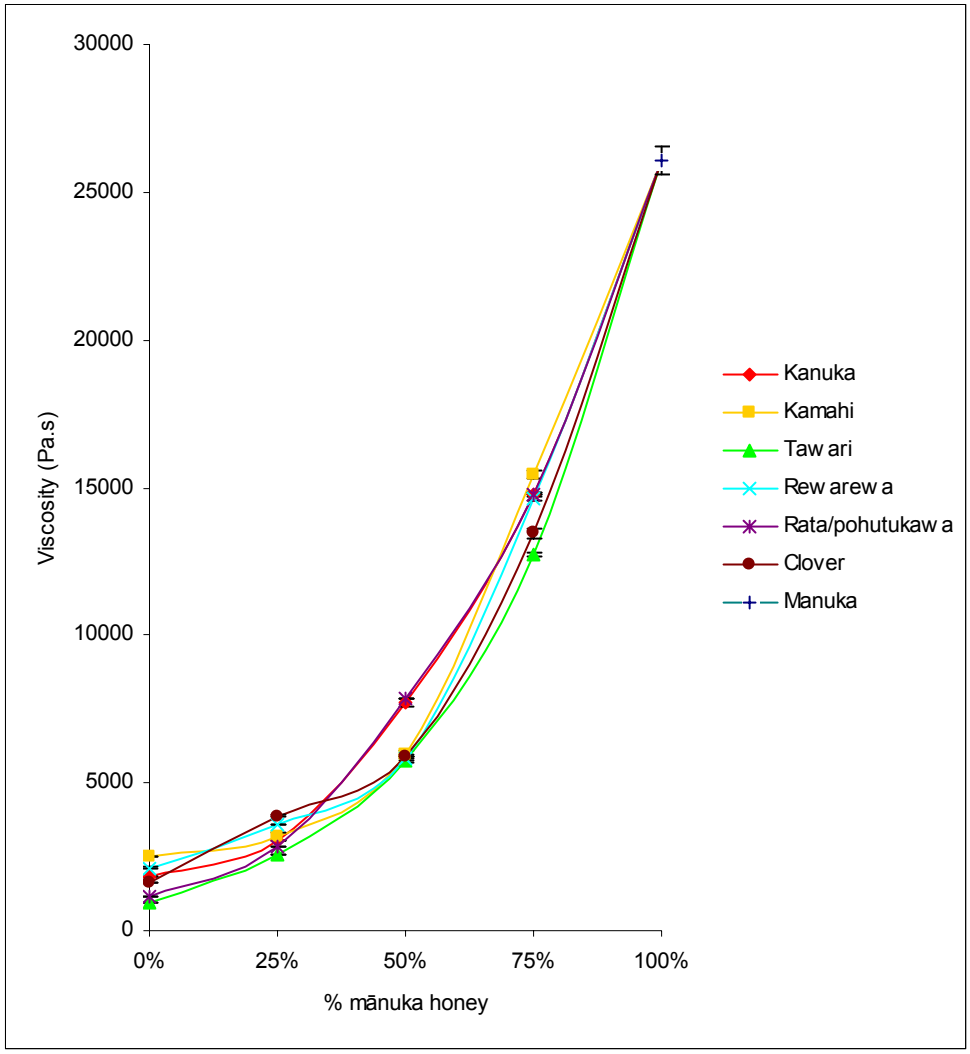

Figure 5.8 Time-independent viscosity of monofloral mānuka honey mixed in different proportions (25%, 50%, 75%) with six other Newtonian monofloral varieties of honey. Viscosity was measured at a constant shear rate of 5 rpm. Mean values from duplicate measurements are shown, error bars represent SEM.

The intention of the method development was to use viscosity as an indicator of the proportion of mānuka honey in an unknown honey sample.

Therefore the overall mean viscosity values for each mānuka honey concentration were re-plotted (Figure 5.9). The relationship of viscosity to the proportion of manuka honey present is logarithmic, with a significant correlation coefficient of (r^2 0.9982).

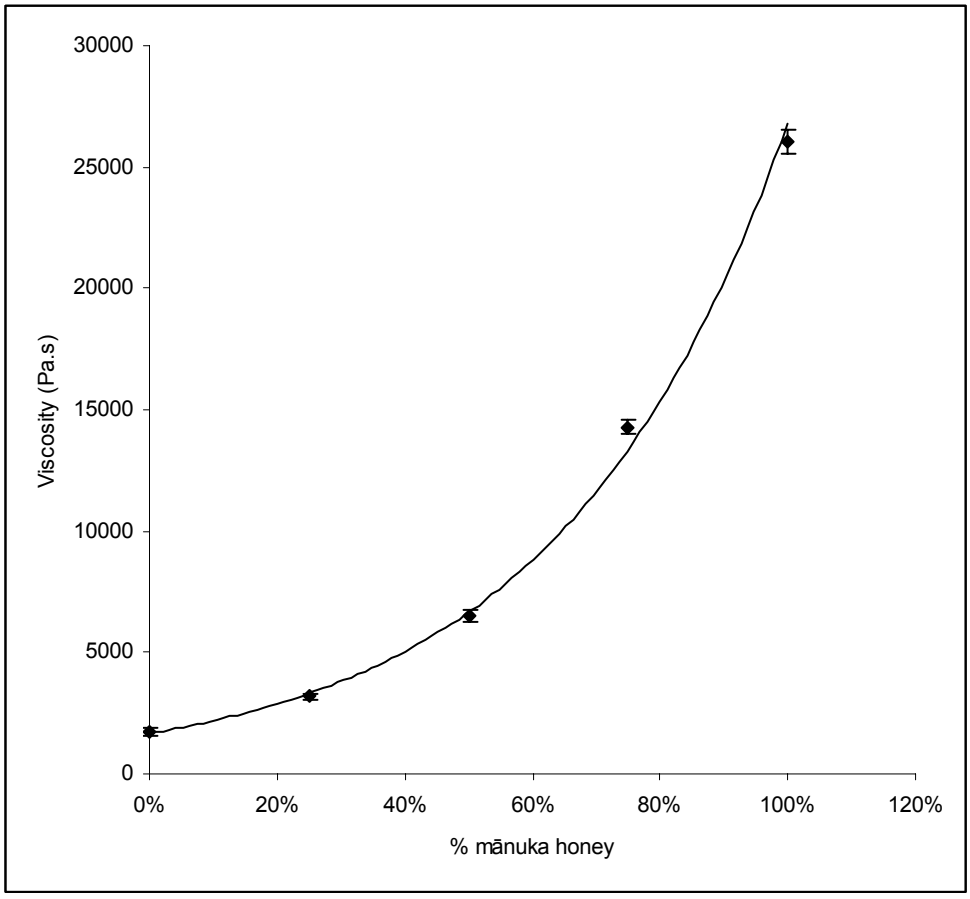

Figure 5.9 Time-independent viscosity of monofloral mānuka honey mixed in different proportions (25%, 50%, 75%) with six Newtonian honey types. Viscosity was measured at a constant shear rate of 5 rpm. Mean values are shown, error bars represent SEM.

A logarithmic transformation of the viscosity data allowed a linear standard curve to be fitted (Figure 5.10), and a reversal of the axes provides a prediction of mānuka honey content in a sample. It is apparent from the 95% confidence intervals shown in Figure 5.10 that the method becomes less sensitive as the percentage of mānuka honey decreases. Therefore the five data points studied are further examined in Table 5.7.

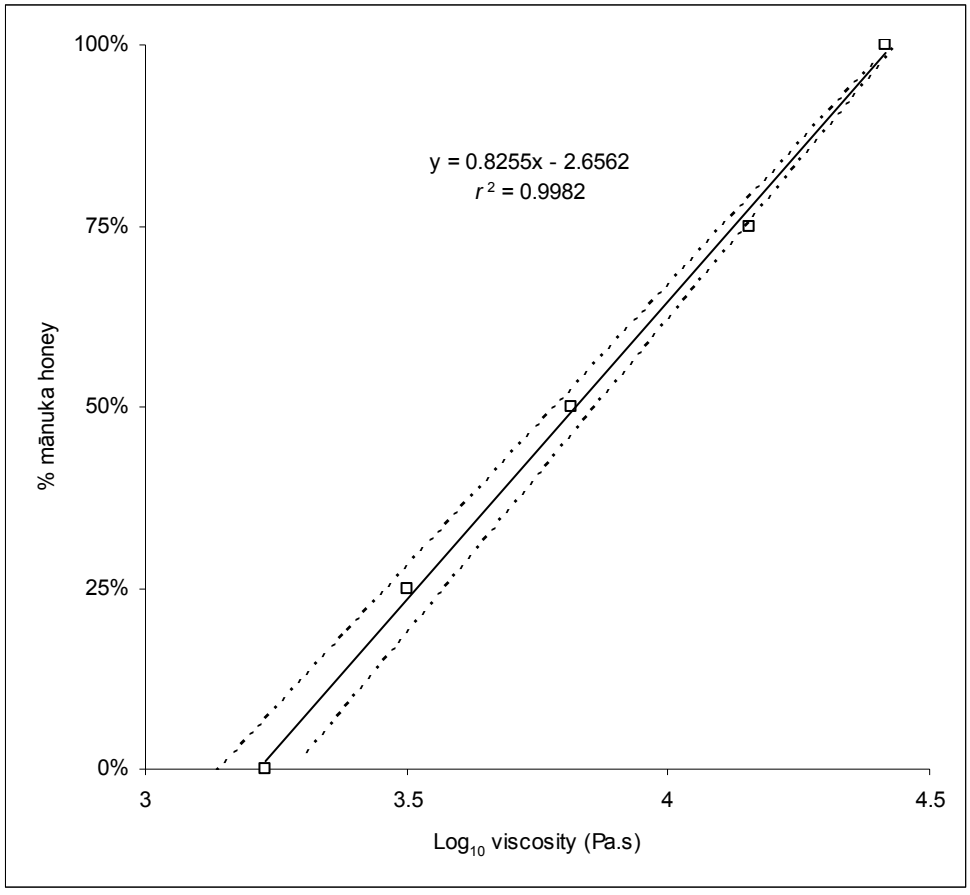

Figure 5.10 Logarithmic transformation of time-independent viscosity of monofloral mānuka honey (–□–) mixed in different proportions (25%, 50%, 75%) with six Newtonian honey types, subject to a constant shear rate of 5 rpm. Mean values, linear trendline, and 95% confidence intervals are shown.

Table 5.7 Time-independent viscosity of various mixes of monofloral mānuka honey measured at a costant shear-rate of 5 rpm, showing mean, SEM, 95% confidence interval, 95% confidence interval as percentage of mean, and range; and percentage mānuka honey in a honey predicted from the standard curve, showing mean, 95% confidence interval, and range.

		Viscosity (Pa.s) at 5 rpm					Predicted % mānuka honey in honey derived from standard curve		
% mānuka honey	*n*	mean	SEM	95% CI	95% CI as % mean	range	mean	95% CI	range
100%[1]	5	26049	481	942	4%	24388–27242	99%	98–101%	97–104%
75%[2]	12	14293	290	568	4%	12709–15422	77%	76–79%	73–80%
50%[2]	12	6512	287	563	9%	5758–7867	49%	46–52%	45–56%
25%[2]	12	3174	142	278	9%	2557–3893	23%	20–26%	16–31%
0%[3]	12	1702	330	330	19%	949–2503	1%	-1–7%	-20–15%

[1]Data from Section 5.5.2.1 and 5.6.
[2]Data from Section 5.6.
[3]Data from Section 5.5.2.1.

The mean 100% mānuka honey viscosity reading is 26 049 Pa.s with a range of 24 400–27 250 Pa.s. The standard curve equates this to a mānuka honey content of 99% and a range of 97–104%. The exponential nature of the curve makes these differences inconsequential. The standard curve calculates for 95–110% mānuka content the viscosity increases from 23 000–35 000 Pa.s.

The viscosity of the monofloral mānuka honey samples described in Table 5.3 ranged between 23 000–30 000 Pa.s. An argument could be made for the 100% mānuka honey viscosity standard to be set at 30 000 Pa.s, approximating the highest recorded figure. However differences in moisture and solid content in the honeys, coupled with the apiarist's differing honey extraction techniques makes this unrealistic. All monofloral mānuka honey will carry some degree of contamination from other floral sources, and the current industry standard, a minimum of 70% of pollen in the honey derived from *Leptospermum scoparium*, leaves the upper range of mānuka honey inadequately defined. Therefore the mean figure of 26 049 Pa.s is representative; and whilst some manuka honey may have a greater viscosity this will probably be due to the factors mentioned above.

The mean 75% mānuka honey viscosity reading is 14 293 Pa.s with a range of 12 700–15 400 Pa.s. The standard curve equates this figure to a mānuka honey content of 77% and a range of 73–80%. The data does not precisely fit the standard curve, but as the exponential gradient remains very sheer in this section of the curve the predictions remain sensitive. The extent of the range is brought about by the mixes of mānuka honey with the other honey types differing in viscosity behaviour. As all honey is a mix of floral sources, the probability of the range extremes being encountered is minimal.

The mean 50% mānuka honey viscosity reading is 6512 Pa.s with a range of 5750–7850 Pa.s. The standard curve equates this to a mānuka honey content of 49% and a range of 45–56%. Again the exponential gradient remains fairly sheer in this section of the curve, the predictions are reasonably sensitive, and the range

is due to the mixes of mānuka honey differing in viscosity behaviour. Again the probability of encountering the range extremes is slight.

The mean 25% mānuka honey viscosity reading is 3174 Pa.s with a range of 2600–3900 Pa.s. The standard curve equates this to a mānuka honey content of 23% and a range of 16–31%. The exponential gradient of the curve has markedly decreased indicating a loss of sensitivity. Once viscosity has fallen below 4000 Pa.s, the honey contains less than approximately 30% mānuka honey. Therefore this honey would be better described as another type. Unfortunately the premium value of mānuka honey encourages these blends to be marketed as mānuka honey, and the strong flavour and odour of mānuka honey masks the other constituents. Consequently 4000 Pa.s would appear to be useful as both the lower value at which the model can be used with confidence and a honey that should be regarded as approximately one-third mānuka.

The mean 0% mānuka honey viscosity reading is 1702 Pa.s with a range of 950–2500 Pa.s. The standard curve equates this to a mānuka honey content of 1% and a range of -20–15%. These figures represent the mean and range of the six Newtonian monofloral honeys. The curve is not sensitive as the range is comparatively large when considered in conjunction with the mean. However this reading is unimportant. This method does not differentiate between the Newtonian honeys, or a honey that has a low content of mānuka honey and behaves virtually as a Newtonian honey. This is in contrast to a high quality mānuka honey, where the high viscosity is logarithmically proportional to the percentage of the thixotropic protein or colloid.

5.7 Conclusion from viscosity measurements

The unique thixotropic nature of mānuka honey may be used to determine the percentage of mānuka honey in an unknown honey sample. The exponential nature of this decrease in viscosity when mānuka honey is diluted with the other

honey types makes this method of measurement particularly sensitive in the region of commercial interest, honey that contains more than 50% mānuka honey. The industry's current melissopalynological method would appear to confirm whether *Leptospermum scoparium* was the major nectar source, however does not determine the mānuka content of a honey. The viscosity method would allow the industry, subject to the creation of an appropriate database, to set the minimum proportion of mānuka in a marketed mānuka honey.

5.8 Dilution of UMF® in mānuka honey by other honey types

The establishment of a method for determining the amount of mānuka honey in a honey allowed the testing of the hypothesis that the mixing of floral sources during honeybee production of honey influences the UMF® activity recorded in a mānuka honey. An experiment was conducted to test whether the level of UMF® in samples of mānuka honey correlates with the percentage of mānuka honey in the samples. Four mānuka honeys were selected from the samples described in Section 5.5.1.1. The agar diffusion method described in Chapter 3 was used to measure the level of UMF® in these samples; unmixed; and mixed at 75%, 50%, and 25% of the original mānuka honey with the blend of the monofloral clover honey created in Section 5.5.1.3.

Three of the mānuka samples, M3, M15 and M18 were monofloral mānuka honeys, and the fourth sample, M27, was practically monofloral with 98% mānuka content, containing a small fraction of other nectar types. The purity of the samples was determined by the viscosity of each sample recorded in Section 5.5.1.1 and the method described in Section 5.6. These samples represented a range of UMF® activities reported from high quality mānuka honeys.

Table 5.8 presents the results of this experiment. All the undiluted mānuka honey samples contained measurable UMF®.

Table 5.8 Relationship of UMF® and concentration of mānuka honey. The level of UMF® was measured for samples of mānuka honey diluted to different degrees with a clover honey

		100% mānuka honey		75% mānuka honey		50% mānuka honey		25% mānuka honey	
Sample	Viscosity (Pa.s)	Predicted % mānuka	UMF®	Calculated % mānuka	UMF®	Calculated % mānuka	UMF®	Calculated % mānuka	UMF®
M3	26735*	100%	16.7	75%	12.7	50%	8.4	25%	ND†
M15	28643*	100%	15.6	75%	11.8	50%	8.1	25%	ND†
M18	28084*	100%	13.2	75%	9.9	50%	ND†	25%	ND†
M27	25311*	98%	16.4	74%	12.3	49%	8.1	24%	ND†
Clover††	1651**	0%	ND†						

*Data from Section 5.5.1.1.
**Data from Section 5.5.2.1.
†UMF® non-detectable.

The subsequent dilutions with the clover honey allowed the calculation of the mānuka content in the honey samples. The clover honey used to dilute the mānuka honeys did not exhibit UMF® activity. The increasing proportion of the clover honey in the original mānuka honey brought about a linear decrease in recorded UMF® (Figure 5.11). Therefore the UMF® in mānuka honey is dependent upon the proportion of mānuka nectar that has been incorporated into the honey.

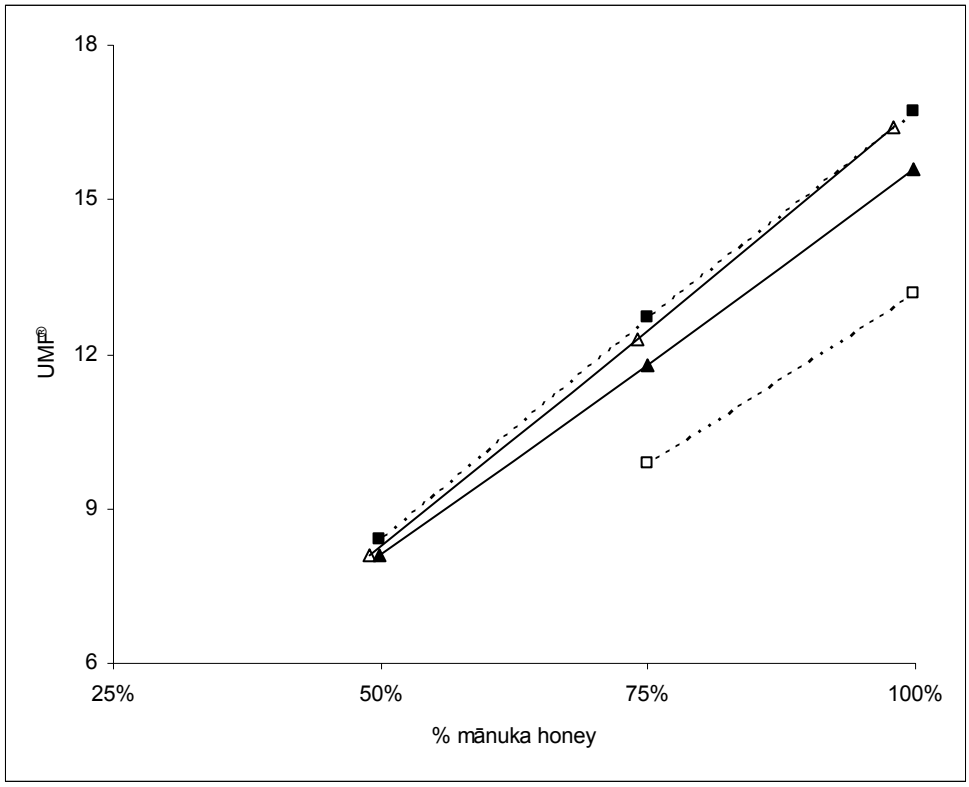

Figure 5.11 UMF® in four mānuka honeys; M3 (−■−), M15 (−▲−), M18 (−□−), and M27 (−△−); mixed in different proportions (25%, 50%, 75%) with a non-detectable UMF® clover honey.

The UMF® contained in each monofloral mānuka honey differed, however viewing the UMF® levels in the blends as a percentage of UMF® in the undiluted mānuka honey revealed a directly proportional linear relationship (Figure 5.12) with a significant correlation coefficient (r^2 0.9988).

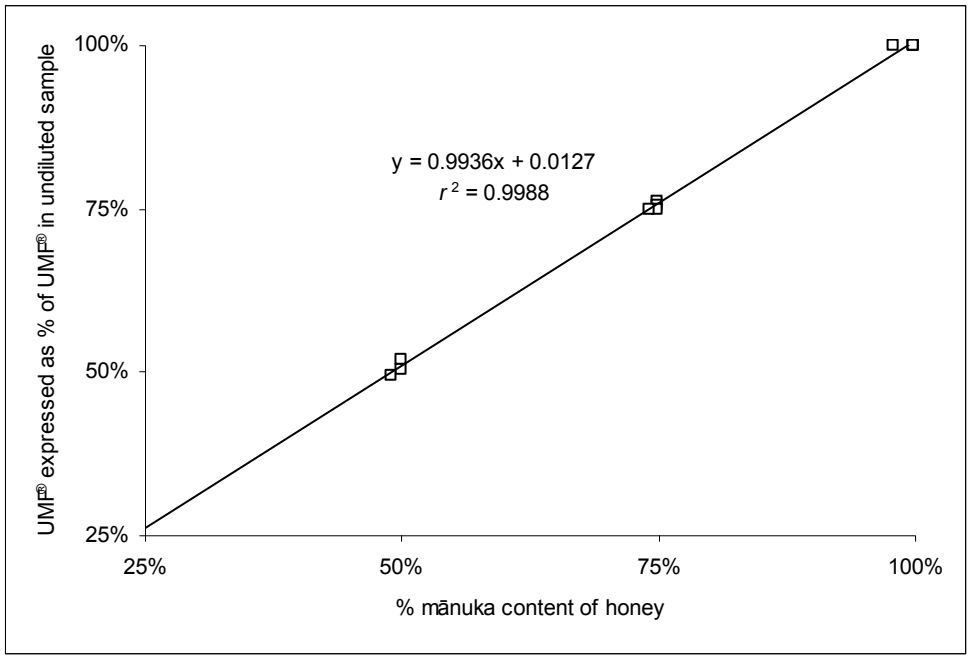

Figure 5.12 Relationship of mānuka honey content and UMF® , UMF® expressed as a percentage of UMF® in undiluted mānuka honey. Linear trendline shown.

5.9 Conclusion

The mānuka content of a honey significantly affects the UMF® recorded in that honey. The thixotropic nature of mānuka honey allows the proportion of mānuka honey in a honey to be determined. The viscosity of mānuka honey reduces logarithmically as the mānuka content of that mānuka honey is diluted with other honey types. The UMF® level in mānuka honey reduces directly proportionally with dilution by other honey types.

Provided the mānuka content and UMF® of a mānuka honey are known, a prediction of the UMF® in that honey, had it been monofloral mānuka honey, can be made. In the next chapter these findings are applied to the field data described in Chapter Three.

Chapter 6

UMF® field results adjusted for purity of mānuka honey samples

This chapter describes the adjustments made to the field results presented in Chapter Three, using the methods developed in Chapter Five to adjust the non-peroxide antibacterial activity (UMF®) recorded in each area of a region to account for the amount of mānuka honey in the samples. An estimate was made of the UMF® activity in the mānuka honey collected in each area if the mānuka honey had not been diluted with nectar from other floral sources.

The effect on the results is described, and comparisons are made between the unaltered and adjusted data. The presence and distribution of three distinct levels of UMF® in New Zealand is described. The geographical regions yielding a high level of UMF® are restricted to four districts. A medium level of UMF® activity is mostly found in mānuka honey harvested from areas contiguous to the high activity districts, and the low activity regions cover the balance of the country. The field data is re-categorised on this basis and presented for the consideration of the remaining three hypotheses outlined in Chapter One.

The material in this chapter has not been published as the thesis is subject to an embargo agreed with the commercial sponsors of this study.

6.1 Method and materials

The samples collected from the beekeepers and described in Chapter Three were used for this analysis. The method used to adjust the results to estimate the true level of UMF® is described in Chapter Five. The standard curve was created using a 35 ml container, and as the immersion depth of the spindle and all other conditions need to be constant for each viscosity test, the decision to bulk together similar field honeys within each geographic area to produce a representative 35 ml sample was considered necessary.

Therefore the honey samples from each area were combined in equal volumes and well mixed to produce a representative sample for viscosity measurement. Granulated sugars were not removed during this process, as the sample heating described in described in Chapter Five re-solubilised these components.

Each area represented samples from a similar environment, recorded similar UMF® ratings, and had similar characteristics of colour, aroma, and flavour, and therefore most probably contained a similar quantity of mānuka honey.

As the samples were disturbed and mixed, each representative area sample was left to re-gel for at least 48 h at 25 °C. This period was shown to be adequate to allow a disturbed thixotropic mānuka honey to re-gel (Section 5.5.2.2) and therefore provide an accurate measure of the proportion of mānuka honey in the sample. The replication of the viscosity measurement was obtained by allowing the samples to re-gel for 48 h at 25 °C following the initial test, and retesting each sample to check that the first measurement was accurate.

A separate test was performed to confirm the UMF® activity in the composite samples represented the mean UMF® area values. After the viscosity measurement had been completed, eleven composite area samples were subjected to the agar diffusion assay to establish the level of UMF®. The UMF® recorded in these composite area samples was equivalent to the mean UMF® value for that

area (data not shown), confirming the mānuka content and UMF® of the samples in an area were very similar, and the composite samples were representative.

All the composite samples also had total soluble solids established (see method Section 5.4.3) following the viscosity test. The moisture content of the samples varied between 15 and 18.5%, an acceptable range for mānuka honey. The moisture content of the composite area samples did not correlate with either the viscosity (r^2 = 0.003) or the level of UMF® adjusted for dilution of the manuka honey by other honey types (r^2 = 0.004). This indicates that neither the viscosity nor the UMF® activity of mānuka honey is reliant upon the total soluble sugar content.

6.2 Adjusted field study results

The regions and areas described in Section 3.7 are presented again here, this time with the UMF® activity adjusted to account for the purity of the mānuka honey from each area. The equations used for these adjustments are provided below.

Equation One – Predicted % mānuka honey in a honey

$$\%\ mānuka\ honey = 0.8255 \times \{\log_{10} viscosity\ (Pa.s)\} - 2.6562$$

Equation Two – Predicted UMF® activity of the pure mānuka honey given that mānuka content (% mānuka honey) and UMF® activity are known for the sample.

$$UMF^{®}\ activity\ of\ pure\ mānuka\ honey = \frac{UMF^{®}\ activity\ of\ sample}{\%\ manuka\ content\ in\ sample}$$

6.2.1 Northland region

As can be seen in Table 6.1, the majority of the Northland areas yielded pure mānuka honey. The honey from Areas 4, 6, 7 and 10 was less pure mānuka honey, and adjustment of the UMF® activity recorded in these areas for the dilution with other honey types increased the UMF® activity to approximately the same range exhibited by the pure mānuka honey in this region. The adjusted UMF® yield from these areas was not significantly different ($p < 0.70$), in comparison to the significant difference ($p < 0.001$) between the areas prior to adjustment.

Table 6.1 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, for areas from the Northland region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
1	14.2	1.2	26553	100	14.2
2	15.3	0.2	26793	100	15.3
3	15.5	1.6	26856	100	15.5
4	13.0	0.4	20836	91	14.3
5	14.7	1.5	26328	100	14.7
6	11.9	0.8	16204	82	14.6
7	13.3	0.2	18512	87	15.4
8	14.4	0.8	26965	100	14.4
9	14.6	0.3	27533	100	14.6
10	12.5	0.2	17657	85	14.7

*From Table 3.2.
**Viscosity of composite of the samples from each area.

Figure 6.1 illustrates the Northland region, and shows the adjusted UMF® activity \where dilution with other honey types had occurred. The UMF® activity of the eight central areas ranges between 14.2–15.5 UMF® points, and the north and south areas 14.6–14.7 UMF®.

Therefore the conclusion can be drawn that the Northland region produces mānuka honey with high UMF® activity, and the differences between areas

recorded in the unadjusted field results are due to dilution of the harvested mānuka honey by other honey types.

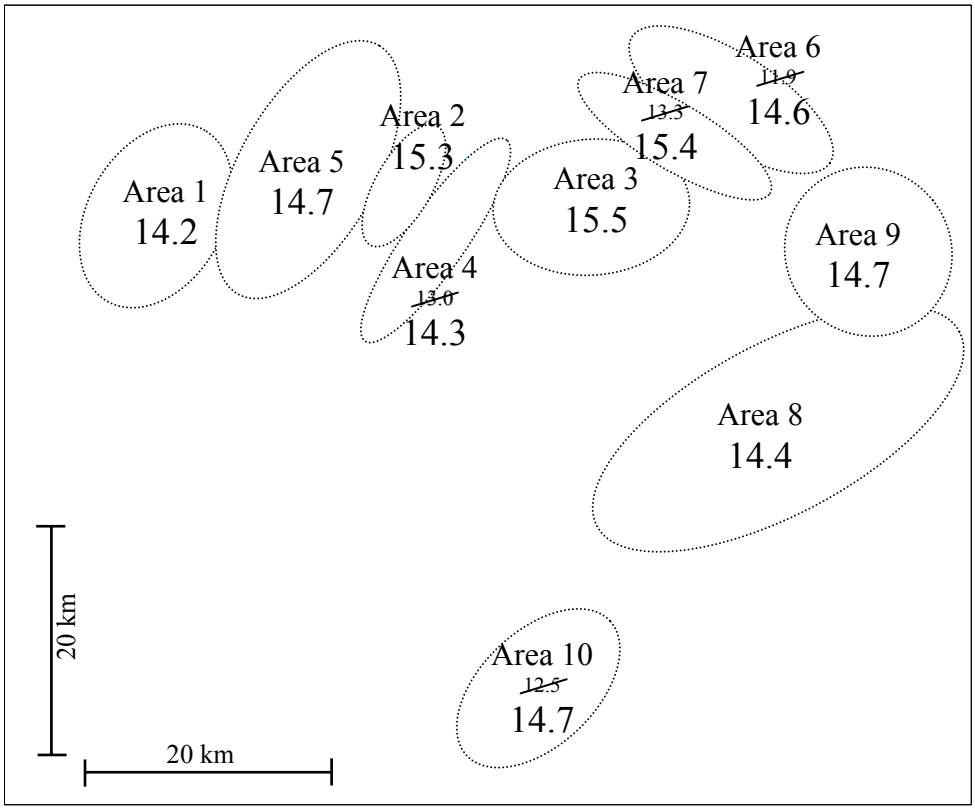

Figure 6.1 The distribution of ten areas producing UMF® mānuka honey in the Northland region, showing the UMF® activity in each area, adjusted where necessary to account for dilution of mānuka honey by other honey types.

6.2.2 Waikato region

Analysis of the two areas of the Waikato region (Table 6.2) revealed a similar situation to that found in the Northland region.

Table 6.2 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded, for areas from the Waikato region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
1	15.6	0.4	27160	100	15.6
2	13.4	0.6	20523	90	14.8

*From Table 3.3.
**Viscosity of composite of the samples from each area.

The area within the central wetlands was a pure mānuka honey and the recorded UMF® activity was unchanged. However the peripheral sites about the central wetland contained a substantial dilution with other honey types, and adjustment increased the recorded UMF® activity. The UMF® activity of these areas was no longer significantly different ($p< 0.1$), in comparison to the unadjusted field results that were significantly different ($p< 0.005$). This region is not illustrated as only the bordering area was altered. Therefore the Waikato wetlands also produces mānuka honey with high UMF® activity, and the difference recorded in the unadjusted field results was a consequence of the dilution of the mānuka honey by other honey types.

6.2.3 Coromandel region

The results for the Coromandel region (Table 6.3 and Figure 6.2) are varied in comparison with the former two regions. The dilution of the mānuka honey by other honey types considerably alters the UMF® activity and explains some of the variability found in the original field study, the region may be divided into three distinct groups of activity levels, and one group without detectable UMF® activity.

Considering the adjusted values, five areas yielded higher UMF® activity than the rest of the region. The activity in Areas 4, 6, 7, 8 and 13 was in the range of 14.4–15.6 UMF® units, and were not significantly different ($p< 0.26$). The five areas contained in this group were significantly different prior to the adjustments

($p< 0.001$). These areas are geographically allied, forming a wedge through the peninsula and south along the east coast of the peninsula.

Associated with the above five high UMF® areas are two areas that yielded 12.5–12.8 UMF® units. Areas 2 and 5 were not significantly different ($p< 0.64$); yet before adjustment these areas were significantly different ($p< 0.001$). Areas 2 and 5 border the geographical wedge of high UMF® activity honeys, to the north and south respectively.

The third group is widespread, and includes Areas 1 and 3 in the far north of the peninsula, Area 9 on the west coast, and Areas 11, 12, 14, 15 and 17 inland and south of the high and mid activity areas described above. The adjusted UMF® activity of these areas ranged between 10.1–10.7 units, and were not significantly different ($p< 0.96$), and cover approximately two-thirds of the peninsula from which mānuka honey is harvested. The unadjusted UMF® data in these areas ranged between 5.3–10.2 points, and before adjustment the eight low activity areas were significantly different ($p< 0.001$). The viscosity measurements show dilution by other honey types was responsible for much of this variation.

The fourth group contained two areas that yielded honey that did not contain detectable UMF®. As there was no recorded activity to adjust for the dilution of the mānuka honey by other honey types an adjustment to UMF® activity could not be made. The viscosity analysis revealed these honeys contained less than 30% mānuka honey. These areas contain unbroken regenerating forest with few environments suitable for *Leptospermum scoparium* colonisation. The strong flavour and odour of mānuka honey would explain the optimistic labelling of these samples as mānuka honey by the apiarists.

Analysis of the data arranged as three discreet groups containing high, mid, and low UMF® revealed a highly significant difference ($p< 0.001$) between these groups.

Therefore whilst the purity of the mānuka honey harvested in the Coromandel region does explain a great deal of the variation in the data initially seen, it does not explain the clearly different geographically based groups reported. A high UMF® group is present, with a range distinct from the low UMF® group. An intermediate group is present as a buffer region between these extremes.

Table 6.3 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the Coromandel region.

	Original field data*		% mānuka content**		Adjusted data
Area	UMF®	SD	Pa.s	%	UMF®
4	14.8	1.5	26527	100	14.8
6	11.1	0.5	13860	76	14.6
7	13.8	0.1	22421	94	14.9
8	15.6	0.8	26609	100	15.6
13	13.8	0.4	23617	95	14.4
2	11.2	0.6	20367	90	12.5
5	8.9	0.6	11495	70	12.8
1	6.9	0.9	11303	69	10.2
3	10.2	0.6	23292	95	10.7
9	7.7	0.6	12113	71	10.7
11	6.2	1.5	8397	58	10.5
12	5.8	0.5	7564	55	10.6
14	5.3	0.3	6951	52	10.4
15	7.7	0.7	13632	76	10.1
17	6.8	0.9	10059	65	10.5
10	ND		3247	24	
16	ND		3650	28	

*From Table 3.4.
**Viscosity of composite of the samples from each area.

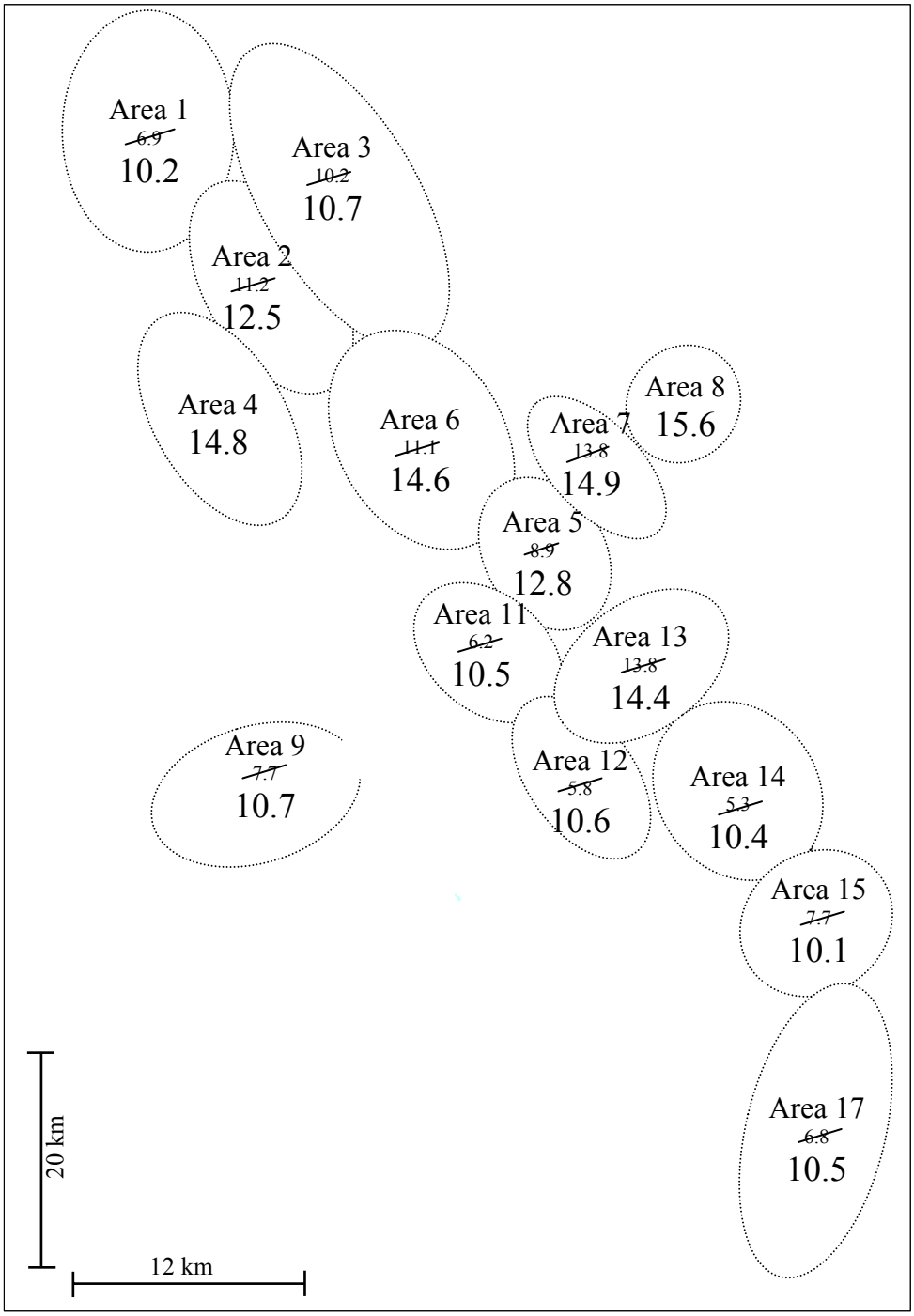

Figure 6.2 The distribution of fifteen areas producing UMF® mānuka honey in the Coromandel region, showing the UMF® activity in each area, adjusted where necessary to account for dilution of mānuka honey by other honey types.

6.2.4 Taranaki Region

Adjustment of the data for the Taranaki region for dilution by other honey types revealed two distinct groups with different UMF® activities (Table 6.4 and Figure 6.3).

Table 6.4 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the Taranaki region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
1	10.1	0.8	24233	96	10.5
2	9.9	1.1	22072	93	10.6
3	10.1	1.0	23665	95	10.6
6	10.9	1.5	25820	99	11.0
4	4.6	0.4	6949	52	8.9
5	7.1	1.5	15086	79	8.9

*From Table 3.5.
**Viscosity of composite of the samples from each area.

Considering the adjusted data Areas 1, 2, 3 and 6, positioned geographically to the west of the Central Plateau, were not significantly different ($p < 0.91$) with a range of 10.5–11.0 UMF® units. The unadjusted UMF® data from theses four areas was also not significantly different ($p < 0.57$), signifying a reduction of variability between these groups. The activity of 8.9 UMF® reported in both Areas 4 and 5, at greater altitude towards the centre of the North Island, were not significantly different ($p < 0.98$), whereas the unadjusted data of Areas 4 and 5 was significantly different ($p < 0.001$).

The two groups remained significantly different in UMF® activity ($p < 0.001$), indicating the dilution of the mānuka honey with other honey types did not completely explain the variability encountered in this region.

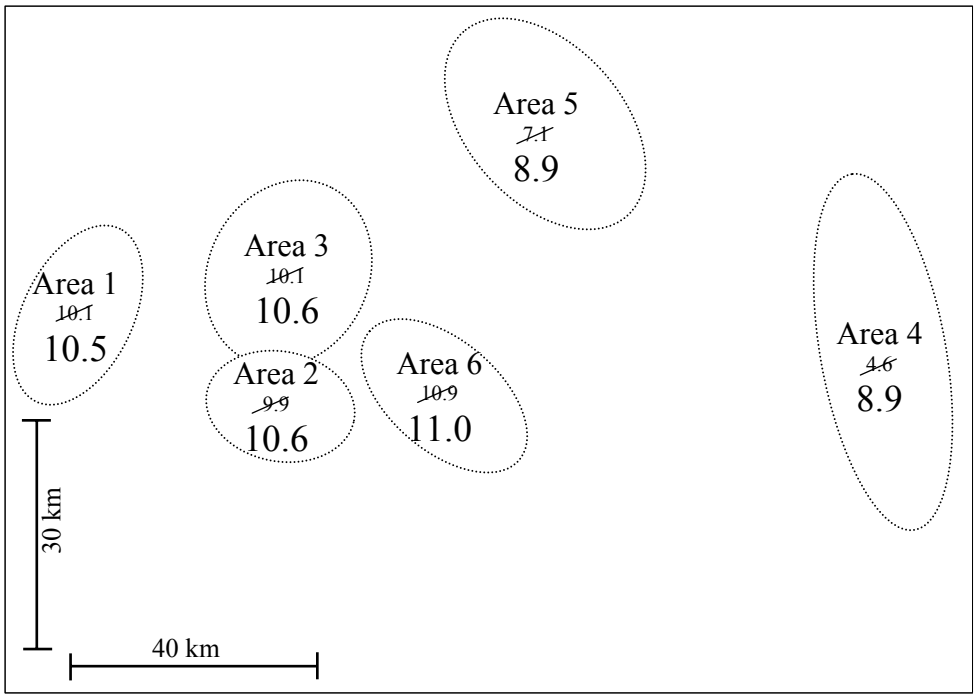

Figure 6.3 The distribution of six areas producing UMF® mānuka honey in the Taranaki region, showing the UMF® activity in each area, adjusted where necessary to account for dilution of mānuka honey by other honey types.

6.2.5 East Coast Region

The adjusted UMF® activity for the eight areas within the East Coast region also fell within two discrete groups (Table 6.5 and Figure 6.4).

The UMF® activity of Areas 3, 6, 7 and 8 ranged between 12.2 and 12.7 units, and was not significantly different ($p < 0.65$). These areas were located on the north-east coast. The unadjusted UMF® activity recorded in these areas was significantly different ($p < 0.001$).

Table 6.5 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the East Coast region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
3	7.5	0.3	8707	60	12.6
6	8.1	0.6	10376	66	12.2
7	9.7	0.4	14125	77	12.6
8	11.4	0.4	19999	89	12.7
1	11.3	1.5	27607	100	11.3
2	10.1	0.4	23245	95	10.6
4	5.1	0.3	6555	49	10.3
5	7.5	0.4	10420	66	11.2

*From Table 3.6.
**Viscosity of composite of the samples from each area.

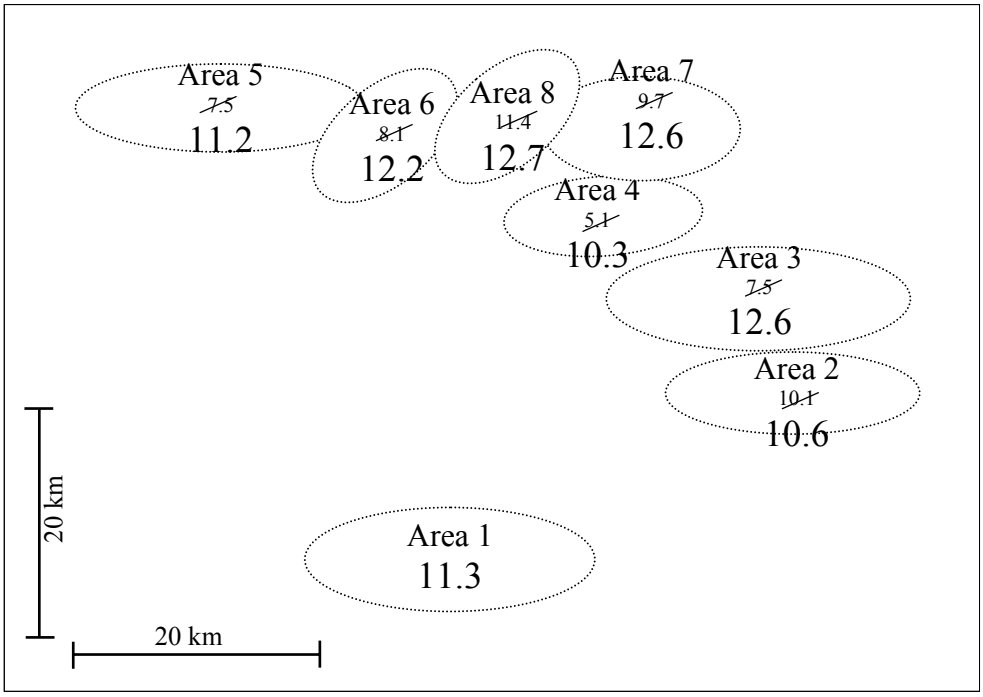

Figure 6.4 The distribution of eight areas producing UMF® mānuka honey in the East Coast region, showing the UMF® activity in each area, adjusted where necessary to account for dilution of mānuka honey by other honey types.

Areas 1, 2, 4 and 5 were more inland, the UMF® activity varied from 10.3 to 11.3 units, and again this group was not significantly different ($p< 0.28$), yet the unadjusted UMF® was significantly different ($p< 0.001$).

Analysis revealed a significant difference ($p< 0.001$) between the two groups in their UMF® activity, again showing dilution of the mānuka honey by other honey types does not completely explain the variability of the field results in this region.

6.2.6 Gisborne Region

The results for the two areas of the Gisborne region are shown in Table 6.6.

Area 1 did not have measurable UMF® activity, so the activity of the undiluted manuka honey could not be estimated. The viscosity analysis revealed that the honey from this area contained less than 30% mānuka honey. However Area 2 was comprised of undiluted mānuka honey, and consequently the UMF® was not adjusted. This region is not illustrated as it is only one area carried UMF® activity.

Table 6.6 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the Gisborne region.

	Original field data*		**% mānuka content****		**Adjusted data**
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
2	10.4	0.6	27335	100	10.4
1	ND		3569	28	

*From Table 3.7.
**Viscosity of composite of the samples from each area.

6.2.7 Hawkes Bay Region

The adjustments for dilution of mānuka honey by other honey types in the Hawkes Bay region are provided in Table 6.7 and Figure 6.5.

The range of UMF® activity recorded in Areas 2, 3, 4 and 5 was increased from 5.1–5.9 to 8.2–8.6 units, indicating the amount of dilution by other honey types was similar across these areas. The UMF® activity of these areas was not significantly different ($p < 0.88$), but the unadjusted data of these four areas was also not significantly different ($p < 0.46$).

Table 6.7 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the Hawkes Bay region.

	Original field data*		% mānuka content**		Corrected data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
2	5.9	0.5	11231	69	8.6
3	5.1	0.5	9545	63	8.2
4	5.5	0.7	9903	64	8.5
5	5.4	0.8	10255	65	8.3
1	ND		3733	29	

*From Table 3.8.
**Viscosity of composite of the samples from each area.

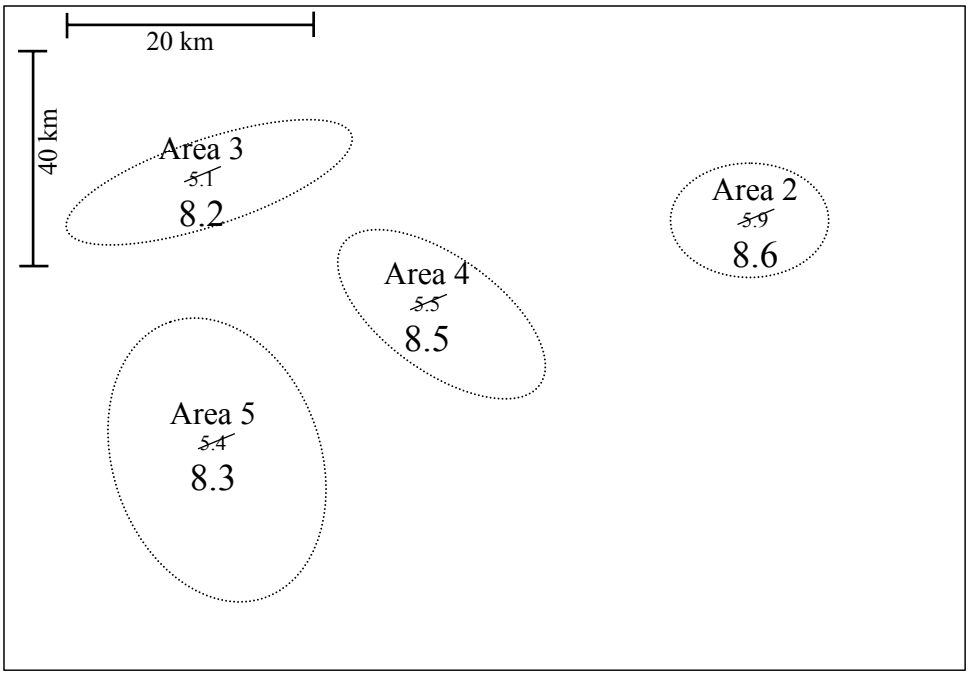

Figure 6.5 The distribution of four areas producing UMF® mānuka honey in the Hawkes Bay region, showing the UMF activity in each area, adjusted to account for dilution of mānuka honey by other honey types.

Therefore the variability of the UMF® data is reduced in this region when the effect of the dilution of the mānuka honey by other honey types is removed from the data. Area 1 contained less than a third mānuka honey and no measurable UMF® activity and consequently was removed from subsequent study.

6.2.8 Wairarapa Region

The adjusted UMF® activity for areas within the Wairarapa region are shown in Table 6.8. This area is not illustrated as only two separated geographical areas yielded UMF® in mānuka honey.

The UMF® in honey harvested from Areas 2 and 3 was diluted by other honey types, and adjustment provided a UMF® rating of 8.9 and 9.3 respectively. These two areas were not significantly different ($p < 0.15$), contrary to the unadjusted UMF® from these two areas that were significantly different ($p < 0.001$). Honey from Area 1 contained less than 20% mānuka honey and did not contain measurable UMF® activity, and was removed from subsequent study.

Consequently the UMF® variability displayed in the Wairarapa region can be explained as mānuka honey dilution by other floral sources.

Table 6.8 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the Wairarapa region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
2	5.7	0.8	9838	64	8.9
3	8.6	0.9	21617	92	9.3
1	ND		2630	17	

*From Table 3.9.
**Viscosity of composite of the samples from each area.

6.2.9 Northern South Island Region

The adjusted UMF® activities for the seven areas of the northern South Island region are shown in Table 6.9 and Figure 6.6.

The adjustment of the UMF® activity for dilution by other honey types in Areas 2–7 revealed that the mean activity ranged between 8.6 and 10.0 units. These areas were not significantly different ($p < 0.23$). The unadjusted UMF® in these areas was significantly different ($p < 0.001$).

Table 6.9 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the northern South Island region.

	Original field data*		% mānuka content**		Adjusted data
Area	**UMF®**	**SD**	**Pa.s**	**%**	**UMF®**
2	5.0	0.4	7485	54	9.2
3	7.2	0.1	16953	84	8.6
4	9.9	0.3	26089	100	9.9
5	5.1	0.4	8129	57	8.8
6	6.6	0.4	12008	71	9.3
7	10.0	1.0	26701	100	10.0
8	5.2	0.9	7873	56	9.3
1	ND		2878	20	

*From Table 3.10.
**Viscosity of composite of the samples from each area.

The honey from Area 1 did not have measurable UMF® activity and contained only 20% mānuka honey, and accordingly was removed from subsequent study.

Therefore the variation of UMF® in the northern South Island can be ascribed to the dilution of mānuka honey by other honey types, and the entire region would for the most part appear to yield manuka honeys with similar UMF® activities.

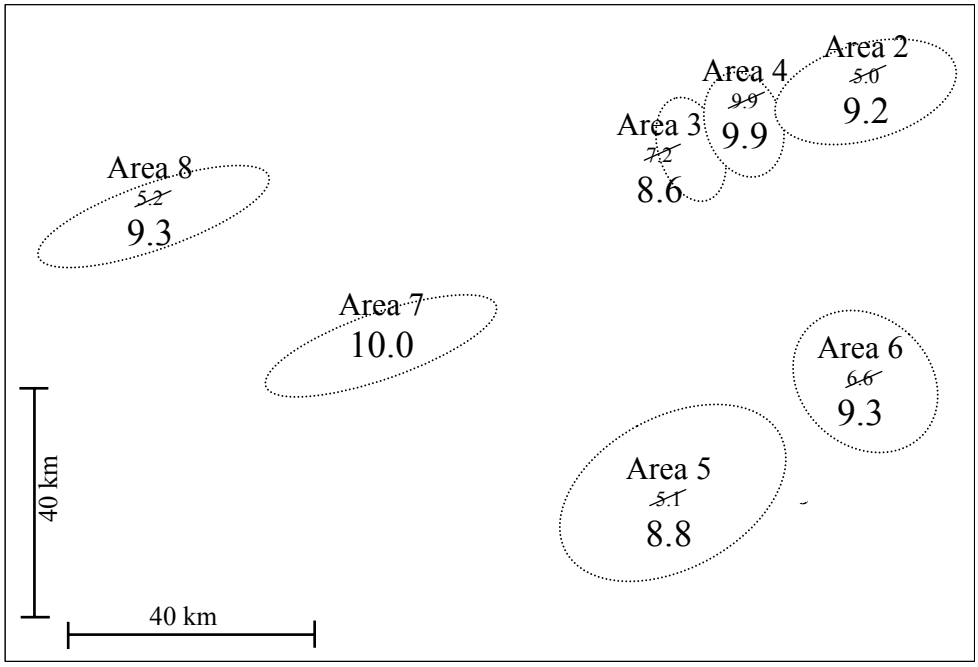

Figure 6.6 The distribution of seven areas producing UMF® mānuka honey in the northern South Island region, showing the UMF activity in each area, adjusted where necessary to account for dilution of mānuka honey by other honey types.

6.2.10 Eastern South Island Region

The adjusted UMF® activity data from the eastern South Island region is presented in Table 6.10. Areas 1 and 2 represent Canterbury sites, and Areas 3 and 4 coastal Otago sites.

Analysis of the UMF® recorded in the Canterbury areas revealed the data corrected for the dilution by other honey types was not significantly different ($p < 0.11$), and likewise neither were the two Otago areas ($p < 0.35$).

A comparison of the four areas revealed the adjusted UMF® activities within this region were not significantly different ($p < 0.43$), unlike the significantly different unadjusted UMF® data ($p < 0.001$), and the variation seen in the field results is therefore attributed to dilution of the mānuka honey.

Table 6.10 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the eastern South Island region.

	Original field data*		% mānuka content**		Adjusted data
Area	UMF®	SD	Pa.s	%	UMF®
1	5.2	0.4	7692	55	9.3
2	10.1	0.2	26767	100	10.1
3	5.3	0.4	7980	56	9.3
4	6.5	0.6	10381	66	9.8

*From Table 3.11.
**Viscosity of composite of the samples from each area.

6.2.11 West Coast Region

The adjusted UMF® data from the West Coast region is detailed in Table 6.11 and Figure 6.7. The adjustment of the data for the West Coast showed a similar pattern to the Coromandel region. The areas were divided into three discreet groups, yielding high, mid and low UMF® activities, and these groups were significantly different ($p < 0.001$) from each other.

Table 6.11 The mean UMF® and standard deviation, the mean viscosity (Pa.s) and predicted mānuka content of honey harvested, and adjusted mean UMF® to account for dilution of sample with other honey types, recorded for areas from the West Coast region.

	Original field data*		% mānuka content**		Corrected data
Area	UMF®	SD	Viscosity	%	UMF®
1	9.2	0.6	10153	65	14.1
3	12.6	0.7	19595	89	14.3
5	15.2	0.8	27373	100	15.2
2	11.1	1.1	15872	81	13.6
4	13.3	0.4	25146	98	13.6
6	10.8	0.7	17554	85	12.8
7	6.8	1.1	7699	55	12.4
12	12.2	1.0	25974	99	12.3
8	5.9	0.8	8813	60	9.8
9	8.2	0.9	19673	89	9.2
10	4.1	0.1	6557	49	8.3
11	6.6	1.3	14495	80	8.2

*From Table 3.12.
**Viscosity of composite of the samples from each area.

Areas 1, 3 and 5 formed a distinct geographic block on pakihi soils through the northern part of this region, and the UMF® adjusted from a range of 9.2 to 15.2 units to a range of 14.1 to 15.2 units when the effect of dilution by other honey types was removed. These areas were not significantly different ($p < 0.06$), compared to the significantly different ($p < 0.001$) unadjusted UMF® data.

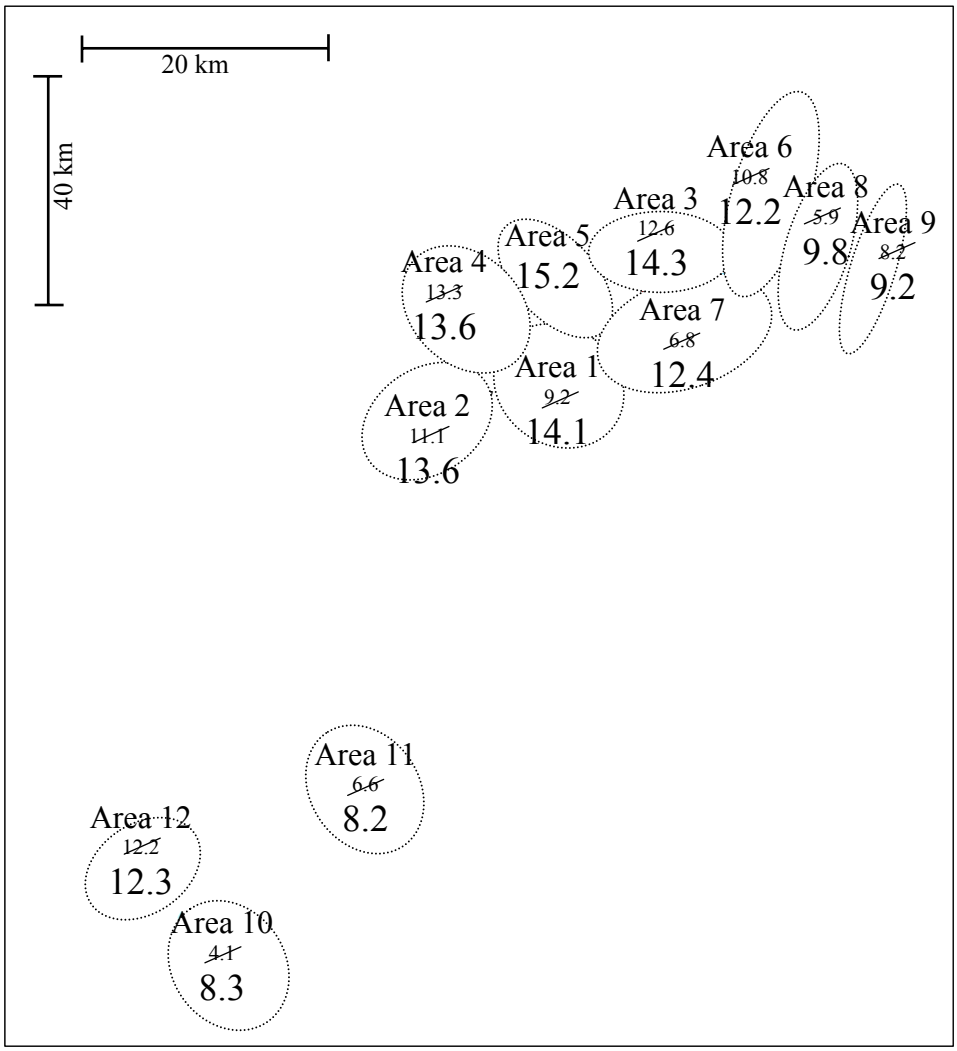

Figure 6.7 The distribution of twelve areas producing UMF® mānuka honey in the West Coast region, showing the UMF® activity in each area, adjusted to account for dilution of mānuka honey by other honey types.

Contiguous to the high activity district were four areas yielding a middle level of UMF® activity. Areas 2, 4, 6, and 7 recorded an adjusted 12.4–13.6 UMF®. In

addition to these areas, the most coastal southern area also contained a mid level 12.3 UMF®. These five areas were not significantly different ($p< 0.22$), however the unadjusted data was significantly different for these areas ($p< 0.001$).

Four areas yielded a low level of UMF®. Areas 8, 9, 10 and 11 were more inland in the north and south of the region, and the UMF® adjusted to account for the dilution of the mānuka honey by other honey types provided a range of UMF® activity of 8.2–9.8 units. This adjusted UMF® of these areas was not significantly different ($p< 0.46$), however the unadjusted data was significantly different ($p< 0.01$).

Therefore the variability of UMF® yield in the West Coast can only be partially explained by the dilution of the mānuka honey by other honey types. A coastal core high activity area exists, surrounded by a ring of medium activity, with the most inland areas yielding the lowest activity.

6.3 Adjusted field results for New Zealand

In view of these adjustments to account for the dilution of mānuka honey by other honey types the data for the whole of New Zealand can be re-summarised (Table 6.12). The groupings of areas within the regions described above that are not significantly different are listed, and areas that did not yield mānuka honey with a measurable UMF® are removed from the analysis.

Four regions/sub-regions yielded a mean UMF® of greater than 14 points. These geographic regions are ideal environments for *Leptospermum scoparium*, the Waikato wetlands, Coromandel seral scrub, and infertile highly-leached Northland gumlands and West Coast pakihi soils. Statistical analysis revealed these four divisions were not significantly different ($p< 0.11$), and accordingly could be treated as a single unit.

Table 6.12 The mean UMF® activity yielded in regions and sub-regions within New Zealand, adjusted to allow for dilution of mānuka honey by other honey types. *n* describes the number of sites included in the division.

Region	Sub-region	Mean UMF®	SD	*n*
Waikato		15.3	0.6	6
Coromandel	High	14.9	0.9	23
Northland		14.8	1.0	35
West Coast	High	14.4	0.9	26
West Coast	Mid	13.1	1.1	27
Coromandel	Mid	12.6	0.7	16
East Coast	High	12.5	0.7	22
East Coast	Low	10.9	0.9	17
Taranaki	High	10.6	1.1	32
Coromandel	Low	10.5	1.1	63
Gisborne		10.4	0.6	2
Eastern South Island		9.6	0.7	14
Wairarapa		9.4	1.4	12
North South Island		9.1	0.8	41
West Coast	Low	9.0	1.2	10
Taranaki	Low	8.9	1.3	19
Hawkes Bay		8.4	1.1	38

The honey from three regions/sub-regions produced a mid-level activity between 12.5–13.1 UMF®. Two of these locations, West Coast and Coromandel, bordered with high activity geographic regions, and both of these areas were also adjacent to districts yielding mānuka honey with low activity UMF®. The third area, East Cape, was adjacent to only low activity UMF® areas and did not border with a high activity region. Analysis showed the three mid-level UMF® divisions were marginally not significantly different from each other ($p < 0.07$). The degree of association with the high activity regions may provide a suitable explanation for two of the mid-level activity regions; hybridisation or mixed stands of different varieties may have been involved. However the reasons for the East Coast mid level activity is not readily apparent.

Low activity of less than 11 UMF® was yielded by ten regions/sub-regions, encompassing sections of the Coromandel and East Coast, Taranaki, Gisborne, Hawkes Bay, Wairarapa, northern and eastern South Island, and sections of the West Coast. Analysed together these ten localities were significantly different ($p< 0.001$), however some geographically justifiable amalgamations were not significantly different.

The UMF® recorded in the four North Island relatively more coastal and low altitude regions/sub-regions; East Coast/Low, Coromandel/Low, Gisborne, and Taranaki/High, were not significantly different ($p<0.60$). These environments are similar, often dominated by seral scrub of which *Leptospermum scoparium* is a prominent member.

The higher altitude areas of the North Island, Taranaki/Low sub-region representing the Central Plateau and the Hawkes Bay hill country were also not significantly different ($p< 0.11$) from each other. However the Wairarapa region was significantly different from the other North Island regions.

A comparison of the four most southern localities yielding low UMF® activity, Wairarapa, northern South Island, eastern South Island, and the West Coast/Low activity showed these districts were not significantly different ($p< 0.31$).

Therefore the regions/sub-regions may be re-grouped together (Table 6.13).

Table 6.13 The mean UMF® yielded in amalgamated regions and sub-regions within New Zealand, adjusted to allow for dilution of mānuka honey by other honey types. Sites list the number of sites/samples included in the division.

Division	Mean UMF®	SD	Sites
New Zealand high activity	14.8	0.9	88
New Zealand medium activity	12.7	0.9	65
North Island low altitude, low activity	10.6	1.1	114
Southern regions, low activity	9.2	1.0	77
North Island high altitude, low activity	8.6	1.2	57

The discrete divisions are significantly different (p <0.001), yet substantial overlap of the data remains (Figure 6.8), and to some extent a continuum of UMF® activity probably exists between these amalgamated groups.

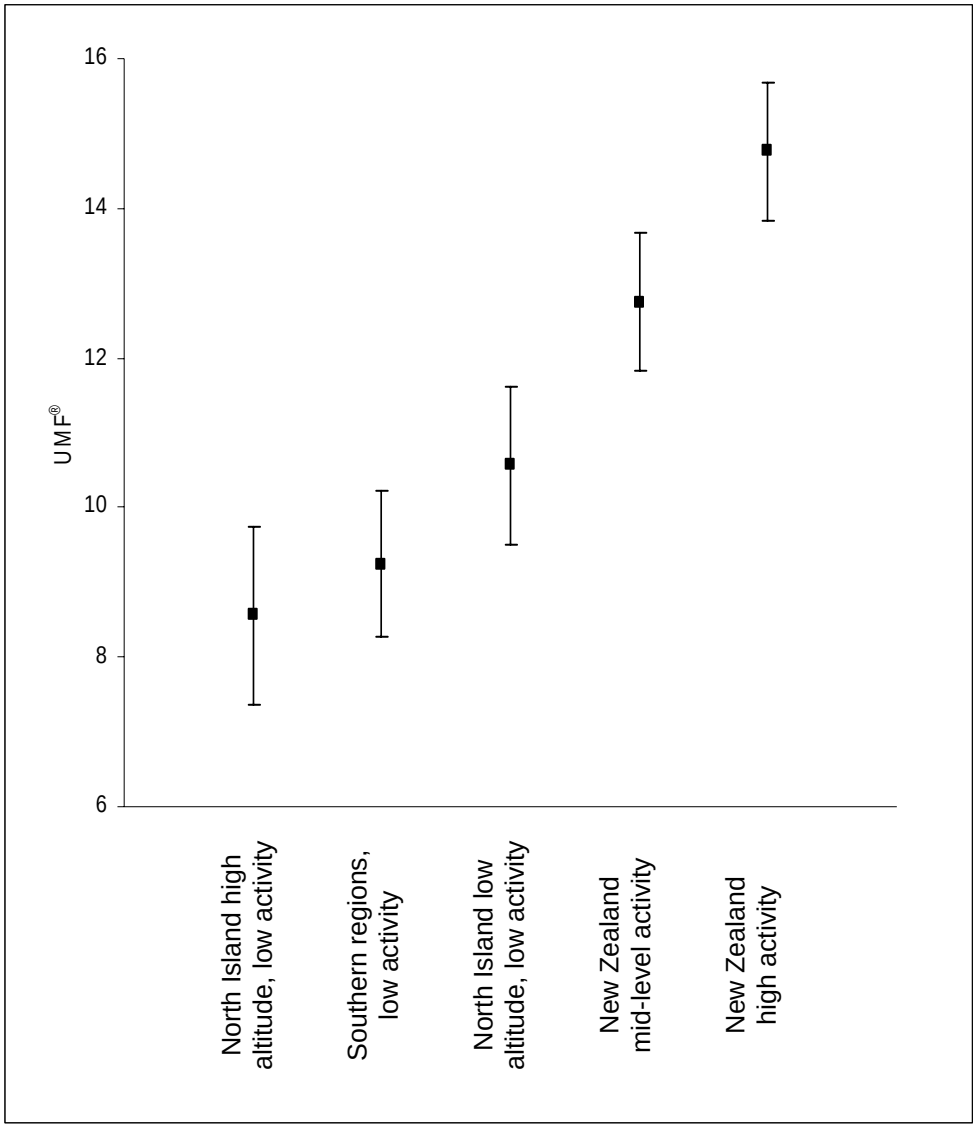

Figure 6.8 Five groups of geographical regions producing similar activity UMF® mānuka honey throughout New Zealand. Mean shown, error bars represent SD.

The regions yielding a high level of UMF® activity are geographically disjointed, and the distribution of these regions in both main islands of New Zealand does not suggest any obvious environmental correlation.

The medium level of UMF® activity is predominately present in association with high activity districts, however the East Coast district is an anomaly to this pattern, isolated from a high-activity district.

The low-activity regions cover the balance of the country from which mānuka honey is harvested. An environmental trend can be recognised; and a response to a climatic gradient would appear to be present.

In summary, the mānuka content of a honey containing measurable UMF® does explain the localised variability in the recorded UMF®. Accordingly discrete localities are seen that are not significantly different from each other.

Equally the mānuka content of the honey does not explain the difference between these localities. This data is used to test the remaining three hypotheses outlined in Chapter One; namely another species' influence on *Leptospermum scoparium*, an environmental influence on *L. scoparium*, or a genetic difference between *L. scoparium* populations, is responsible for the UMF® variability encountered between localities.

Chapter 7

Another species influences *Leptospermum scoparium* UMF® production

This chapter develops the hypothesis that the variability of UMF® in mānuka honey arises from an association of *Leptospermum scoparium* and another species, and that relationship influences *L. scoparium* UMF® production. For this hypothesis to be correct the candidate species would need to fulfil a number of conditions that are outlined. The candidate species are described and divided into four groups; plant, fungal, arthropoda, and nematoda associations. The candidate species distribution and degree of association with *L. scoparium* are considered.

None of the candidate species meet the required criteria; the distinct and clearly defined distribution of different levels of UMF® activity in mānuka honey eliminates the probability of any of the candidate species influencing *L. scoparium* UMF® production.

Sections of this chapter have been published in an amended form in the New Zealand Journal of Botany (Stephens, J. M. C.; Molan, P. C.; Clarkson, B D. 2005: A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany 43*: 431–449).

7.1 Hypothesis

The hypothesis that the variability encountered in UMF® in mānuka honey is a result of the impact of another species on *Leptospermum scoparium* is explored.

The proposed species must fulfil a number of requirements –

(i) Be commonly associated with *L. scoparium*.

(i) Be present in all regions where UMF® is recorded in mānuka honey.

(ii) Be present in varying densities that explain variability of UMF® reported in honey.

An indigenous species is not a prerequisite condition, as a relatively recently introduced species may affect *L. scoparium* giving rise to UMF®. The non-peroxide antibacterial activity of mānuka honey was first described by Molan and Russell (1988); accordingly an introduced species that had become widespread by that date may have caused the UMF® phenomenon.

7.2 Plant associations

Three direct plant associations occur with *Leptospermum scoparium* (Table 7.1). The bark of *L. scoparium* is frequently shed, and therefore this species is not a host for epiphytes (Molloy 1975).

The Orchidaceae are represented by one species, the very rare non-green orchid *Gastrodia minor*. *G. minor* shares mycorrhizae with *L. scoparium* as well as other plant species (Moore & Edgar 1970) and is distributed throughout New Zealand (Wardle 1991).

Table 7.1 Plant species reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Family	Species	Distribution	Occurrence	Comments
Orchidaceae[1]	*Gastrodia minor*[1]	NI, SI[1]	Uncommon[2]	Often reported under *L. scoparium*[1]
Loranthaceae[3]	Ileostylus micranthus[3]	NI, SI north of Lat 46°[3]	Common[3]	Many host species, rarely on *L. scoparium*[1,3]
Loranthaceae[3]	Korthalsella salicornioides[3]	NI, SI[3]	Common[3]	Many host species, lowland distribution[3]

[1]Moore & Edgar 1970; [2]Molloy 1975; [3]Allan 1961.
Distribution abbreviations. NI – North Island; SI – South Island.

L. scoparium acts as a host for two members of Loranthaceae, the mistletoe family. The widely distributed large-leafed mistletoe *Ileostylus micranthus* (Molloy 1975) exhibits low host specificity and is most frequent found in association with *Coprosma* spp. (Patel 1991) rather than *L. scoparium*. The widespread lowland parasitic dwarf leafless mistletoe *Korthalsella salicornioides* attaches preferentially to *Leptospermum* and *Kunzea* (Stevenson 1934), but is also found with other indigenous species and has been recorded in association with introduced *Erica* spp. (Bannister 1989).

The orchid *Gastrodia minor* is rare, and the mistletoe *Ileostylus micranthus* does not frequently utilise *L. scoparium* as a host and *Korthalsella salicornioides* is not found throughout the entire UMF® range. Therefore an association with another plant species cannot be responsible for the UMF® in mānuka honey.

7.3 Fungal associations

The New Zealand fungi have not been fully classified and the complete number of species is unknown. Many fungi species have been noted in association with *Leptospermum scoparium*, and a search of the New Zealand Fungal Herbarium database (NZFungi 2004) revealed 699 *L. scoparium*-hosted specimens.

The taxonomic arrangement of fungi is subject to debate; however this discussion follows the divisions outlined by Ainsworth et al. (1973). In this treatment the fungi are initially divided into two major divisions, the Myxomycota (slime moulds and allies) and the Eumycota (true fungi); and both divisions are further subdivided. The *L. scoparium*-hosted specimens are classified within the Ascomycotina, Basidiomycotina, and Deuteromycotina subdivisions of the Eumycota; and the Myxomycetes order of the Myxomycota.

Fungal nutrition falls into three strategies, saprobic, parasitic, or symbiotic. Saprobic organsisms grow on dead organic matter. The obligate parasitic fungi grow on living organisms and cause some degree of disease, whereas the facultative parasites adopt both the saprobic and parasitic strategies. The symbiotic fungi adopt mutualism as a nutritional strategy, and are notably represented by the mycorrhizal associations with plants. As the taxonomy of the fungi is determined by morphology, taxa within the subdivisions adopt varying nutritional strategies.

Accordingly the fungal associations with *L. scoparium* can be divided into saprobic, having no effect on the plant; facultative parasitism, generally saprobes with the ability to also invade the host; parasitism; and symbiotic mutualism. The saprobic fungi associated with *L. scoparium* are classified as the Myxomycota; and the Ascomycotina, Basidiomycotina, and Deuteromycotina subdivisions of the Eumycota. The facultative parasitic saprobes are found in the Ascomycotina subdivision of the Eumycota. The symbiotic fungal taxa belong within the Ascomycotina and Basidiomycotina of the Eumycota.

The facultative parasitic fungi inhabiting *L. scoparium* foliage have been studied, and a narrow but characteristic range of *L. scoparium* endophytic fungi was reported from a study in the Auckland Province utilising sites about Auckland Isthmus and the northern Coromandel Peninsula (Johnston 1998). *Phyllosticta* spp. (Ascomycotina, Dothideales), in association with *Diploceras leptospermi* (Ascomycotina, Sphaeriales) and Coelomycetes (Deuteromycotina), dominated natural populations, with a range of other species present in insignificant and variable proportions. Within natural sites neither the species diversity nor the variability of infection rate could be explained by any obvious correlation with plant age or any environmental factor (Johnston 1998). *Phyllosticta* spp. were specific to *L. scoparium* and not recorded on *Kunzea ericoides*, yet *D. leptospermi* was present on both plant species (Johnston 1998) in contradiction to an earlier report (Bagnall & Sheridan 1972). The fungal species' pathogenicity is unknown

though six of the families represented grow as leaf epiphytes and are associated with leaf wounds (Johnston 1998).

The symbiotic fungi form two types of relationship with *L. scoparium*. The ectomycorrhizal and endomycorrhizal (vesicular-arbuscular) associations are frequent yet the number of fungal partners is unknown, and endomycorrhizae infection appears to be more common (Moyersoen & Fittler 1999). Although Hawksworth et al. (1995) identified the order Glomales as the most common endomycorrhizal symbiont, the herbarium collection (NZFungi 2004) does not include any specimen from this order, probably due to the difficulties associated with classification and laboratory growth. However Baylis (1971) successfully infected *L scoparium* with an endomycorrhiza in laboratory conditions, and in a study of five South Island sites dominated by *Nothofagus* spp., *Pinus radiata*, or podocarp/broad-leaved forest, five of the twelve endomycorrhizal symbionts described were found in association with *L. scoparium*, whereas only 10-36% of the infections were ectomycorrhiza at four of these sites (Cooper 1976). *L. scoparium* is one of the principal indigenous ectomycorrhizal hosts in New Zealand's indigenous flora, along with *Kunzea ericoides* and *Nothofagus* spp. (Hall et al. 1998). *L. scoparium* ectomycorrhizal infection appears to be determined by the presence of the appropriate inoculum and alternative host plant species, particularly *Nothofagus* spp. (Moyersoen & Fittler 1999).

The principal role of *L. scoparium* mycorrhizal partners is the improvement of phosphorus uptake (Baylis 1971; Johnson 1976; Hall 1977) allowing rapid growth and exploitation of available light (Wardle 1991). The level of mycorrhizal infection correlates with available phosphorus and growth conditions (Baylis 1975; Cooper 1975; Hall 1975). The development of the *L. scoparium* ectomycorrhizae may also facilitate the growth and succession of *Nothofagus* spp. seedlings (Baylis 1980).

7.3.1 Ascomycotina

Eight orders of the Ascomycotina (Table 7.2) are recorded in association with *Leptospermum scoparium*, representing 71 taxa (NZFungi 2004). The nutritional strategies employed are saprobic, parasitic, and facultative parasitism.

The Discomycetes are represented by four orders. The Tuberales are mycorrhizal with angiosperm plants (Hawksworth *et al*. 1995) and have been found beneath *L. scoparium* (Stevenson 1994) in an ectomycorrhizal association (Orlovich & Cairney 2004). The Helotiales, Pezizales, and Phacidiales are considered primarily saprobes with the ability to become facultative parasites (Korf 1973). The Helotiales were common endophytes on *L. scoparium* foliage in the Auckland Province (Johnston 1998). Orlovich and Cairney (2004) list both the Helotiales and Pezizales as possible ectomycorrhizal symbionts of *L. scoparium*. The Phacidiales contain families that are obligate parasites (Lancaster 1955), and one of these, the Rhystismaceae, are recorded three times in association with *L. scoparium* (NZFungi 2004). The distribution of these orders in New Zealand on *L. scoparium* is not recorded.

The Loculascomycetes are represented by two orders. The saprobic Doithideales typically inhabit plant and animal wastes in tropical climates (Luttrell 1973) but temperate species also exist. The most common family represented is the Capnodiaceae that include the sooty moulds (Stevenson 1994), which is widespread on *L. scoparium* foliage as a result of Hemiptera (scale insect) invasion (Hoy 1961), but does not directly influence the plant. The Pleosporales are saprobic and facultative parasites, and some genera are known to cause scab diseases of plants (Stevenson 1994) but this condition has not been reported on *L. scoparium*.

Table 7.2 Ascomycotina taxa reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Class	Order	Nutritional strategy	Comments
Discomycetes[1]	Helotiales[2]	Saprobic and parasitic[2]	Facultative parasites[2], ectomycorrhizal[5]
	Pezizales[2]	Saprobic and parasitic[2]	Facultative parasites[2], ectomycorrhizal[5]
	Phacidiales[2]	Saprobic and parasitic[2]	Facultative and obligate parasites[2]
	Tuberales[2]	Ectomycorrhizal[2,5]	Truffle producing order[6]
Loculoascomycetes[1]	Dothideales[3]	Saprobic[3]	Sooty moulds[6]
	Pleosporales[3]	Saprobic and parasitic[3]	Genera may cause plant scab[6]
Pyrenomycetes[1]	Meliolales[4]	Parasitic[4]	Obligate parasites, subtropical-tropical, black mildews[4]
	Sphaeriales[4]	Saprobic and parasitic[4]	Common endophytes of *L. scoparium*[7]

[1]Ainsworth et al. 1973; [2]Korf 1973; [3]Luttrell 1973; [4]Muller & von Arx 1973; [5]Orlovich & Cairney 2004; [6]Stevenson 1994, [7]Johnston 1998.

The Pyrenomycetes are represented by two orders. The Meliolales are obligate parasites of higher plants, and are common in tropical and sub-tropical climates (Muller & von Arx 1973). The Sphaeriales are saprobic and facultative parasites (Muller & von Arx 1973). The Sphaeriales are recorded as common endophytic fungi of *L. scoparium* foliage (Johnston 1998). Three families, the Xylariaceae, Hypocreaceae, and Sordariaceae were the dominant members of the fungal community in a natural stand of *L. scoparium* in the Auckland Province (Johnston 1998). The distribution of these orders on *L. scoparium* in New Zealand is not recorded, however little difference in fungal populations was found between sites on the Auckland Isthmus and the northern Coromandel Peninsula (Johnston 1998).

7.3.2 Deuteromycotina

The Coelomycetes, Hyphomycetes, and anamorphs classified within both of these divisions, are listed in association with *Leptospermum scoparium* (Table 7.3), representing 29 taxa (NZFungi 2004). The species are microscopic, and while usually saprobic many are also capable of facultative parasitism (Kendrick and Carmichael 1973; Sutton 1973). Two unidentified Coelomycetes were recorded as endophytes of *L. scoparium* foliage in the Auckland Province (Johnson 1998), however the number of fungal taxa inhabiting the plant species, and the distribution of those taxa, are unknown in New Zealand.

Table 7.3 Deuteromycotina and Myxomycota taxa reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Class	Order	Nutritional strategy	Comments
Coelomycetes[1]	Melanconiales[2]	Saprobic and parasitic[2]	Microscopic facultative parasites[2]
	Sphaeropsidales[2]	Saprobic and parasitic[2]	Microscopic facultative parasites[2]
	Anamorph[3]	Saprobic and parasitic[2]	Microscopic facultative parasites[2]
Hyphomycetes[1]	*Anamorph*[3]	Saprobic and parasitic[4]	Microscopic facultative parasites[4]
Myxomycetes[1]	Physarales[3]	Saprobic[1]	Saprobe only[1]

[1]Ainsworth et al. 1973; [2]Sutton 1973; [3]NZFungi 2004; [4]Kendrick &Cairney 1973.

7.3.3 Myxomycota

One order of the Myxomycetes (Table 7.3) is listed in association with *L. scoparium* in the New Zealand Fungal Herbarium (NZFungi 2004). However as the Myxomycota are saprobic and do not influence living organisms, the Physarales listed would have subsisted on non-living organic material.

7.3.4 Basidiomycotina

The Basidiomycotina in New Zealand have received more attention than the other fungal groups discussed. Ten orders (Table 7.4) are recorded in association with *Leptospermum scoparium*, representing 195 taxa (NZFungi 2004). The nutritional strategies employed are saprobic or mycorrhizal mutualism.

Four orders are saprobes subsisting on timber. The Dacrymycetales and Tremellales inhabit well decayed timber (Lancaster 1955). The Hymenocheatales and Polyporales cause damage to standing timber, both causing rot staining (Lancaster 1955; Hawksworth et al. 1995).

Six orders are recorded as ectomycorrhizal in association with *L. scoparium*. New Zealand ectomycorrhiza have recently been reviewed, and 22 families from these orders utilised *L. scoparium* as a host species, often also with *Kunzea ericoides*, *Nothofagus* spp. and introduced higher plant species (Orlovich & Cairney 2004). The Agaricales represent six widespread families (Orlovich & Cairney 2004), including the invasive *Amanita muscaria* (Pluteaceae) (Ridley 1991), which is considered to displace the indigenous species (Orlovich & Cairney 2004). The Boletales are also common and represent seven families (Orlovich & Cairney 2004). The Cantharellales represent three families and are less common, being present under only *Nothofagus* spp., *L. scoparium* and *K. ericoides* (Orlovich & Cairney 2004).

Table 7.4 Basidiomycotina taxa reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Order	Nutritional strategy	Comments
Dacrymycetales[1]	Saprobic[4]	On decaying wood[3]
Hymenochaetales[1]	*Saprobic*[4]	Standing timber decay, white rot[4]
Polyporales[1]	*Saprobic*[4]	Standing timber decay[3]
Tremellales[1]	Saprobic[4]	On decaying wood[3]
Agaricales[2]	Ectomycorrhizal mutualism[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp. and numerous introduced hosts[2]
Boletales[2]	*Ectomycorrhizal mutualism*[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp. and numerous introduced hosts[2]
Cantharelles[2]	*Ectomycorrhizal mutualism*[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp. hosts[2]
Phallales[2]	Ectomycorrhizal mutualism[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp., *Eucalyptus* spp. hosts[2]
Russulales[2]	*Ectomycorrhizal mutualism*[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp. and several introduced hosts[2]
Thelephorales[2]	Ectomycorrhizal mutualism[2]	*L. scoparium*, *K. ericoides*, *Nothofagus* spp. and several introduced hosts[2]

[1]NZFungi 2004; [2]Orlovich & Cairney 2004; [3]Lancaster 1955; [4]Hawksworth et al. 1995.

The Phallales represent three families, and are found growing with *Eucalypus* spp. as well as the three indigenous plant taxa (Orlovich & Cairney 2004). The Russulaceae is the single family within the Russulales, and is widespread, growing under many hosts (Orlovich & Cairney 2004). The Thelephorales are represented by two families, both of which are fairly widespread (Orlovich & Cairney 2004).

7.3.5 Fungal association conclusions

As the distribution and density of these fungal taxa is not recorded it is impossible to completely dismiss the possibility of an influence on *Leptospermum scoparium* leading to UMF® production.

Nevertheless some generalised observations may be made, indicating the probability that a fungal species is responsible for influencing *L. scoparium* UMF® production is minimal. The saprobic species do not influence the living plant, and the symbiotic species bring about a beneficial mutualism. The facultative and obligate parasitic species undoubtedly have a detrimental effect, to some degree, on the host species.

The UMF® distribution and variability are described in Chapter 6. UMF® is encountered in mānuka honey harvested throughout the country, wherever *L. scoparium* is present in sufficient density for a relatively pure mānuka honey to be harvested. Many of these fungal species are not present in relatively cooler environments, yet UMF® is present in mānuka honey harvested from the Central Plateau and Otago.

Consideration of the northern Coromandel Peninsula clearly illustrates the difficulty in attributing UMF® production by *L. scoparium* to the influence of a phytopathogenic fungal species. Within a small geographic range that has a relatively constant set of environmental conditions and hence not limiting the distribution or density of a fungal species, three distinct levels of UMF® are

encountered. Therefore if a fungal species was causing this effect, it would be present throughout this region, yet have a field density that altered from low, to medium, to high, to medium, to low again on a north-south axis, within the distance of approximately 50 km, and this density variance would not be caused by an environmental gradient. This distribution pattern would have to be reproduced on an east-west axis in the West Coast through a shorter distance, where environmental conditions are very different from the northern Coromandel. Moreover if the fungal species influencing *L. scoparium* was soil borne, a high species density would be required in the Waikato peat wetlands and the Coromandel volcanic-derived clay soils, very different substrates with extreme environmental conditions, particular water status.

Therefore the probability of a fungal species influencing *L. scoparium* UMF® production is very unlikely.

7.4 Arthropoda associations

The Arthropoda phylum in New Zealand has received more attention than the fungal phylum, and has been divided into eight sections for this discussion. The species listed have been recorded feeding on *Leptospermum scoparium* leaves, flowers, sap, or wood.

7.4.1 Hemiptera

The principal insect pests associated with *Leptospermum scoparium* in New Zealand are the sap-feeding scale insects, order Hemiptera (Table 7.5). Five families from this order are listed, representing twenty-three species.

One species of the Asterolecaniidae, the pit scales, is listed. The distribution of *Asterolecanium vitrem* is through the drier regions of the North Island and

Table 7.5 Hemiptera species reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Order		Species	Distribution	Occurrence	Comments
Hemiptera[2]	Asterolecaniidae[1]	*Asterolecanium vitreum*[1]	East NI, north SI[1]	Rare[1]	*L. scoparium* not principal host[1]
	Coccidae[2]	Ceroplastes sinensis[2]	Aus, NI, north SI[2]	Common north NI[2]	*L. scoparium* not principal host[2]
	Coccidae[2]	*Crystallotesta insignia*[2]	NI, north SI[2]	Common in drier regions[2]	*K. ericoides* & *L. scoparium* hosts[2]
	Coccidae[2]	*Crystallotesta ornatella*[2]	NI, north SI[2]	Common in drier regions[2]	*K. ericoides* & *L. scoparium* hosts[2]
	Coccidae[2]	Plumichiton flavus[2]	NI, north SI[2]	Common[2]	Uncommon on *Leptospermum*[2]
	Coccidae[2]	*Plumichiton pollicinus*[2]	NI, north SI[2]	Common[2]	*K. ericoides* & *L. scoparium* hosts[2]
	Coccidae[2]	Umbonichiton bullatus[2]	Central NI[2]	Rare[2]	*K. ericoides* & *L. scoparium* hosts[2]
	Coccidae[1]	*Eriococcus leptospermi*[1]	AUS, NI, SI[1]	Rare[1,2]	*K. ericoides* & *L. scoparium* hosts[1]
	Coccidae[1]	*Eriococcus orariensis*[1]	AUS, NI, SI[1]	Common in drier regions[1]	*L. scoparium* principal host in NZ[1]
	Diaspididae[3]	*Chionaspis angusta*[3]	NI, SI[3]	Common[3]	*L. scoparium* not principal host[3]
	Diaspididae[3]	*Hemiberlesia rapax*[3]	NI, north SI[3]	Common[3]	*L. scoparium* not principal host[3]
	Diaspididae[3]	*Lindingaspis rossi*[3]	NI, north SI[3]	Common[3]	*L. scoparium* not principal host[3]
	Diaspididae[1]	*Lepidosaphales leptospermi*[1]	NI, north SI[1]	Common[1]	*K. ericoides* & *L. scoparium* hosts[1]
	Pseudococcidae[4]	*Crisicoccus tokaanuensis*[4]	Central NI north[4]	Rare[4]	*L. scoparium* principal host[4]
	Pseudococcidae[4]	*Dysmicoccus viticis*[4]	NI, SI[4]	Common[4]	*L. scoparium* not principal host[4]
	Pseudococcidae[4]	Paracoccus leptospermi[4]	South SI[4]	Rare[4]	*L. scoparium* principal host[4]
	Pseudococcidae[4]	*Paracoccus miro*[4]	NI, SI[4]	Common[4]	*L. scoparium* not principal host[4]
	Pseudococcidae[4]	*Paracoccus zealandicus*[4]	NI, north SI[4]	Common[4]	*L. scoparium* not principal host[4]

[1]Hoy 1961; [2]Hodgson & Henderson 2000; [3]Charles & Henderson 2002; [4]Cox 1987.
Distribution abbreviations. AUS – Australia; NI – North Island; SI – South Island.

northern South Island. This species is uncommon, being very rare on *L. scoparium* and occasionally found living on *Kunzea ericoides* (Hoy 1961).

The Coccidae, sap sucking soft-bodies scale insects, are represented by eight species. The introduced cosmopolitan *Ceroplastes sinenis* is common on *Citrus* spp. in the far north of the North Island, and is found as far south as the northern South Island. However this species is rarely found in association with the indigenous flora (Hodgson & Henderson 2000).

Two endemic species of the *Crystallotesta* genus associate with *L. scoparium*. Both species are distributed through the drier regions of the North Island and northern South Island (Hodgson & Henderson 2000). *Crystallotesta leptospermi* is common on both *L. scoparium* and *Kunzea ericoides*, and *Crystallotesta ornatella* is also usually found on these plant species as well as *Leucopogon fasciculatus* (Hodgson & Henderson 2000).

Two endemic species of the *Plumichiton* genus inhabit *L. scoparium*, both being fairly common and widespread throughout the North Island and northern South Island. *Plumichiton flavus* is not specific to a limited range of hosts, and sixteen host plant taxa are described (Hodgson & Henderson 2000). *Plumichiton pollicinus* feeds exclusively on *L. scoparium* and *Kunzea ericoides* (Hodgson & Henderson 2000).

Umbonichiton bullatus is also endemic, and is found in the central North Island. In addition to *L. scoparium* and *Kunzea ericoides*, this species also feeds on at least two other plant families including a podocarp (Hodgson & Henderson 2000).

The *Eriococcus* genus contains two species that associate with *L. scoparium*, both of which appear to have been introduced from Australia (Hoy 1961). *Eriococcus leptospermi* is widespread throughout both islands, and *Eriococcus orariensis* is locally common in the drier regions of both islands (Hoy 1961) but absent in wetter regions (Wardle 1991). The distribution of *E. orariensis* has been reduced

by the subsequent spread of the entomogenous fungus *Myriangium thwaitesii*, found in warmer wet climates (Hoy 1961). *E. leptospermi* does not appear to cause plant death and is not infected by *M. thwaitesii*, and is widely distributed in low frequency (Hoy 1961).

The Diaspididae, the armoured scale insects, are represented by seven species. Three introduced species are recorded in association with *L. scoparium*. *Hemiberlesia rapax* is found throughout the North Island and the north South Island, and inhabits at least forty-two native plant species (Charles & Henderson 2002). *Lindingaspis rossi* is also present through the North Island and north South Island, and has been recorded on seventeen native plant species (Charles & Henderson 2002). *Chioaspis angusta* is distributed throughout both main islands apart from the west of the South Island, and is found on *Callistemon* spp. and *Kunzea ericoides* as well as *L. scoparium* (Charles & Henderson 2002). Four endemic Diaspididae species have been recorded in association with *L. scoparium*. *Lepidosaphales leptospermi* is distributed throughout the North Island and the north of the South Island, and inhabits both *L. scoparium* and *Kunzea ericoides*, and is considered common (Hoy 1961). However the three other species mentioned by the same author, *Lepidosaphales intermedia*, *Phenacaspis dubia*, and *Poliaspis argentosis*, have a single earlier reference (Hoy 1961) and the author may be describing isolated occurrences of an association with *L. scoparium*.

Two species of the Margarodidae, the giant coccids, have been recorded with *L. scoparium*. Hoy (1961) suggests *Coelostomidia wairoensis* commonly inhabits *L. scoparium* throughout New Zealand, however later research has disagreed with this observation (Morales 1991). Likewise *L. scoparium* is not currently considered to act as a host of *Coelostomidia zealandica* (Morales 1991).

Five species represent the Pseudococcidae, the mealybugs recorded feeding on *L. scoparium*. Two species have been exclusively recorded with *L. scoparium*, *Crisicoccus tokaanuensis* is present north of the central North Island and

considered rare, and *Paracoccus leptospermi* is present in southern South Island (Cox 1987). Two species are found throughout both islands, *Dysmicoccus viticis* feeds on eleven plant taxa including *L. scoparium*, and *Paracoccus miro* is usually found on Podocarpaceae but has been recorded on *L. scoparium* (Cox 1987). *Paracoccus zealandicus* is present in the North Island and the north of the South Island, and is also a generalised feeder of at least seven taxa (Cox 1987).

Therefore two Hemiptera species are found throughout the distribution of UMF® and utilise *L. scoparium* as the principal host. Both *Eriococcus leptospermi* and *E. orariensis* were introduced from Australia approximately 50 years ago, and have spread throughout the country. However the incidence of *E. orariensis* has decreased dramatically in the warmer northern climates since the introduction of an entomogenous fungus, contrary to the UMF® distribution pattern. *E. leptospermi* is also widely distributed, but is not common and no areas of increased density have been recorded. Accordingly neither of these species presents a distribution pattern that would account for the variability of UMF® recorded in mānuka honey.

7.4.2 Coleoptera

Four families of Coleoptera are recorded in association with *Leptospermum scoparium*, representing seven species (Table 7.6).

The Cerambycidae, longhorn beetles, are represented by four indigenous species. *Calliprason elegans* is a rare beetle restricted to shrub in a few areas of the North Island, where the adult feeds on *L. scoparium* and *Dracophyllum* spp. (Crowe 2002). *Gastrosaurus nigracollis* is common in both main islands and the adult is reported grazing on *L. scoparium* with other species, and the larvae inhabiting a number of hosts (Crowe 2002). The kānuka longhorn beetle *Ochrocydus huttoni* is found throughout New Zealand and feeds on *Nothofagus* spp., *Kunzea ericoides*, and *L. scoparium* live wood (Hosking 1978; Crowe 2002). The lemon tree borer *Oemona hirta* is common in the range of the North Island and northern South

Table 7.6 Coleoptera, Heteroptera, Homoptera, Orthoptera, Thysanoptera and Arachnida species reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Order	Family	Species	Distribution	Occurrence	Comments
Coleoptera[1]	*Cerambycidae*[1]	Calliprason elegans[1]	NI[1]	Rare[1]	Leaf-grazing adult[1]
	Cerambycidae[1]	Gastrosaurus nigracollis[1]	NI, SI[1]	Common[1]	*L. scoparium* not principal host[1]
	Cerambycidae[1]	Ochrocydus huttoni[1]	NI, SI[1]	Common[1]	*L. scoparium* not principal host[1]
	Cerambycidae[1]	Oemona hirta[1]	NI, north SI[1]	Common	*L. scoparium* not principal host[1]
	Chrysomelidae[1]	*Eucolaspis brunnea*[1]	NI[1]	Abundant[1]	*L. scoparium* not principal host[1]
	Scarabaeidae[2]	Pyronota festiva[1]	NI, SI[1]	Abundant[2]	Root and leaf-grazing [1,2]
	Scolytidae[3]	Amasa truncates[3]	NI, SI[3]	Common[3]	Wood borer, *L. scoparium* not principal host[5]
Heteroptera[4]	Miridae[4]	*Romna* spp.[4]	NI, SI[4]	Common[4]	Sap-feeding [4]
	Ricaniidae[1]	*Scolypopa australis*[1]	AUS, NI, north SI[1]	Common[1]	*L. scoparium* not principal host[1]
Homoptera[5]	Cicadellidae[5]	*Zygina zealandica*[5]	AUS, NI, SI[5]	Common[5]	*L. scoparium* not principal host[5]
Orthoptera[1]	Tettigoniidae[1]	*Caedicia simplex*[1]	AUS, NI, SI[1]	Common[1]	Leaf-grazing adult[1]
Thysanoptera[6]	Aeolothripidae[6]	*Scirothrips pan*[6]	SI[6]	Rare[6]	Sap-feeding thrips[6]
	Aeolothripidae[6]	*Thrips obscuratus*[6]	NI, north SI[6]	Common[6]	Sap-feeding thrips[6]
Arachnida[7]	Eriophyoidae[7]	*Aceria manukae*[7]	NI[7]	Common[7]	Gall-forming mite[7]

[1]Crowe 2002; [2]Hudson 1892; [3]Brockerhoff & Bain 2000; [4]Eyles & Carvalho 1988; [5]Knight 1976; [6]Mound & Walker 1982; [7]Manson 1984.
Distribution abbreviations. AUS – Australia; NI – North Island; SI – South Island.

Island, however *L. scoparium* is not the usual host and this species is often found on *Citrus* spp. (Crowe 2002).

The Chrysomelidae, the leaf-feeding beetles, are represented by one species, *Eucolaspis brunnea*. This indigenous beetle is abundant in the northern North Island. Typically the adult grazes on orchard fruit tree leaf material that has become the species' preferred food (Crowe 2002), however an association with *L. scoparium* has been recorded (Spiller & Wise 1982).

One species of Scarabaeidae, named for the morphological similarity to the Egyptian scarab, is recorded in association with *L. scoparium*. *Pyronota festiva*, the mānuka chafer, is widespread throughout New Zealand (Crowe 2002) often found on light sandy montane soils associated with grassland on forest margins. The larvae appear to be non-specific feeders, often preferring grass root material to *L. scoparium* (Thomson et al. 1979), and the adult is recorded feeding on *L. scoparium* foliage (Hudson 1892). However this species is not present in large numbers in the heavy waterlogged soils of the Waikato wetlands, contrary to the distribution of UMF®.

One introduced species of woodborer, *Amasa truncates*, represents the Scolytidae. This species inhabits the dead wood of *L. scoparium* and at least twelve other plant taxa, and is found throughout New Zealand (Brockerhoff & Bain 2000).

7.4.3 Heteroptera

Two families of Heteroptera are recorded in association with *Leptospermum scoparium* (Table 7.6), the Miridae and Ricaniidae.

The Miridae are sap-feeding insects and are often considered crop pests (Crowe 2002). Spiller and Wise (1982) list the *Romna* spp. associating with *L. scoparium*. The genus has subsequently been re-examined by Eyles and Carvalho (1988), and

eleven endemic species described. Six species are found in the North Island, two in the South Island, and three in both islands, however *L. scoparium* was not listed as a host plant species.

The Rocaniidae are also sap feeding insects, characterised as plant hoppers. *Scolypopa australis*, the passion vine hopper, arrived from Australia in the late 19th century, and has become a common garden pest throughout the North Island and northern South Island (Crowe 2002). Whilst recorded on *L. scoparium* (Spiller & Wise 1982), this plant species is not the usual host for this cosmopolitan insect.

7.4.4 Homoptera

The Homoptera are represented by one family, the Cicadellidae (Table 7.6). The leaf hopper *Zygina zealandica* has been recorded on *Leptospermum scoparium* along with at least sixteen endemic plant species. The species is present through both islands of New Zealand and Australia, is described as predominately grass inhabiting (Knight 1976), but is not present at high altitudes where UMF® is yielded.

7.4.5 Orthoptera

One family and species of Orthoptera, characterised by large rear legs, are recorded in association with *Leptospermum scoparium* (Table 7.6). *Caedicia simplex,* Tettigoniidae, is widespread throughout New Zealand at lower altitudes and is also present in Australia. The adult katydid grazes on young foliage and flowers of numerous plant species (Crowe 2002), and has been reported assuming the red tinge of the flowers when feeding on the brightly coloured *L. scoparium* cultivars (Natusch 1967).

7.4.6 Thysanoptera

One family of the Thysanoptera is recorded in association with *Leptospermum scoparium* (Table 7.6). The Thysanoptera are usually polyphagous sap feeders with approximately 34 species in New Zealand, many of which are endemic (Crowe 2002). Mound and Walker (1982) re-examined the New Zealand Aeolothripidae family and recorded two species inhabiting *L. scoparium*. *Scirothrips pan* was restricted to the northern South Island, and *Thrips obscuratus* was common throughout the North Island and northern South Island. Both species are generalised feeders, utilising other hosts as well as *L. scoparium*.

7.4.7 Arachnida

One species of the Arachnida is recorded as a *Leptospermum scoparium* associate (Table 7.6). Classified in the Eriophyinae family, a group of parasitic mites causing hypertrophy in plant species, *Aceria manukae* is widespread throughout the North Island and more common in the central and eastern areas (Manson 1984). Recorded only in association with *L. scoparium*, this gall-forming mite sometimes also forms witches brooms (Manson 1984).

7.4.8 Lepidoptera

Five families of Lepidoptera are recorded in association with *Leptospermum scoparium*, representing eleven species (Table 7.7). Spiller and Wise (1982) list a further four species, however these relationships are tenuous, having been drawn from early references that described *Kunzea ericoides* as a *Leptospermum* species, or have been contradicted by later research.

Table 7.7 Lepidoptera species reported in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Family	Species	Distribution	Occurrence	Comments
Geometridae[3]	*Declana floccosa*[2]	NI, SI[2]	Abundant[2]	Caterpillar feeds on *L. scoparium*[2]
Geometridae[3]	*Declana junctilinea*[2]	NI, SI[2]	Common[2]	Caterpillar feeds on *L. scoparium*[2]
Geometridae[3]	*Declana leptomera*[2]	NI, SI[2]	Common[2]	Caterpillar feeds on *L. scoparium*[2]
Geometridae[3]	*Poecilasthena subpurpureata*[2]	NI, SI[2]	Common[2]	Caterpillar feeds on *L. scoparium*[2]
Geometridae[4]	*Zermizinga indocilisaria*[4]	Central NI south[4]	Common[4]	Caterpillar feeds on *L. scoparium*[4]
Hepialidae[3]	*Aenetus virescens*[3]	NI[1]	Common northern regions[2]	Larvae feeds on living wood, rarely *L. scoparium*[2]
Oecophoridae[3]	*Hierodoris atychoides*[3]	NI, SI[5]	Common[5]	Caterpillar feeds on *L. scoparium*[5]
Psychidae[3]	*Liothula omnivora*[2]	NI, SI[2]	Common[2]	*L. scoparium* not principal host[2]
Tortricidae[3]	*Planotortrix excessana*[4]	NI, SI[2]	Common[2]	*L. scoparium* not principal host[2]
Tortricidae[3]	*Strepsicrates ejectana*[4]	NI, SI[4]	Common[4]	Caterpillar feeds on *L. scoparium*[4]
Tortricidae[4]	*Holocola zopherana*[4]	NI, SI[4]	Rare[4]	Caterpillar feeds on *L. scoparium*[4]

[1]Parkinson & Patrick 2000; [2]Gaskin 1966; [3]Dugdale 1988; [4]White 2002; [5]Hudson 1928.
Distribution abbreviations. NI – North Island; SI – South Island.

The Geometridae, of which the caterpillars move in a characteristic looping manner, are represented by five species. The caterpillar of the three *Declanna* species feed on *L. scoparium* foliage, and these species are commonly referred to as mānuka moths (Gaskin 1966; Molloy 1975). *Declanna floccosa* is abundant throughout both islands, and also feeds on other plant species (Gaskin 1966). *Declanna junctilinea* is widely distributed throughout the North Island but is only locally common in Marlborough and Southland in the South Island, and it also utilises other host plants (Gaskin 1966). *Declanna leptomera* is not found exclusively on *L. scoparium*, and is common throughout the North Island and reported from the eastern side of the South Island (Gaskin 1966). *Poecilasthena subpurpureata* is common throughout both islands and the caterpillar has been recorded on *Pinus radiata* as well as *L. scoparium* (Gaskin 1966). *Zermizinga indocilisaria* inhabits montane environments of the North Island Volcanic Plateau southwards including the South Island, and has been recorded on eight host plant species as well as *L. scoparium* (White 2002).

The Hepialidae, being swift and erratic fliers, are represented by one species, *Aenetus virescens*. This moth is found throughout the North Island, but is significantly more common in northern regions (Gaskin 1966). The larvae feeds on living wood and whilst *L. scoparium* is occasionally utilised *Vitex lucens* is the preferred host (Walker 2000), hence the common name puriri moth.

Hierodoris atychoides represents the Oecophoridae, the caterpillars of which feed in the confines of a silk web. This webworm is widespread throughout both islands and the caterpillar has been recorded feeding on *L. scoparium* and at least two other plant hosts (Hudson 1928).

The Psychidae, the larvae living and pupating in often-elaborate cases, are represented by one species, *Liothula omnivora*. This bagworm moth, as the species name suggests, feeds on almost any plant species and is common throughout New Zealand (Gaskin 1966).

The Tortricidae are represented by three species, and are named for the leaf-rolling habit of the larvae. *Planotortrix excessana* is common and found throughout New Zealand, and the caterpillar is more often found on introduced fruit trees rather than indigenous plant species such as *L. scoparium* (Gaskin 1966). *Holocola zopherana* is present throughout both islands but is uncommon, and is reported to feed exclusively on *L. scoparium* and *K. ericoides* (White 2002). *Strepsicrates ejecta* is also present throughout New Zealand, the caterpillar being a generalised feeder utilising Myrtaceae and Ericaceae species as hosts.

7.5 Nematoda associations

The nematodes are classified by gross morphology, and consequently different nutritional strategies are found within an order. However the two nematode orders that contain families considered to have a direct influence on plants are both present in New Zealand. The Tylenchida order contains free living plant root feeders and plant parasitic taxa, and the Dorylaimida are large free living nematodes that contain families that feed on plant roots (Goodey 1963). The endemic New Zealand nematodes and introduced species are listed by Wouts (1996), and the nomenclature in this discussion follows Goodey (1963). The forest nematodes have received less attention than the species parasitic on pasture plants, though a study of four forests within the North Island has been completed (Egunjobi 1971). Nematode infestation of *Leptospermum scoparium* has not been recorded, and was probably not present in the root material studied by Cook et al. (1980). However the Australian Myrtaceae are susceptible to infestation, and *Eucalyptus* spp. are utilised as hosts by plant parasitic nematodes (Ruehle 1973).

Therefore it is probable that *L. scoparium* may also be subject to nematode parasitism. However whilst the distribution and frequency of these parasites has not been fully described in New Zealand, the same criteria apply as demonstrated with the fungal associations.

Table 7.8 Nematode taxa in New Zealand that may influence *Leptospermum scoparium* UMF® production.

Order	Family	Habitat	Comments
Dorylaimida[1]	Belondiridae[1]	Forest and pasture soils[2]	Generalised root feeders[1]
	Dorylaimidae[1]	Forest and pasture soils[2]	Generalised root feeders[1]
Tylenchida[1]	Aphelenchoididae[1]	Forest and pasture soils[2]	Generalised root feeders[1]
	Criconematidae[1]	Forest and pasture soils[2]	Generalised root feeders[1]
	Heteroderidae[1]	Pasture soils[3]	Obligate host specific parasites[4]
	Hoplolaimidae[1]	Forest and pasture soils[2]	Obligate host specific parasites[4]
	Neotylenchinae[1]	Forest and pasture soils[2]	Generalised root feeders[1]
	Tylenchidae[1]	Forest and pasture soils[2]	Generalised root feeders[1]

[1]Goodey 1963; [2]Egunjobi 1971; [3]Yeates 1973; [4]Mercer 1994.

In a small geographic range, the nematode population would need to alter substantially without the effect of a strong environmental gradient. This pattern would need to be repeated in more than one region. Furthermore, the soil-dwelling nematode parasite would need to be prevalent in both the Waikato peat wetlands and the Coromandel clay soils. Consequently it is very unlikely that the influence of a nematode species infection of *L. scoparium* accounts for the variability reported UMF® in manuka honey.

7.5.1 Dorylaimida

The Dorylaimida order (Table 7.8) are large free living nematodes (Yeates 1975), and two plant feeding families are recorded in New Zealand. The Belondiridae and Dorylaimidae are found in pasture and forest soil (Egunjobi 1971), but are not as widespread or common as members of the Tylenchida order.

7.5.2 Tylenchida

Six families of the Tylenchida order (Table 7.8) are reported in New Zealand. The Heteroderidae and Hoplolaimidae are obligate parasites with specific hosts (Goodey 1963) The species within these families cause the root-knots, cysts, and root lesions often reported in pasture plant species (Mercer 1994), and have not been reported as parasites of *Leptospermum scoparium*.

The Heteroderidae are only reported beneath pasture whereas the Hoplolaimidae are also found in forest soils (Egunjobi 1971; Yeates 1975). The remaining four families are non-specific root feeders. The Tylenchidae are common beneath pasture and forest, and the Aphelenchoididae, Neotylenchinae, and Criconematidae are more often found in forest soils (Egunjobi 1971).

7.6 Conclusion

A number of candidate species form an association with *Leptospermum scoparium*. However a review of these species distribution, frequency within that distribution, and degree of association with *L. scoparium*, eliminates most possibilities.

The plant and Arthropoda taxa and ranges are well described in New Zealand, and no candidate species fulfils the requirement of a distribution and frequency that is identical to the geographic pattern exhibited by the UMF® in mānuka honey, and commonly utilises *L. scoparium* as a preferred host.

The groups that are not so well described, the fungi and nematode phyla, cannot be eliminated on the basis of reported distribution, frequency, and degree of association. However the geographic distribution of UMF® shows the improbability of a species influencing *L. scoparium* variably in a pattern that is identical to the distribution of UMF® throughout New Zealand.

Therefore a candidate species was not identified, and the hypothesis that another species influences the UMF® production of *Leptospermum scoparium*, giving rise to the variable UMF® in mānuka honey, is not supported.

Chapter 8

UMF® in mānuka honey and environmental variables

This chapter describes the multivariate analysis completed with Landcare Research to establish whether the geographic variability of UMF® recorded in mānuka honey, derived from *Leptospermum scoparium*, may be attributed to environmental factors.

The probability of the environment influencing nectar production of *L. scoparium* is summarised, and the environmental variables included in the analysis introduced. The statistical method is described.

The results indicated that the environmental parameters considered did not fully explain the variability of UMF® reported in mānuka honey, yet the regional factor overwhelmingly accounted for the UMF® range. This indicated *L. scoparium* found in different regions was distinct and producing different levels of UMF® in the nectar. Accordingly the hypothesis that an environmental factor was responsible for the variability of UMF® was rejected.

The material contained in this chapter has not been submitted for publication, as the thesis is subject to an embargo agreed with the commercial sponsors of this study.

8.1 Hypothesis

The hypothesis that the geographic variability of UMF® in mānuka honey arises from the influence of one or more environmental factors is explored.

The influence of the environmental factor or factors would affect either nectar yield or components, and differ geographically in accordance with the variability of recorded UMF® activity.

8.2 Nectar production

The floral nectar production of plants has been recognised to vary within a species, and much of this variability has been ascribed to environmental influences through a species' distribution. Generally, differences in secretion of nectar are attributed to irradiance, temperature, and soil water balance (Kearns & Inouye 1993). Field research and controlled experiments have limitations in determining the principal causes of the differences in nectar production between the flowers from one plant, between individual plants, or between populations of the species. The flower age, collection technique, diurnal production fluctuations, nectar re-absorption, and response to pollinator visits may confound nectar production responses to environmental influences (Kearns & Inouye 1993).

A significant genotypic control of nectar production and components has also been recorded. Flower nectar functions only as a reward for pollinators, and is considered to be controlled by pollinator selection pressure (Kearns & Inouye 1993). Therefore a considerable portion of nectar production variability within a species is probably genotypic, often masked by the overriding environmental influences (Mitchell 2004). Two recent studies of *Echium vulgare* confirmed both phenotypic and genotypic responses to water availability (Leiss et al. 2004; Leiss & Klinkhamer 2005). Whilst both the low and high nectar-producing varieties increased nectar yield when watered, thereby illustrating an environmental

influence, genotypic response was shown in dry field conditions where the yield of the high nectar-producing variety was significantly greater than the low nectar-producing variety (Leiss & Klinkhamer 2005).

The nectar components vary between plant species. A number of studies investigating the floral sources of honeys have attempted to seek correlation of the floral source with compounds originating in the nectar. A unique set of extractable organic substances have been described from mānuka honey (Wilkins et al. 1993). Where the same chemical substances are present in honey harvested from allied plant species the concentrations differ, illustrated by comparisons of mānuka honey with *Kunzea ericoides* (kānuka) (Tan et al. 1988) and *Leptospermum polygalifolium* (jelly-bush) honeys (Yao et al. 2003).

However the concentration of these floral components in mānuka honey also differs. Wilkins et al. (1993) compared mānuka honey samples from two seasons, and noted whilst the range of chemical constituents remained constant the concentration of those constituents differed significantly. The mānuka honey samples used were confirmed to be monofloral by pollen analysis: if this was correct, the differences in the concentration of nectar components may be due to either the honey being harvested from different varieties of the species or the influence of the environment.

In summary, a number of conclusions may be drawn. The total nectar yield is influenced by the environment, but yield may be also genotypically affected. Whilst the constituents of a species' nectar remain constant, considerable variability in the amount of those constituents is reported in monofloral honeys derived from the same floral source. This second level of variability may be phenotypic or genotypic. It is assumed that the environment influences *L. scoparium* nectar production is accepted, with the qualification that a genotypic component is also involved.

8.3 UMF® activity data

The UMF® data used in this section of the study is described in Chapter Six, and is detailed in Table 8.1. The UMF® activity data that had been adjusted for the dilution of mānuka honey by other honey types was utilised, as the analyses in this chapter were performed to compare the UMF® yields in monofloral mānuka honey with a set of environmental factors.

Table 8.1 The mean UMF® activity yielded in regions and sub-regions within New Zealand, adjusted to allow for dilution of mānuka honey by other honey types, upper and lower quartiles, and number of observations within divisions.

Region/sub-region	Mean UMF®	UMF® quartiles	Sites
1 - Waikato	15.3	15.1–15.7	6
2 - Coromandel/high	14.9	14.6–15.2	23
3 - Northland	14.8	14.2–15.4	35
4 - West Coast/high	14.4	13.7–15.1	24
5 - West Coast/mid	13.1	12.6–13.6	27
6 - Coromandel/mid	12.6	12.2–12.9	16
7 - East Coast/high	12.5	12.2–12.7	22
8 - East Coast/low	10.9	10.4–11.3	17
9 - Taranaki/high	10.6	9.6–11.3	32
10 - Coromandel/low	10.5	9.8–11.1	63
11 - Gisborne	10.4	10.2–10.6	2
12 - Eastern South Island	9.6	9.1–10.0	14
13 - Wairarapa	9.4	8.4–10.1	12
14 - North South Island	9.1	8.5–9.6	41
15 - West Coast/low	9.0	8.3–9.4	10
16 - Taranaki/low	8.9	8.0–9.5	19
17 - Hawkes Bay	8.4	7.6–9.1	38

The unadjusted UMF® activity data recorded the UMF® of the honey harvested at a site, and did not account for dilution by other honey types. The correlation of the unadjusted UMF® data, where dilution by other floral sources at harvest or dilution caused by the apiarists' extraction technique had occurred, would establish the relationship between the abundance of *Leptospermum scoparium* at a site or the monofloral purity of mānuka honey after extraction by the apiarists with environmental variables rather than the potential UMF® yield at that site. For example, a site that carried principally *L. scoparium* and *Kunzea ericoides* would yield mānuka and kānuka honey, the former species flowering before the latter. If the sample from the site represented the earlier honey flow the honey would be comprised of monofloral mānuka, however if all honey from that site was extracted together and combined the sample would represent a mānuka/kānuka mixture.

8.4 Environmental factors

Irradiance, temperature, and water balance are the environmental factors reported to influence nectar production, and therefore the components of these factors were analysed. These environmental components have been shown to demonstrate functional links with plant physiological processes (Landsberg 1986), and are listed in Table 8.2.

The climatic variables were estimated for points on a 100 m grid across New Zealand from thin plate splines (Hutchinson & Gessler 1994) fitted to meteorological data (Leathwick & Stephens 1998).

Mean annual temperature represents the mean of 12 monthly averages, a parameter that strongly affects plant productivity (Leathwick et al. 2003). The mean daily minimum temperature of the coldest month, July, is considered to influence bud development phenological processes (Jones 1994).

Table 8.2 The definition and derivation of the environmental factors employed in the study.

Name of variable	Abbreviation	Definition	Units	Category
Mean annual temperature	Mat	Mean annual temperature	°C	Climate
Minimum temperature	Tmin	Mean July minimum temperature	°C	Climate
Mean annual solar radiation	Mas	Mean annual solar radiation	$MJ.m^{-2}.day^{-1}$	Climate
Minimum solar radiation	Smin	Mean June minimum solar radiation	$MJ.m^{-2}.day^{-1}$	Climate
Soil water deficit	Def	Annual rainfall minus evaporation	mm	Climate
Rain to potential evaporation	R/pev	Mean monthly average of rainfall to potential evaporation	Ratio	Climate
Vapour pressure deficit	Vpd	Mean October vapour pressure deficit	kPa	Climate
Slope	Slope	Slope estimated from New Zealand Land Resource Inventory	7 classes[1]	Landform
Drainage	Drain	Derived from New Zealand Land Resource Inventory and New Zealand Soil Classification	5 classes[2]	Landform
Soil particle size	Spart	Derived from New Zealand Land Resource Inventory and New Zealand Soil Classification	5 classes[3]	Landform

[1]Flat (0-3°), undulating (4-7°), rolling (8-15°), strongly rolling (16-21°), moderately steep (21-25°), steep (26-35°), very steep (>35°).
[2]Very poor, poor, imperfect, moderate, good.
[3]Clay/silt (<0.06 mm), sand (0.06-2 mm), gravel (2-60 mm), coarse gravel (60-200 mm), boulders-massive (>200 mm)

Plant productivity, which is largely determined by solar energy in temperate latitudes, was described by mean daily annual solar radiation (Leathwick et al. 2003), and seasonal variation was described as the minimum mean daily winter solar radiation in June (Leathwick et al. 2003).

Water balance was described by a combination of annual soil water deficit, vapour pressure deficit, and rainfall to potential evaporation. Annual soil water deficit was derived from the water balance model between rainfall and potential evaporation (Leathwick et al. 2002), whereas the rainfall to potential evaporation variable was a monthly indicator of water balance determined as a mean annual ratio (Leathwick et al. 2002), and whilst these measurements correlate for areas experiencing a water deficit, at many sites rainfall exceeds potential evaporation and influences plant characteristics (Lehmann et al. 2002b). The mean 9 am vapour pressure deficit in October was used to determine relative humidity during the flowering season of *Leptospermum scoparium*, when the predominately westerly winds promote a strong difference of vapour pressure deficit in New Zealand (Leathwick et al. 2002).

Landform factors were also considered. The landform characteristics influence the climatic variables, and therefore provide a further layer of definition. Slope, drainage, and soil particle size influence soil water deficit. Slope and drainage were derived from the New Zealand Land Resource Inventory database, the former measured by a seven-step scale and the latter a five-step scale (Leathwick et al. 2003). Likewise soil particle size was also derived from the New Zealand Land Resource Inventory database, and assessed on a five-step scale (Milne et al. 1995).

Other climatic/landform variables were considered but were not included in the analysis as a convincing correlation with one or more of the above variables existed. Elevation and mean annual temperature were similar, and soil parent materials and soil fertility variables were not included as no evidence of an

influence existed, exploratory analysis included these variables however the models proposed did not utilise these environmental characteristics.

Region coding was also considered, and Table 8.1 details the codes (1 - 17) assigned. The inclusion of region codes allowed analysis in discrete groups, and was also used to determine whether the geographical distribution of the UMF® activity in the regions/sub-regions explained more of the variability in the generalised additive models than the climatic and landform parameters discussed.

Table 8.3 describes the correlation of the environmental factors. The most significant correlations were between mean annual and minimum winter temperature (*r* 0.96) and mean annual and minimum winter solar radiation (*r* 0.85) however neither variable was dropped as these measures of seasonality account for low readings that are dissimilar to averaged minimum figures in some geographic regions (Lehmann et al. 2002a). The high correlation of these parameters indicates *L. scoparium* scrub from which mānuka honey is harvested is for the most part not subject to climatic winter extremes.

Table 8.3 Correlations of the environmental factors.

	Mat	Tmin	Mas	Smin	Def	R/pev	Vpd	Slope	Drain	Psize
Mat	–	0.96	0.69	0.85	-0.08	-0.34	0.23	-0.26	-0.38	0.16
Tmin	–	–	0.56	0.78	-0.16	-0.25	0.11	0.31	-0.42	0.13
Mas	–	–	–	0.87	0.14	-0.62	0.63	0.32	0.08	0.33
Smin	–	–	–	–	0.01	-0.52	0.47	0.02	-0.17	0.21
Def	–	–	–	–	–	-0.49	0.60	0.13	-0.26	-0.32
R/pev	–	–	–	–	–	–	-0.79	-0.31	-0.26	-0.32
Vpd	–	–	–	–	–	–	–	0.39	0.31	0.34
Slope	–	–	–	–	–	–	–	–	0.65	0.51
Drain	–	–	–	–	–	–	–	–	–	0.04

8.5 Method

The statistical analyses were completed by the Generalised Regression Analysis and Spatial Prediction (GRASP) model system developed by Landcare Research (Lehmann et al. 2002a). The general concept of generalised regression analysis and the processes followed in these analyses is presented in the following section.

8.5.1 Generalised regression analysis

The generalised regression model develops statistical relationships between the variable of interest (UMF®) and a set of environmental variables. The process can be described as the behaviour of the response variable (UMF® activity) in relation to climate and landform. This process may be illustrated with a simple example. The relationship between mean annual temperature and UMF® activity is a simple regression technique, and allows a correlation to be drawn. However, here many variables are included, and the model predicts the proportion of variability that can be assigned to each response environmental variable. Both continuous and categorical variables can be included in the analysis, the model treating the former as a regression and the latter as an analysis of variance (Fraser et al. 2004). The GRASP system uses a generalised additive model, considered the most appropriate method for the exploration of environmental gradients in accordance with ecological theory (Lehmann et al. 2002a).

8.5.2 Environmental variables

The data for environmental variables described in Section 8.4, representing each 100 m grid site, were transformed to allow the analyses to be completed, and two difficulties were simultaneously overcome. Firstly a level of homogeneity at the grid sites was created, and secondly the environmental data at all grid sites represented the environmental conditions found in nearby scrub from which mānuka honey would have been produced.

Mānuka honey is created by honeybees from the nectar produced by *Leptospermum scoparium*. Therefore the locations of *L. scoparium* throughout New Zealand determine the environmental conditions that are applicable to the UMF® in the honey.

Much of New Zealand's geographic space no longer supports *L. scoparium*. The Land Cover Database was employed to select those areas containing scrub. The relevant set of environmental variables was mined for each 100 m grid site within these scrub areas. Two transformations were then completed. The environmental data from each grid site that carried scrub was averaged to represent the mean values of the environmental data from grid sites within a 10 km radius. Grid sites that did not carry scrub were converted to reported the environmental data from the most proximate sites with scrub. These transformations created a neighbourhood smoothing effect; the grid sites represented the mean values for the environmental variables of nearby scrub.

Honeybees will travel up to 10 km from hives to a nectar source, and consequently range widely around the hive sites. Furthermore, for ease of access many apiarists place hives in developed land adjacent to undeveloped indigenous scrub. Therefore the geographical locations reported with the samples often represented proximate agriculturally developed land, and not nearby scrub. Mānuka honey produced from such a site was sourced by the honeybees flying to *L. scoparium*, and not at the hive sites. Accordingly the UMF® site data was not re-positioned within the nearby scrub, and the data of all grid sites was transformed to represent a mean value of the environmental data from the nearest scrub that may produce mānuka honey.

8.5.3 Model selection, interpretation and validation

A Gaussian model was selected as the UMF® data followed an equal binominal distribution. The model evaluated the environmental factors and selected those factors that correlated with the UMF® data (Lehmann et al. 2002a). The model results were analysed by three sets of data.

Two contribution graphs were produced for each multiple regression. The second contribution graph details the drop contribution. The drop contribution describes the difference between the variability explained by the full final model and the model with a variable excluded. In effect the drop contribution illustrates the amount of variability that the other contributing factors can explain for any given variable when that variable is removed from the model, and is calculated by dropping out single variables, one at a time, and recording the decrease in amount of variability explained. If a variable is excluded from the final model, that variable does not account for any variability (Overton & Lehmann 2003). The second contribution graph details the alone contribution. The alone contribution describes the significance of each environmental variable. Alone contribution is calculated by having no variables in the model, each variable is then put into the model alone and the amount of variation explained is recorded (Overton & Lehmann 2003).

The partial response curves for each environmental predictor can also be examined to determine the response of UMF® yield to each environmental variable (Lehmann et al. 2002a). This data set allows comparison of trend behaviour between the response variable and the predictor variables alone.

The validation involved testing for a correlation of the observed and predicted UMF®, indicating the overall validity of the generalised additive model obtained. To test reliability of the model, cross-validation was performed using five random subsets of the data (Lehmann et al. 2002a). New data was not employed for cross-

validation, as the data set represented one season of mānuka honey production and collection variability may have been introduced (Lehmann et al. 2002a).

8.6 Results and discussion

The results of the multiple regression analyses of New Zealand UMF® data are presented in detail. To assist with interpretation two analyses are compared, the first using the environmental factors discussed above, and the second the environmental factors and the region coding together.

8.6.1 Contribution of predictors

The drop contribution graphs (Figure 8.1) of the environmental analysis illustrated mean annual temperature was the most important variable. Any combination of the other six variables selected by the model, minimum temperature, mean annual solar radiation, minimum solar radiation, rain to potential evaporation, vapour pressure deficit, slope and drainage, did not account for the variability explained by mean annual temperature. Therefore the conclusion may be drawn that mean annual temperature is the most important factor in this set of environmental variables.

However the inclusion of the region code, in addition to the environmental factors analysed above, significantly alters this conclusion. Only two factors are shown to be significant; the region code accounts for virtually all of the variability, and mean annual temperature, whilst still present, becomes less important.

This indicates that whilst the mean annual temperature may contribute a little to the UMF® variability reported, the overriding factor is the region/sub-region from which the honey is harvested. Consequently the environmental factors associated with nectar yield and any gradients of these factors between regions are not

important; the variability results from differences between the *Leptospermum scoparium* populations within these regions.

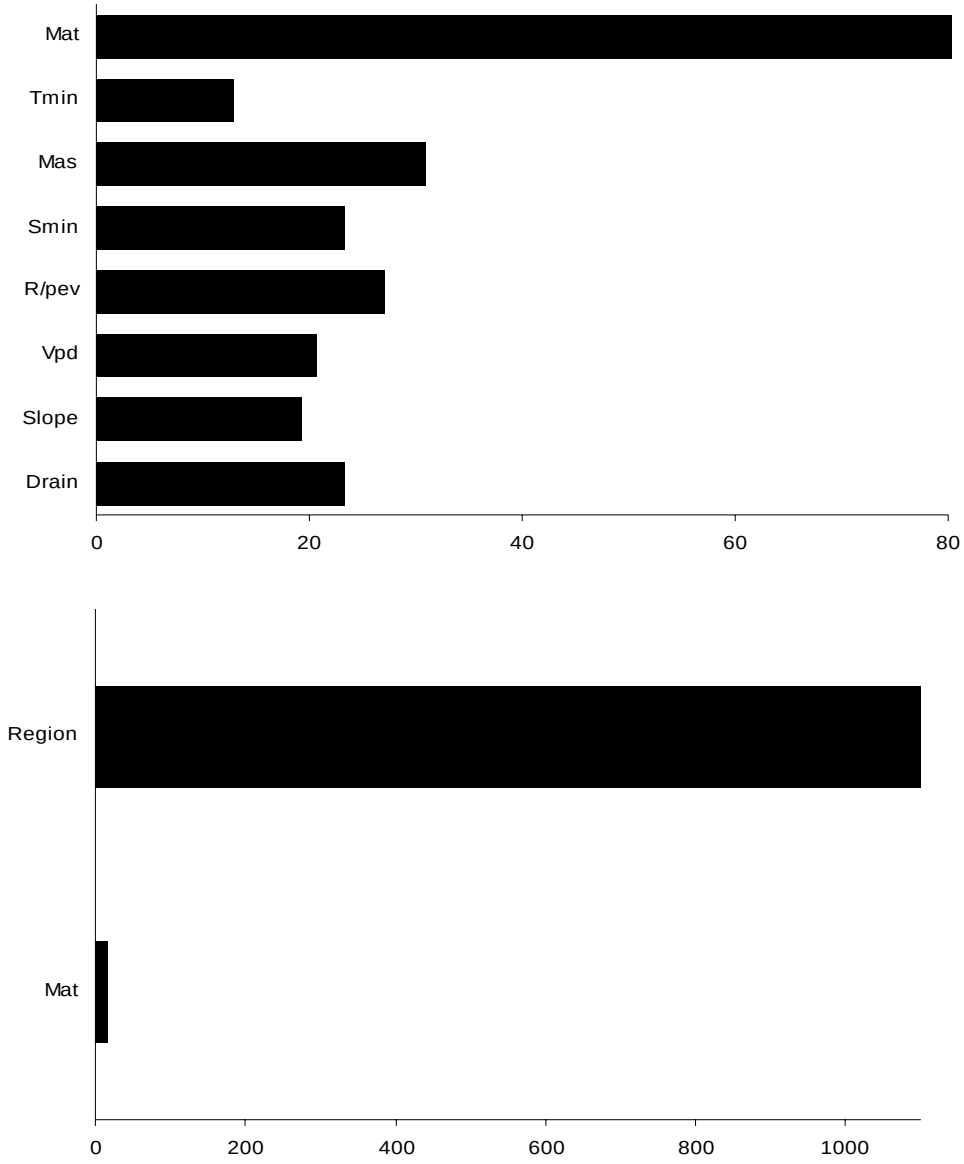

Figure 8.1 Model drop contribution graphs, showing relative degree of contribution of selected factors, environmental factors (above), and environmental factors and region code (below). Abbreviations defined in Table 8.2.

The alone contribution analyses (Figure 8.2) demonstrate the potential of each variable to explain the distribution of UMF® activity. The environmental factors ranked in decreasing order, drainage, minimum solar radiation, mean annual temperature, minimum winter temperature, slope, mean annual solar radiation, vapour pressure deficit and rain to potential evaporation are important until the region code is introduced as additional factor. The region code alone accounts for most of the variability, and the environmental factors other than mean annual temperature and minimum winter temperature are no longer important alone contributors.

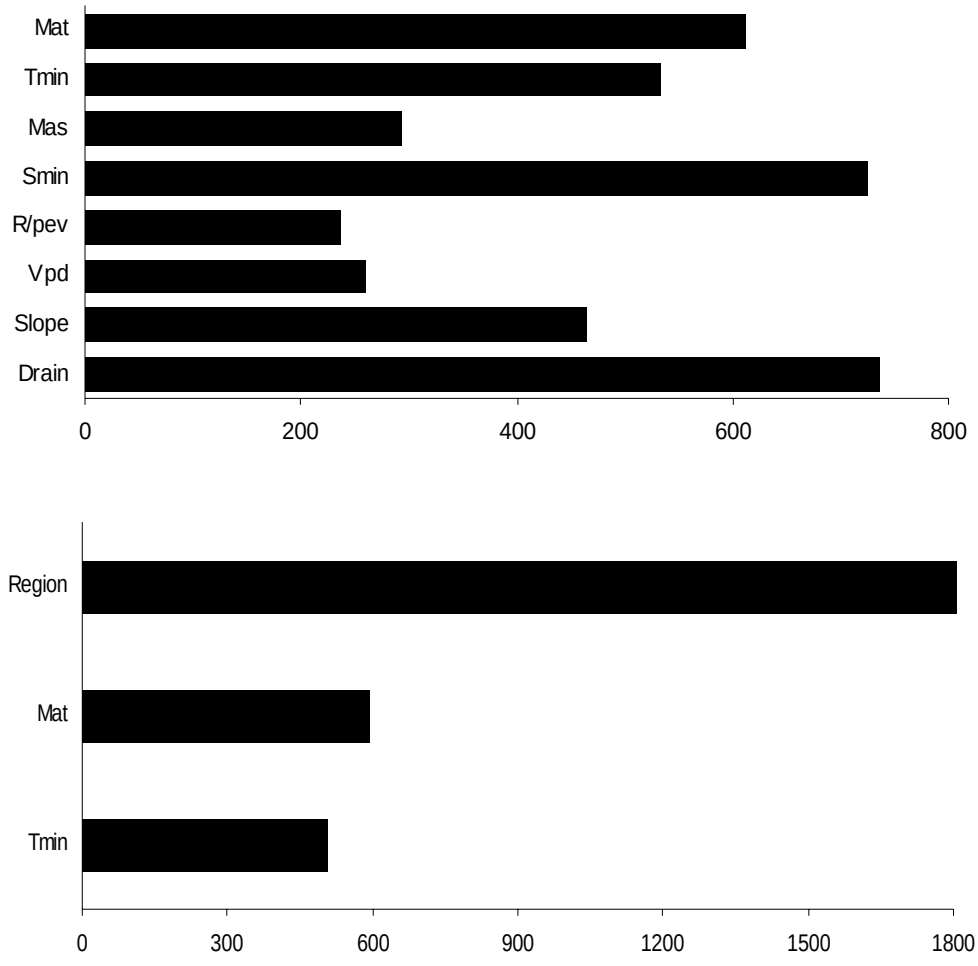

Figure 8.2 Model alone contribution, showing relative degree of contribution of selected factors, environmental factors (above), and environmental factors and region code (below). Abbreviations defined in Table 8.2.

8.6.2 Partial response curves

The partial response curves require cautious interpretation as the region code, a non-environmental categorical factor, has been shown to account for the most variability. Nonetheless the two environmental factors (Figure 8.3) included in the final model are initially reported with accompanying clarification. The dotted lines parallel with the *x*-axis on the graphs represent an indication of the number of observations in that portion of the prediction, and dashed lines with the partial response curve the upper and lower 95% confidence intervals.

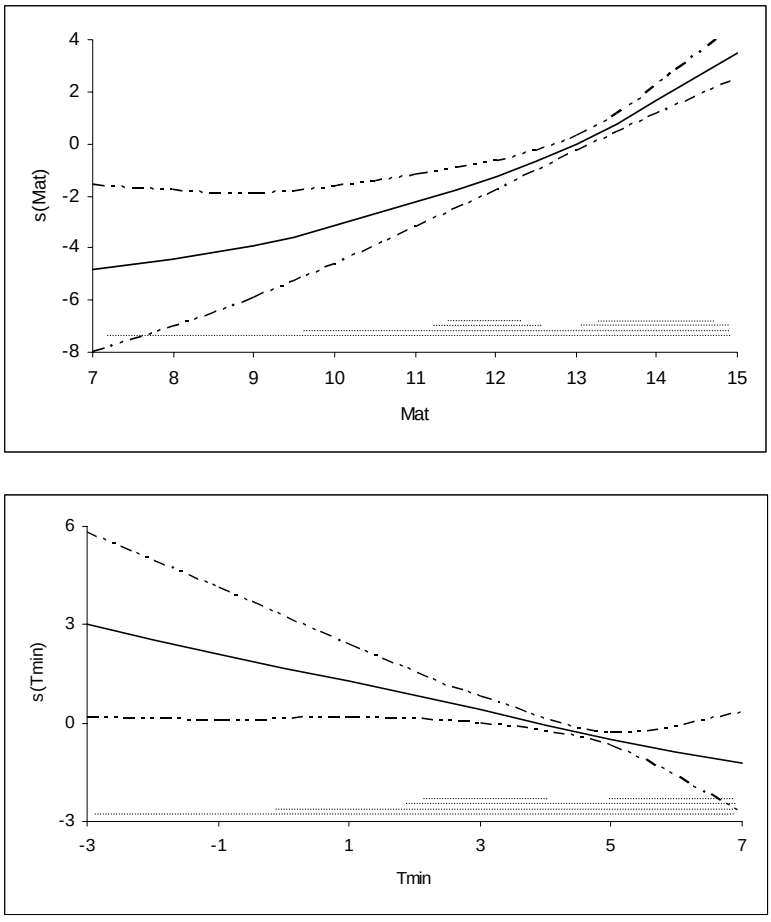

Figure 8.3 Partial response curves showing model relationship between UMF® activity and mean annual temperature (above) minimum winter temperature (below) derived from the model. Abbreviations are defined in Table 8.2.

UMF® activity was characterised by a positive response to mean annual temperature, confirming the general relationship between nectar production and temperature. However the relationship between minimum winter (July) temperature and UMF® contradicted the partial response curve of mean annual temperature. As the minimum winter temperature increased a linear decline of UMF® was recorded. This pattern was partially interpreted as the influence of the regions that carry *Leptospermum scoparium* producing high activity UMF® mānuka honey yet are subject to cold winter temperatures, for example the Waikato wetlands and West Coast pakihi sites. Furthermore the model provided large confidence intervals at either end of the range.

Further consideration of the other six partial response curves derived from the model containing environmental factors without the region code is worthwhile. For the most part the water balance environmental factors responded as expected, wetter more favourable conditions for nectar production and *L. scoparium* growth and enhanced UMF® production. Increasing October vapour pressure deficit lead to decreased UMF® yield, and better drainage negatively influenced UMF® production. The rain to potential evaporation ratio increased yield until the ratio 0.8 was reached, then UMF® yield began to decrease. Low to moderate mean annual solar radiation correlated with a greater UMF® yield, however higher solar radiation led to a decrease. This was a response to location, *L. scoparium* in high mean annual solar radiation regions, Gisborne, Hawkes Bay and the north South Island, yielded low activity UMF® mānuka honey and consequently the general correlation of UMF® and increasing solar radiation was curtailed. The minimum winter solar radiation partial response curve fitted with latitude, the region/sub-regions providing high UMF® from the South Island West Coast and northern North Island and the low activity honeys harvested in the centre of the New Zealand latitudinal range meant a parabolic curve was produced, another example of region distribution affecting the model built on environmental factors alone. The slope partial response curve did not reveal an interpretable pattern, slope between 0–10° linearly reduced yield, however this was reversed for locations with a slope >10°.

Notwithstanding the replacement of the environmental factors model with a model containing region codes, a number of observations may be made. Mean annual temperature, along with the water relations at a site, positively influence UMF® yield. However excessive rainfall leads to a decrease in yield, understandable in terms of the gross morphology of *L. scoparium* flowers that are exposed to rain allowing the rinsing of nectar. The UMF® yield decline in excess rainfall is contrary to the general improvement of nectar yield by improving water relations and despite the enhancement of *L. scoparium* growth in such areas.

8.6.3 Validation and cross-validation of models

Correlations between the actual and predicted UMF® activity was used to test the goodness of fit for the Gaussian models, describing the Pearson coefficient. The model utilising environmental factors correlated observed and predicted UMF® with lower accuracy (r 0.748) than the model including the region code (r 0.91). The former model explains approximately half the variability, whereas the latter accounts for slightly more than four-fifths of the variability. Therefore the model including region code is the better fit, and the region from which the mānuka honey was harvested is of primary importance, rather than environmental gradients.

The cross validation of the models, using five random subsets of data, recorded the environmental data model correlation (r 0.717) as explaining a little less of the variability. However the model that included the region coding cross-validation correlation value (r 0.902) remained virtually identical to the original model, indicating good model stability.

Therefore region determines the UMF® yield to the greatest extent, and coupled with the temperature environmental factors explains much variability (r^2 0.8281).

To confirm the importance of the region code an analysis was completed using the region code as the only predictive factor. The validation model (*r* 0.907) and the cross-validation (*r* 0.901) correlation values validate the opinion the region from which the mānuka honey is harvested explains the most variability.

8.7 Conclusions

The environmental factors considered do not explain most of the variability of UMF® activity in mānuka honey harvested throughout New Zealand. Another parameter, which is closely correlated with the region/sub-regions outlined in Table 8.1, is responsible for the range in UMF® activity. Nectar from another plant species or the by-product of another species has been shown to be incorrect (see Chapter Four), and a causative relationship between *Leptospermum scoparium* and another species is highly unlikely (see Chapter Seven).

Therefore a difference between the *L. scoparium* populations growing in these regions may be present, and that difference explains the variability of UMF®. Previous studies have shown nectar production and composition may be influenced genotypically, and the variety status of *L. scoparium* growing in these regions may be responsible for the UMF® variability. These varieties of *L. scoparium* may be better adapted to certain environments, and therefore assume the status of ecotypes. The environmental preferences of these ecotypes may account for the partial contribution of climatic factors to the model that included the region/sub-region coding. This hypothesis is explored in Chapters Nine, Ten, and Eleven.

Chapter 9

The morphology of *Leptospermum scoparium* in the North Island

This chapter describes the morphology and morphological analysis of the *Leptospermum scoparium* populations growing in five regions in the North Island of New Zealand.

The genotypic morphological traits of *L. scoparium* are reviewed, and the characteristics chosen for analysis described. Analysis of those traits in *L. scoparium* populations of the North Island revealed three distinct varieties, in accordance with previous literature, and the presence of these varieties in the study region is discussed.

The distribution of the varieties is considered and the hypothesis that *L. scoparium* varieties account for the variable UMF® distribution reported in Chapter Six is developed. A relationship between the distribution of the varieties and the UMF® activity in mānuka honey is described.

The material contained in this chapter has not been submitted for publication, as the thesis is subject to an embargo agreed with the commercial sponsors of this study.

9.1 Hypothesis

The hypothesis that the variability of UMF® activity in mānuka honey arises from the presence of different varieties of *Leptospermum scoparium* in geographical regions of New Zealand is explored. This chapter details the morphological research; Chapters Ten and Eleven describe the chemotaxonomic and population genetics analyses respectively. These three methods were employed to ascertain whether distinct varieties exist, to describe geographical distribution of the varieties, and to establish whether the different methods revealed similar *L. scoparium* populations.

The *Leptospermum scoparium* varieties must fulfil one requirement; have a geographic range that explains the variable distribution of UMF® in mānuka honey between and within regions.

9.2 Morphological traits of *Leptospermum scoparium*

The morphology of *Leptospermum scoparium* has received attention, and the species is recognised as a variable taxon. The early botanists in New Zealand (Hooker 1867; Cockayne 1919; Cheeseman 1925) considered the species to contain varieties but the classifications differed. More recently Allan (1961) described two varieties and mentioned a further four, suggesting that the forms may result from either environmental modification or be genetically determined.

For a morphological analysis of *L. scoparium* to be used to determine variety status it requires the employment of characteristics that are determined genetically. A number of characteristics have been shown to be genetic traits by common garden experiments. The leaves of seedlings transplanted by Burrell (1965) into a more favourable position immediately increased in size but the ratio of dimensions remained typical of the ecotype. Others have also found that leaf dimensions have had a significant genetic component (Yin et al. 1984; Harris

2002). The frequency of capsule-splitting illustrated a genetic response (Harris 2002). Growth form has also been shown to be genetically determined (Harris 1994): the prostrate form found in sub-alpine positions described by Allan (1965) has been developed ornamentally (Dawson 1997b). Furthermore, tolerance of soil acidity (Berninger 1992), soil fertility response (Lyon et al. 1971), root anatomy (Cook et al. 1980), and freezing resistance (Greer et al. 1991; Decourtye & Harris 1992) are all characteristics containing a degree of genotypic control.

This section of the study was carried out on five regions within the North Island that yielded a range of UMF® in mānuka honey. Therefore the morphological characteristics utilised were chosen to illustrate differences between these populations. Leaf dimensions were selected as the regions studied contained four of the varieties previously described having different leaf characteristics (Allan 1961). *Leptospermum scoparium* var. *scoparium*, *L. scoparium* var. *myrtifolium*, and *L. scoparium* var. *linifolium* were considered widespread; and *L. scoparium* var. *incanum* is common especially in the North Auckland Botanical District. Plant growth dimensions were not utilised, as field experience suggested that in the study regions little difference would be encountered, and the varieties with different growth form previously described (Allan 1961; Harris 2002) were not present. However capsule splitting has been described as being significantly different within the study regions (Harris 2002), and was therefore included for analysis. The balance of the characteristics described above subject to genetic control was not considered suitable for field measurement.

9.3 Site descriptions

Leptospermum scoparium populations identified from previous publications, topographic maps and field observation throughout the North Island of New Zealand were sampled (Table 9.1). These regions represented the range of UMF® activity recorded in mānuka honey from the study. The mean UMF® yield in mānuka honey shown in Table 9.1 for each region/sub-region was adjusted to account for the dilution by other honey types, as reported in Chapter Six. The site

coordinates and names represent the collection details of *L. scoparium* material, and only broadly correspond to the apiarist's sites.

Table 9.1 Regions, mean adjusted UMF® activity, sites, location and site descriptions of *Leptospermum scoparium* populations sampled.

Region	Adjusted UMF®	Site	Coordinates	Habitat description	Altitude (m)
Northland	14.8	Omanaia	35°27' S, 173°31' E	Lowland scrub	20-40
		Takehe	35°28' S, 173°40' E	Lowland scrub	40
		Ngawha Springs	35°25' S, 173°51' E	Hill scrub	100
		Maromaku	35°29' S, 174°05' E	Hill scrub	150
		Kawakawa	35°27' S, 174°04' E	Hill scrub	100
Coromandel	14.9	Colville	36°39' S, 175°29' E	Coastal hill scrub	20-100
		Kuaotunu	36°43' S, 175°45' E	Coastal hill scrub	150
		Hahei	36°55' S, 175°41' E	Coastal hill scrub	100-200
	10.5	Waikawau	36°57' S, 175°29' E	Coastal hill scrub	20-100
		Whenuakite	36°57' S, 175°48' E	Coastal hill scrub	20-200
Waikato	15.3	Meremere	37°20' S, 175°10' E	Lowland swamp	20
		Whangamarino	37°20' S, 175°10' E	Lowland swamp	20
		Te Kauwhata	37°23' S, 175°08' E	Lowland swamp	20
		Orini	37°34' S, 175°16' E	Lowland swamp	20
		Torehape	37°20' S, 175°28' E	Lowland swamp	20
Central North Island	8.6	Rotorua	38°27' S, 176°03' E	Highland scrub	500
		Rangipo	39°12' S, 175°45' E	Montane scrub	900
		Raetihi	39°23' S, 175°15' E	Highland scrub	600
		Kuripapango	39°25' S, 176°20' E	Montane scrub	800
		Esk Valley	39°16' S, 176°42' E	Hill scrub	400
East Coast	10.9	Ruatoria	37°52' S, 178°12' E	Hill scrub	120
		Te Araroa	37°39' S, 178°20' E	Coastal scrub	10
		Whanarua Bay	37°41' S, 177°47' E	Coastal hill scrub	100
	12.5	Hicks Bay	37°34' S, 178°19' E	Coastal hill scrub	100
		Whangaparoa	37°35' S, 178°02' E	Coastal scrub	20

The Northland sites represented the infertile highly-leached podzol gumlands soils dominated by *L. scoparium*; and the Waikato sites were peat wetlands again dominated by *L scoparium*. The Coromandel and East Coast sites were regenerating seral scrub growing on either volcanic or sedimentary derived clays, and neither region was dominated by *L. scoparium*. The Central North Island sites were a range of higher altitude environments often on recent volcanic substrates and not dominated by *L. scoparium*.

9.4 Materials and methods

The sites described above were visited during spring 2003. Authority was sought from the District Councils to collect *Leptospermum scoparium* material from roadsides. Site details of location, altitude, habitat, and associated species were recorded on a collection sheet. Five plants were sampled at each site, and the sampling was at least 100 m apart to minimise the possibility of using closely related individuals. To reduce within-tree variation, one-year-old apical branches 2 m from the ground were selected for analysis. Approximately 30 mature leaves were stripped from the branches, the largest ten mature leaves were selected visually, and the greatest length and width of each of these were measured (Mitutoyo Digimatic Calipers Series 500). The proportion of unsplit capsules was recorded from ten capsules from the two-year-old wood of the same branch for each plant sampled.

Ecological differences may influence genotypic morphological characteristics, however the ratio of dimensions of the *L. scoparium* leaf is thought to remain fairly constant regardless of environmental pressures (Burrell 1965). Whilst leaves may display phenotypic plasticity (Blue & Jensen 1988), leaf morphology may reveal differences within (Jensen et al. 1984) and between populations of a species (Dickinson et al. 1987). The leaves of *L. scoparium* are linear–lanceolate–ovate with entire margins and not complex. The simple structure of the leaves meant size and shape were the most appropriate analyses (Jensen et al.

2002), and these were completed utilising leaf length and width, leaf length/width ratio, and shape which was visually determined from the leaf shape diagram in Allan (1961). The shape was recorded on a scale of 1–5, 1 being the most linear, 2 linear–lanceolate, 3 representing lanceolate, 4 lanceolate–ovate, and 5 ovate. This comparison was completed after all the leaf material from the sites had been collected.

Accordingly the data used in the morphological analysis was leaf length, leaf width, leaf length/width ratio, leaf shape, and proportion of unsplit capsules. Assumptions of normality were accepted for statistical analyses, principal component analysis and dendrograms were produced using StatSoft Statistica (Ver. 6). Euclidean distance was employed to define the geometric distance between sites and therefore the relatedness, and complete linkage selected as the data illustrated clustering behaviour prior to analysis.

9.5 Morphological descriptions

The results of the morphological analyses are summarised in Table 9.2. The morphological characteristic data suggested *Leptospermum scoparium* exhibits a range of forms in accordance with previous authors. The leaf characteristics and retention of unsplit capsules in the separate populations was distinct for the most part, and where one characteristic was shared between populations other features would allow a separation to be made.

This section includes illustrations of the *L. scoparium* plant material from populations in each region (Figures 9.1 – 9.6). These have been produced to display the morphological differences between the populations in the regions studied, and where applicable, differences within a region.

Table 9.2 Morphological analysis of five *Leptospermum scoparium* populations in the North Island, detailing mean (±SD) leaf length and width (mm), leaf length/width ratio, proportion of unsplit capsules, and leaf shape. The variety is shown where naming is established.

	Site	Length	Width	Unsplit capsules ratio	Length/ width ratio	Shape*	Variety
Northland	Omanaia	15.12±0.84	2.28±0.24	0.96±0.05	6.68	lan	*incanum*
	Takehe	14.47±0.84	2.17±0.2	0.98±0.04	6.74	lan	*incanum*
	Ngawha	14.84±0.79	2.26±0.21	0.88±0.08	6.62	lan	*incanum*
	Maromaku	12.94±0.4	2.12±0.17	0.98±0.04	6.13	lan	*incanum*
	Kawakawa	14.36±0.44	2.26±0.17	0.9±0.1	6.37	lan	*incanum*
Waikato	Meremere	9.82±0.7	1.26±0.11	0.46±0.09	7.84	lin	*linifolium*
	Whangamarino	9.5±0.82	1.31±0.13	0.44±0.09	7.29	lin	*linifolium*
	Te Kauwhata	9.11±0.85	1.26±0.12	0.42±0.13	7.28	lin	*linifolium*
	Orini	9.26±0.73	1.26±0.09	0.5±0.1	7.38	lin	*linifolium*
	Torehape	9.29±0.57	1.29±0.09	0.52±0.08	7.21	lin	*linifolium*
Coromandel	Waikawau	9.97±0.67	1.87±0.15	0.3±0.07	5.35	lin-lan	
	Colville	13.06±0.52	2.08±0.11	0.76±0.11	6.29	lan	*incanum*
	Kuaotunu	13.66±0.35	2.25±0.15	0.84±0.09	6.11	lan	*incanum*
	Hahei	13.76±0.34	2.26±0.11	0.84±0.05	6.09	lan	*incanum*
	Whenuakite	9.81±0.39	1.82±0.1	0.22±0.4	5.4	lin-lan	
East Coast	Ruatoria	6.38±0.32	1.43±0.1	0.26±0.05	4.46	lin-lan	
	Te Araroa	6.74±0.21	1.58±0.08	0.24±0.04	4.27	lin-lan	
	Hicks Bay	12.62±0.48	2.26±0.2	0.84±0.05	5.61	lan	*incanum*
	Whangaparoa	13.37±0.51	2.46±0.09	0.94±0.05	5.45	lan	*incanum*
	Whanarua Bay	7.92±0.4	1.77±0.1	0.32±0.09	4.49	lin-lan	
Central North Island	Rotorua	6.56±0.51	2.28±0.23	0.36±0.05	2.89	lan-ov	
	Rangipo	5.96±0.44	2.37±0.23	0.06±0.05	2.53	ov	*myrtifolium*
	Raetihi	5.82±0.36	2.48±0.17	0.04±0.05	2.35	ov	*myrtifolium*
	Kuripapango	5.62±0.29	2.32±0.22	0.26±0.05	2.44	ov	*myrtifolium*
	Esk Valley	6.47±0.43	2.46±0.28	0.38±0.08	2.67	lan-ov	

*Shape abbreviations. lin – linear; lin-lan – linear lanceolate; lan – lanceolate; lan-ov – lanceolate ovate, ov – ovate.

9.5.1 Northland population

The Northland *Leptospermum scoparium* population represented the description of *L. scoparium* var. *incanum* provided by Allan (1961). The plant leaf was lanceolate in shape with distinctive grey/silver-coloured tomentum on juvenile growth. Mature leaves were glabrous, with a mean length of (13)–14.3–(15) mm, and a mean width of (2)–2.2–(2.5) mm. The mean leaf length/width ratio in the Northland sites was 6.5. Petal colour was pink/white–pink/white, with a gradient of more pink colours in the north and more white in the south of the region and often blended. The capsules were overwhelmingly unsplit with only approximately 5% split, in agreement with Harris (2002). Figures 9.1 and 9.2 illustrates *L. scoparium* var. *incanum* with these characteristics.

9.5.2 Waikato population

The Waikato populations were also homogeneous, with a linear leaf shape and sparse tomentum on juvenile growth. Mean leaf length was (9)–9.4–(10) mm. Mean leaf width was (1.1)–1.3–(1.4) mm, the leaf length/width ratio was 7.4. Therefore the *Leptospermum scoparium* leaves in the Waikato wetlands' population were approximately two-thirds of the length and half the width of the Northland populations.

Petal colour was uniformly white, and approximately half the capsules were unsplit. In the wetlands these plants have an erect growth habit, however this may be due to the density rather than a genetic control as isolated individuals were more branched. The Waikato *Leptospermum scoparium* populations represented the description of *L. scoparium* var. *linifolium* (Allan 1961), and is illustrated in Figure 9.3.

Figure 9.1 *Leptospermum scoparium* var. *incanum* collected from Northland gumlands near Kaitaia.

Figure 9.2 *Leptospermum scoparium* var. *incanum* collected from Northland gumlands near Kaikohe.

Figure 9.3 *Leptospermum scoparium* var. *linifolium* collected from the Waikato wetlands near Meremere.

9.5.3 Coromandel population

The morphology of *Leptospermum scoparium* differed in the Coromandel region.

Three locations, Colville, Kuaotunu and Hahei carried the *L. scoparium* var. *incanum* variety with lanceolate leaves. These populations exhibited a mean leaf length (12.5)–13.5–(14.3) mm, a mean width (1.8)–2.2–(2.4) mm, and a leaf length/width ratio of 6.2. These dimensions are similar to the Northland population, however the leaves were slightly shorter but width remained constant, indicating a marginally more lanceolate-ovate shape. Tomentum was present on juvenile growth, and flower colour was mostly white, however pink tinged petals were occasionally present. Another shared characteristic with the Northland variety was that 80% of the capsules were unsplit.

However in contrast the other two locations sampled, Waikawau and Whenuakite, carried a different variety of *L. scoparium*. At both locations the leaves were smaller, mean length (9.5) 9.9–(10.5) mm, and width (1.6)–1.8–(2.0) mm, with a leaf length/width ratio of 5.4. The leaf shape was linear-lanceolate. Petal colour was universally white, and tomentum was less pronounced on juvenile foliage. The capsules were mostly split, with approximately 25% remaining unsplit.

A cluster analysis confirmed the Coromandel populations branched, the populations with *L. scoparium* var. *incanum* variety characteristics grouping separately from the other two populations sampled.

Both of these types are illustrated in Figure 9.4, and particular attention is drawn to the differences in foliage.

Figure 9.4 *Leptospermum scoparium* collected from the Coromandel, illustrating *L. scoparium* var. *incanum* traits (left and middle) in plant material near Kuaotunu, and the variety found further south (right) near Whenuakite.

9.5.4 East Coast populations

The East Coast *Leptospermum scoparium* populations were also shown to be heterogeneous.

In two locations, Whangaparoa and Hicks Bay, the plants again had *L. scoparium* var. *incanum* characteristics. Mean leaf length was (12)–13–(13.8) mm, mean width (2)–2.3–(2.5) mm, and the leaf length/width ratio was 5.5. The leaves were lanceolate in shape, however slightly shorter and wider dimensions than the Northland population revealed a moderately lanceolate-ovate shape. Tomentum was apparent on juvenile growth, and 90% of capsules were unsplit. Further evidence of *L. scoparium* var. *incanum* was provided by pink-coloured petals, particularly about the coast west of Whangaparoa, however the white petals predominated in these sites.

In contrast, the other three sites, Ruatoria, Te Araroa, and Whanarua Bay contained *L. scoparium* populations that had smaller, narrower leaves. Mean leaf length was (6)–7–(9) mm, and mean leaf width was (1.3)–1.6–(1.9) mm. The leaf length/width ratio was 4.4, and the foliage from these plants was linear-lanceolate. Tomentum was sparse on juvenile growth. The capsules were predominately split, with 30% remaining unsplit, and petal colour was universally white. The leaf dimensions range is comparatively large in these three locations because plant material from Whanarua Bay had considerably longer and wider leaves than the material collected from Ruatoria and Te Araroa.

A cluster analysis of the data from the East Coast region revealed two branches, the dendrogram placed the populations with *L. scoparium* var. *incanum* variety traits separately from the other three locations. These varieties are contrasted in Figure 9.5.

Figure 9.5 *Leptospermum scoparium* collected from the East Coast, illustrating *L. scoparium* var. *incanum* traits (left) in plant material from Whangaparoa, and the unnamed variety (right) sampled in Whanarua Bay.

9.5.5 Central North Island populations

The *Leptospermum scoparium* populations from the Central North Island also were different from each other, but the characteristics of *L. scoparium* var. *myrtifolium* described by Allan (1961) were evident.

The two populations positioned on the Volcanic Plateau in the centre of the North Island, Rangipo and Raetihi, and the other relatively higher altitude location, Kuripapango, exhibited the characteristics of *L. scoparium* var. *myrtifolium* illustrated in Figure 9.6. The mean leaf length was (5.4)–5.8–(6.2) mm, and mean leaf width (2.2)–2.4–(2.6) mm. The leaves were distinctly ovate, with a leaf length/width ratio of 2.4. Tomentum was sparse on juvenile foliage, 95% of capsules were split, and the petal colour was white. These plants did not exhibit the prostrate growth form of *Leptospermum scoparium* var. *prostratum* described by Allan (1961) as a montane variety.

In contrast, the other two populations, located near Rotorua and in the Esk Valley, did not exhibit such pronounced *L. scoparium* var. *myrtifolium* characteristics. Mean leaf length was slightly longer, (6) 6.5–(7) mm, however the mean width of (2.1)–2.4–(2.6) mm was similar to the more elevated populations. The variability of these two populations was greater than recorded in the higher altitude locations. The leaf length/width ratio was 2.8, and the shape lanceolate-ovate. Again petal colour was always white and tomentum not particularly obvious on juvenile material. Approximately 60% of the capsules were unsplit at these two locations.

Accordingly the *L. scoparium* populations situated at higher altitude exhibited the characteristics of the variety *L. scoparium* var. *myrtifolium*, and the populations in lower altitude positions, whilst retaining some of those traits, revealed characteristics of the low altitude populations elsewhere.

Figure 9.6 *Leptospermum scoparium* var. *myrtifolium* collected from the Central North Island near Rangipo.

9.6 Morphological traits analyses

Within the area of the North Island studied, four distinct varieties of *Leptospermum scoparium* were noted. The comparison of the most distinct populations defines these character states.

In Northland *L. scoparium* var. *incanum* is found. The leaves are lanceolate, usually longer than 13 mm and about 2.2 mm wide. Capsules on two year old stems remain mostly unsplit. Whilst pink petal colour is common, a range of petal colouration was noted, varying from insignificantly tinged to completely coloured. Clearly *L. scoparium* var. *incanum* readily hybridises with the white-petal variety, and whilst the pink-petal variety may be prevalent further north (Enright 1989) and breed true (Allan 1961), the leaf shape of this type allows a more meaningful regional separation.

In the Waikato wetlands *L. scoparium* var. *linifolium* is present. The leaves are linear, about 9.5 mm long and 1.3 mm wide. This variety carries white blossoms, and approximately half capsules on two year old wood are split.

In the most eastern sections of the East Coast an undefined variety is present, though *L. scoparium* var. *linifolium* may be an appropriate variety name determined by morphology. The leaves are fairly short and narrow, about 6.5 mm long and 1.5 mm wide. The short length provides a linear-lanceolate shape. The petals are white, and about 75% of the capsules on two year old stems are split.

In the Central North Island *L. scoparium* var. *myrtifolium* is encountered. The leaves are very short and comparatively broad, about 5.8 mm long and 2.4 mm wide. The leaf shape is therefore ovate. Petal colour is white and virtually all the capsules present on two year old wood are split.

Principal component analysis revealed that 97% of the variability was explained by the first two eigenvalues, 54.7% and 42.5%. Discernable clusters in two dimensional ordinate space were apparent when geographical source was overlaid (Figure 9.7). Three *L. scoparium* populations were well separated on both axes, the Central North Island, Northland, and Waikato. However the Coromandel and East Coast populations were split. The populations located in the East Coast and Coromandel that had *Leptospermum scoparium* var. *incanum* characteristics grouped with Northland population, whereas the populations from those regions without the characteristics prevalent in Northland grouped together in the intermediate space approximately equidistant from the other three varieties. The x-axis may be interpreted as defining leaf characteristics, the variety with more ovate leaf is positioned to the left, and the variety with the most linear leaf is found in the extreme right.

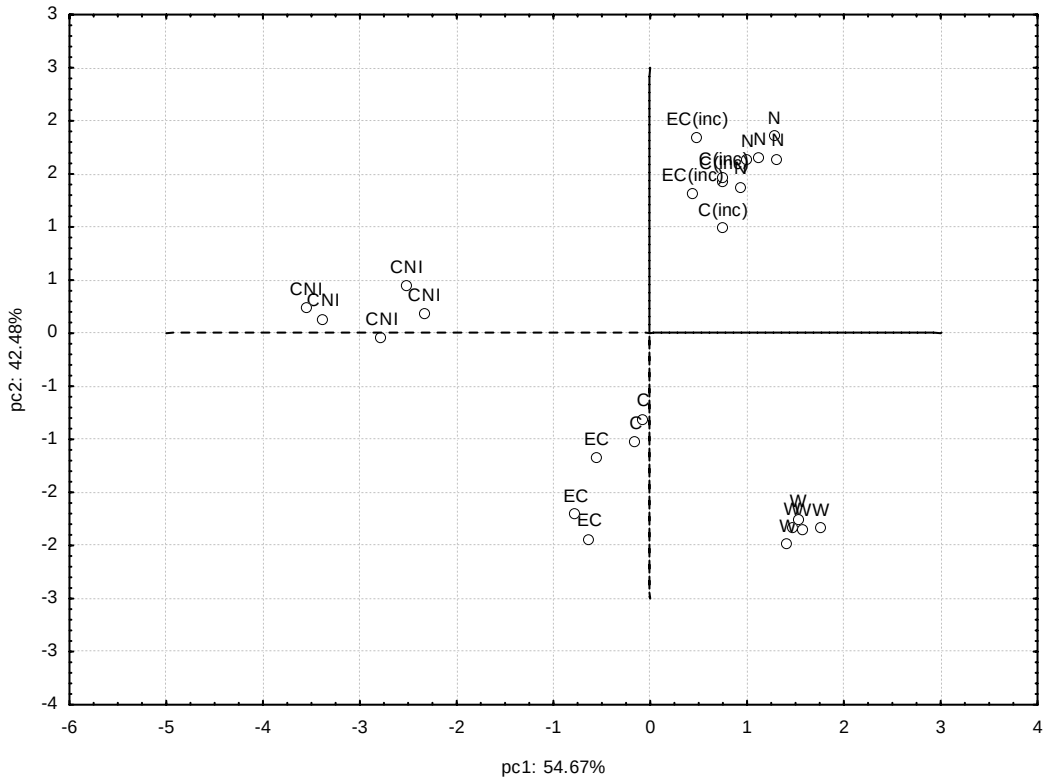

Figure 9.7 Principal component analysis of morphological characteristics utilised for *Leptospermum scoparium*. N, Northland; W, Waikato; C(inc), Coromandel *L. scoparium* var. *incanum*; C, Coromandel other varieties; EC(inc), East Coast *L. scoparium* var. *incanum*; EC, East Coast other varieties; CNI, Central North Island.

A relationship was established between the apparent core populations defined by morphological characteristics. The cluster analysis (Figure 9.8) revealed an initial branching between the northern and southern populations. *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* grouped together indicating a degree of relatedness, likewise *L scoparium* var. *myrtifolium* and the unnamed variety from the East Coast also grouped together. The southern varieties were more closely related than their northern counterparts.

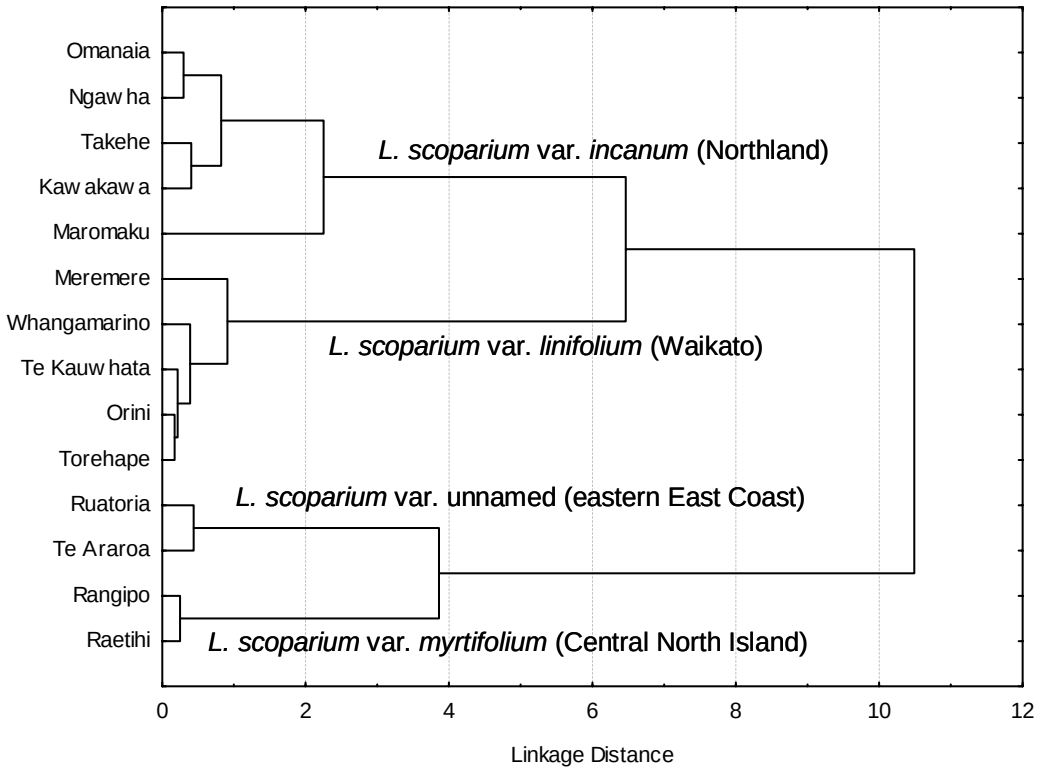

Figure 9.8 Dendrogram of cluster analysis of core populations of *Leptospermum scoparium* varieties utilising morphological characteristics.

Inclusion of all populations studied in a cluster analysis (Figure 9.9) does not alter the major divisions outlined above. *L. scoparium* var. *incanum* encompasses Northland, and three populations of the Coromandel and two populations of the East Coast that exhibited characteristics of this variety. *L. scoparium* var. *linifolium* is represented by the Waikato wetlands populations, and the two southern Coromandel populations share characteristics with this. *L. scoparium* var. *myrtifolium* includes all the populations sampled in the Central North Island. The peripheral sites, Rotorua and Esk Valley, share characteristics with the three core populations found at higher altitude. The East Coast populations that did not display *L. scoparium* var. *incanum* traits clustered together, aligned with but separate from *L. scoparium* var. *myrtifolium*.

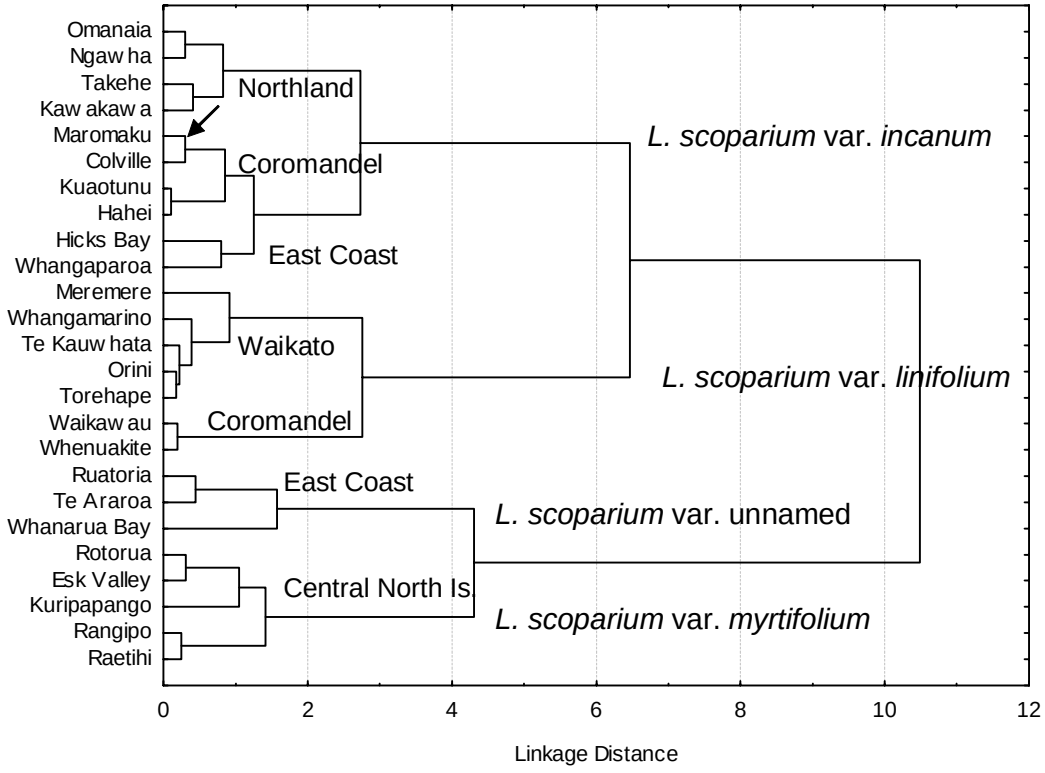

Figure 9.9 Dendrogram of cluster analysis of sampled populations of *Leptospermum scoparium* utilising morphological characteristics.

Therefore the *Leptospermum scoparium* populations analysed by morphological characteristics show both evidence of distinct varieties and the possible extension of those varieties range. Characteristics of *L. scoparium* var. *incanum* are recorded through the middle of the Coromandel Peninsula and on the north-facing coast of the East Coast, and this variety would appear to have an extended range. Yet the clustering of the Waikawau and Whenuakite Coromandel populations with the core Waikato *L. scoparium* var. *linifolium* does not appear justified from field observations.

In contrast, the ovate foliage characteristic of the Central North Island populations brings about a homogenous cluster bearing the traits of *L. scoparium* var. *myrtifolium*. The three populations present on the East Coast without *L. scoparium* var. *incanum* characteristics appear closely related to each other and more similar to *L. scoparium* var. *myrtifolium* present in the Central North Island rather than the two northern varieties.

9.7 Morphological population clusters and regional UMF®

A comparison of the varieties determined by morphological characteristics and the UMF® yield from the appropriate region reveals a number of relationships.

Leptospermum scoparium var. *incanum* is present in Northland, the Coromandel, and the East Coast. These areas are represented by a UMF® region/sub-region mean activity rating of 14.8, 14.9, and 12.5 respectively. The Northland and Coromandel values represent two of the high UMF® regions in the North Island, and the East Coast sub-region mid-activity along the north facing coast of that region.

The Waikato wetlands also produced high activity mānuka honey; a mean 15.3 UMF® was recorded from the locality where *L. scoparium* var. *linifolium* is predominant. The relatedness between *L. scoparium* var. *incanum* and this variety

is noted, branching separately from the southern varieties. However the two Coromandel sites positioned in that cluster, Waikawau and Whenuakite, returned an activity rating of a mean 10.5 UMF®.

L. scoparium var. *myrtifolium* is present in the Central North Island, a region that yielded mānuka honey with a low rating of a mean 8.6 UMF®. The unnamed variety recorded on the East Coast also produced low mean activity mānuka honey 10.9 UMF®, and grouped with *L. scoparium* var. *myrtifolium*.

Beyond the geographical range of this study but of particular interest is the description of the *L. scoparium* variety present on the pakihi soils of the West Coast. This sub-region yielded high activity mānuka honey rated with a mean of 14.4 UMF®. The *L. scoparium* population was reported to be serotinous, retaining capsules unsplit in contradiction to the rainfall correlation, the usual parameter for fire adaptation (Bond et al. 2004). However this area has been subjected to regular fires and therefore serotiny is a selective advantage.

Therefore a relationship between the presence of *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* in a region/sub-region, two varieties aligned by this morphological analysis of genotypic traits, and the high UMF® yielded in those regions is established. The northern coast of the East Coast, where some *L. scoparium* var. *incanum* traits are observed, yielded a medium level of UMF® activity.

This cluster analysis is determined by the analysis of leaf size, ratio, and shape, and the proportion of split capsules. Whilst these traits have been shown to be genotypic and adequately illustrate the presence of different varieties throughout the study area, further evidence is required to show relatedness between the geographically separate populations. The varieties' method of range extension accounting for the spread of varieties into geographically isolated areas, and an explanation of the replacement of *in situ L. scoparium* populations by the incoming variety is also required.

Chapter Ten and Eleven re-evaluate the *Leptospermum scoparium* variety status within the study utilising chemotaxonomy and population genetics respectively, and a general account and explanation of the current range of the varieties is provided in Chapter Twelve.

Chapter 10

Chemotaxonomy of *Leptospermum scoparium* in the North Island

This chapter describes the essential oils and chemotaxonomic analysis of the *Leptospermum scoparium* populations growing in five regions in the North Island of New Zealand.

The work in this chapter was completed in conjunction with the Department of Chemistry, University of Waikato. Professor A. Wilkins supervised the essential oil extractions and analyses, and the chemistry laboratory techniques were performed by M. Senanayake on *L. scoparium* samples supplied by the author. The data analyses and consideration of this material was completed by the author.

Three leaf oil types were recognised from six groups of components; the components are described, and the distribution of the chemotypes is discussed. Population clusters are described, which are in agreement with the morphological analysis previously described, and the hypothesis that the variable UMF® activity reported in mānuka honey and the distribution of *L. scoparium* varieties are correlated is considered.

The material contained in this chapter has not been submitted for publication, as the thesis is subject to an embargo agreed with the commercial sponsors of this study.

10.1 Hypothesis

The hypothesis that the variability of UMF® activity in mānuka honey arises from the presence of different varieties of *Leptospermum scoparium* in geographical regions of New Zealand is explored. This chapter details the chemotaxonomic analysis: Chapters Nine and Eleven describe the morphological and population genetics analyses, respectively. A concurrence of proposed varieties deduced by this method and the varieties of *L. scoparium* deduced from the morphological analysis is necessary to validate the earlier findings.

10.2 Chemotaxonomy of *Leptospermum scoparium*

The chemotaxonomy of New Zealand *Leptospermum scoparium* has already received attention, stimulated by the commercial value of the plant's steam-distilled essential oils. Analysis of commercial samples has revealed major divisions within the oil (Perry et al. 1997b; Porter & Wilkins 1998; Christoph et al. 1999).

A common garden experiment confirmed *L. scoparium* essential oils differed between natural populations; the East Cape population contained a high triketone level, high levels of α-pinene and β-pinene monoterpene hydrocarbons were found in Northland populations, and the balance of populations sampled contained a complex mix of sesquiterpene and oxygenated sesquiterpene hydrocarbons (Perry et al. 1997b; Porter & Wilkins 1998). This genotypic effect is in agreement with the reported genetic rather than environmental control of Australian Myrtaceae *Melaleuca alternifolia* oil composition (Homer et al. 2000; Shelton et al. 2002).

Australian *L. scoparium* samples grown in the same experiment had a higher monoterpene level than the New Zealand populations (Perry et al. 1997b). Triketones were not reported in the Australian *L. scoparium* var. *scoparium* and

L. scoparium var. *eximum* (Brophy et al. 1999, 2000), yet were found in the closely allied *Leptospermum* divisions in Australia described by Thompson (1989).

The most recently published study of New Zealand *L. scoparium* chemotypes confirmed monoterpene-enriched areas in Northland and the West Coast, triketone-enriched in East Cape and Marlborough, and sesquiterpene-rich oils throughout the rest of the country (Douglas et al. 2004).

Therefore to complement the morphological analysis of the *L. scoparium* populations studied in Chapter Nine a chemotaxonomic analysis of the essential oils was performed.

10.3 Sites, materials, and methods description

The sites from which leaf material was taken are those described in Section 9.2.

The oil profiles were determined from additional leaf material taken with the morphological material. To avoid age-dependent variability within a *Leptospermum scoparium* population reported by Porter et al. (1988), and to minimise the cyclical and environmental variation that may alter oil production (Butcher et al. 1994), mature leaves were selected from one-year-old wood during the spring of 2003. The leaves were bagged and identified, and stored at 4°C until analysed. Equal quantities of leaf material from the five plants representing a site was bulked together and a sub-sample analysed.

An adaptation of the micro-scale technique developed by Brophy et al. (1989) was used to characterise leaf oil constituents from *L. scoparium* leaves. This technique has been found to afford a GC-FID or GC-MS profile that closely matches the profile of the commercial steam-distilled oil. Twenty to forty leaves were soaked in ethanol:dichloromethane (1:1) (~2 ml) in glass vials for 18–20 h at

room temperature. Extracts of 2 ml were transferred to GC vials and analysed using GC–MS.

Extracts were analysed using helium as the carrier gas (column inlet pressure 16 psi; carrier gas flow, 1.5 ml/min) and a 30 m x 0.25 mm internal diameter ZB-5 column (Phenomex) installed in a HP6890 (Hewlett Packard) GC coupled to a HP5973 mass selective detector (MSD). The GC injector, MS interface and MS ion source were maintained at 250^{o}C, 280^{o}C and 200^{o}C respectively. Mass spectral data was acquired in total ion chromatogram mode, scanning the range *m*/*z* 42–500 Da. Aliquots of the extractive solutions (typically 1-2 µl) were injected using a HP7683 auto-sampler and the Grob split/splitless technique (splitless time, 6 s). The GC-MS oven temperature was programmed from isothermal at 45 ^{o}C (20 s hold) to 65 ^{o}C at rate of 20 ^{o}C/min, and then to 285 ^{o}C at 6 ^{o}C/min. The final temperature was maintained for 10 min to elute flavanoids, wax hydrocarbons and other higher boiling point components from the column. Compounds were identified using a combination of retention time, mass spectral data, and previously characterised *L. scoparium* leaf oil samples available in our laboratory. Integration of peaks was performed using Hewlett Packard ChemStation software (GB1701BA Version B.01.00).

The GC-MS retention windows and principal components of the six classes of essential oil compounds recognised in this investigation are presented in Table 10.1. Integrated peak areas, expressed as % total ion current (TIC) contributions, were calculated as follows, using group A as an example.

$$\%\ \text{group A contribution} = \frac{\text{peak area (group A)}}{\text{total peak area (group A–F)}} \times 100$$

Where: peak area (group A) = sum of peak areas in Group A
total peak area (group A–F) = sum of all groups peak areas

Table 10.1 Six chemical groups of *Leptospermum scoparium* leaf essential oils.

Group	Chemotype	Major constituents
A	Monoterpenes-rich	α-pinene, β-pinene, myrcene
B	Sesquiterpenes-rich	α-ylangene, α-copaene, β-caryophyllene, α-humulene, α-muurolene
C	Oxy-sesquiterpenes-rich (excluding eudesmols)	β-nerolidol, spathulenol, caryophyllene epoxide, virifloral, ledol
D	Eudesmols-rich	α-eudesmol, β-eudesmol, γ-eudesmol
E	Triketones-rich	leptospermone, isoleptospermone, flavesone
F	Nor-triketones-rich	nor-leptospermone, nor-isoleptospermone, nor-flavesone

Assumptions of normality were accepted for statistical analysis, and the principal component and cluster analyses were produced by StatSoft Statistica Ver. 6. Euclidean distance was utilised to define the geometric distance between sites and therefore the relatedness, and complete linkage was employed as the data illustrated clustering behaviour prior to analysis.

10.4 Chemotaxonomic results

In this investigation six major groups of volatile (steam distillable) compounds were recognized in the leaf oil components, based on GC peak retention time windows and the presence or absence of specific compounds: Group A: monoterpenes; Group B: sesquiterpene hydrocarbons; Group C: oxygenated sequiterpenes (excluding eudesmols); Group D: eudesmols; Group E: triketones; and Group F: nor-triketones.

Consideration of the %TIC contributions of the Group A-F contribution showed that while generally similar levels of sequiterpenes hydrocarbons (≈40%), oxygenated sequiterpenes (≈30%), and low to moderate levels of monoterpenes were present in all of the leaf extracts, there were appreciable variations in the levels of eudesmols, triketones, and nor-triketones. This lead to the formulation of three chemotype classifications based on considerations other than monoterpenes and sesquiterpenes contributions; Type 1: eudesmols rich; Type 2: triketones rich; and Type 3: nor-triketones rich.

Table 10.1 lists the principal components of each chemical grouping. Group A was comprised of monoterpene hydrocarbons and some oxygenated analogues, and Group B sesquiterpene hydrocarbons. Group C was comprised of oxygenated sesquiterpenes, excluding eudesmol isomers that were classified as group D compounds. Group E was comprised of triketones and Group F nor-triketones. Whilst further divisions of these chemical groups can be made, particularly within the sesquiterpene and oxygenated sesquiterpene hydrocarbons (Douglas et al. 2004), for the purpose of this study subdivision was unnecessary.

The three nor-triketones (Group F) are reported for the first time here from New Zealand *L. scoparium* essential oil extracts. These nor-triketones, described here as nor-leptospermone, nor-isoleptospermone and nor-flavesone, are believed to be the nor-methyl analogues of leptospermone, and isoleptospermone and flavesone respectively (A. Wilkins pers. comm.). The nor-triketones were eluted from the GC column after the corresponding triketone peaks, presumably because the dominant solution and vapour phase forms of these compounds are nor-diketo-dienol and triketo-enol tautomeric forms. Nor-diketo-dienol analogues would be expected to elute from a GC column later than the corresponding triketo-enol analogue, due to their increased polarity, and presumably also higher boiling points (A. Wilkins pers. comm.). The presence of triketones in *Myrtaceae* and *Eucalyptus* species has been reported previously (Hellyer et al. 1968; Ghisalberti et al. 1996; Van Klink et al. 1999), and the synthesis of triketones has been carried out by the C-methylation of phloroglucinol (Bick et al. 1965). Perry et al. (1997b)

reported synthesis of triketones, and like Hellyer et al. (1968) showed that enol tauomers are the dominant solution forms of these compounds.

Table 10.2 details the *L. scoparium* essential oil groups recognised in this study and recorded at the twenty five sites.

Eudesmols-enriched Type 1 plants were found in Northland, Waikato, and Colville, Kuaotunu and Hahei localities in the Coromandel Peninsula. These populations, particularly the Northland one, also contained a higher level of monoteperenes than those in the other regions. Eudesmols are also present to a lesser degree in a ring to the east of the centre of the Central North Island. Plants from the Rotorua, Kuripapango and Esk Valley populations contained moderately enhanced levels of eudesmols, and a much lesser enrichment was also recorded in material from the East Coast. Eudesmols were absent in *L. scoparium* plants growing on the Central Volcanic Plateau and one location in Coromandel.

Triketones-enriched Type 2 plants were found principally in the East Coast population in accordance with the literature. *L. scoparium* leaf from three central Coromandel localities, Waikawau, Kuaotunu and Whenuakite, also contained triketones, and the Waikawau population contained a level (≈20%) sufficient for classification as triketones-enriched. A slight trace of triketones was also found in the Rotorua and Rangipo locations, both west of the East Coast. Triketones were not present in the Northland and Waikato populations, the most western and southern Central North Island locations; or Colville, the most northern population samples in the Coromandel.

Nor-triketones-enriched Type 3 plants were found in the Central North Island and Coromandel. The Central North Island leaf material contained the greatest content of nor-triketones, and a smaller yield was present in the Coromandel populations. Nor-triketone was also recorded at Whanarua Bay, the East Coast site closest to the Central North Island. Likewise the Northland Kawakawa site also displayed a trace of nor-triketones, illustrating a proximity to the

Coromandel; however the balance of the Northland localities, Waikato, and more eastern East Coast locations did not contain nor-triketones.

Table 10.2 Essential oil profiles of *Leptospermum scoparium* leaf material from the North Island. The proportion of monoterpenes, sesquiterpenes, oxy-sesquiterpenes, eudesmols, triketones, and nor-triketones are shown as a percentage of total ion count.

Type	Region	Site	Mono-terpenes	Sesqui-terpenes	Oxy-sesqui-terpenes	Eudes-mols	Tri-ketones	Nor-tri-ketones
Type 1	Waikato	Meremere	2.6%	30.6%	39.3%	27.5%	0.0%	0.0%
	Waikato	Whangamarino	3.4%	25.7%	37.4%	33.5%	0.0%	0.0%
	Waikato	Te Kauwhata	2.7%	23.7%	40.7%	32.9%	0.0%	0.0%
	Waikato	Orini	2.7%	24.4%	45.6%	27.3%	0.0%	0.0%
	Waikato	Torehape	3.8%	24.1%	46.7%	25.3%	0.0%	0.0%
	Northland	Takehe	9.9%	37.8%	19.2%	33.1%	0.0%	0.0%
	Northland	Maromaku	16.6%	30.8%	17.8%	34.7%	0.0%	0.0%
	Northland	Kawakawa	26.7%	32.1%	26.7%	14.4%	0.0%	0.2%
	Northland	Omanaia	7.1%	45.1%	26.4%	21.4%	0.0%	0.0%
	Northland	Ngawha	10.7%	50.8%	23.7%	14.9%	0.0%	0.0%
Type 2	East Coast	Whanarua Bay	0.6%	37.9%	28.3%	0.05%	30.8%	2.3%
	East Coast	Whangaparoa	0.3%	43.5%	23.2%	0.09%	33.0%	0.0%
	East Coast	Hicks Bay	0.6%	37.3%	30.3%	0.1%	31.7%	0.0%
	East Coast	Te Araroa	0.3%	37.3%	29.7%	0.03%	32.7%	0.0%
	East Coast	Ruatoria	0.7%	41.5%	25.7%	0.05%	32.1%	0.0%
	Coromandel	Waikawau	2.5%	44.7%	22.5%	0.0%	22.7%	7.6%
Type 3	Coromandel	Colville	2.7%	41.4%	31.6%	17.1%	0.0%	7.2%
	Coromandel	Kuaotunu	1.6%	37.5%	26.9%	13.0%	9.1%	11.9%
	Coromandel	Hahei	1.6%	39.9%	36.8%	12.6%	0.3%	8.8%
	Coromandel	Whenuakite	2.8%	46.7%	30.2%	0.4%	6.4%	13.5%
	Central	Rotorua	0.5%	44.2%	21.4%	5.9%	0.7%	27.1%
	Central	Rangipo	0.4%	46.0%	27.5%	0.0%	1.7%	24.4%
	Central	Raetihi	1.3%	41.3%	29.4%	0.0%	0.0%	27.9%
	Central	Kuripapango	1.0%	43.0%	32.0%	4.0%	0.0%	19%
	Central	Esk Valley	2.0%	37.0%	36.0%	7.0%	0.0%	17.0%

10.5 Varieties determined by chemotaxonomic analysis

Principal component analysis showed 90% of the variability was explained by the first three eigenvalues, 44.1%, 23.9% and 22%. Clusters in two dimensional ordinate space were apparent when geographical source was overlaid (Figure 10.1). Two *Leptospermum scoparium* populations, Northland and Waikato, were separated by both axis and from the other populations. However the other three populations were amalgamated. The populations located in the East Coast and Central North Island were closely positioned, yet *L. scoparium* collected from the Coromandel that carried the morphological characteristics of *L. scoparium* var. *incanum* was positioned closer toward the Northland and Waikato populations.

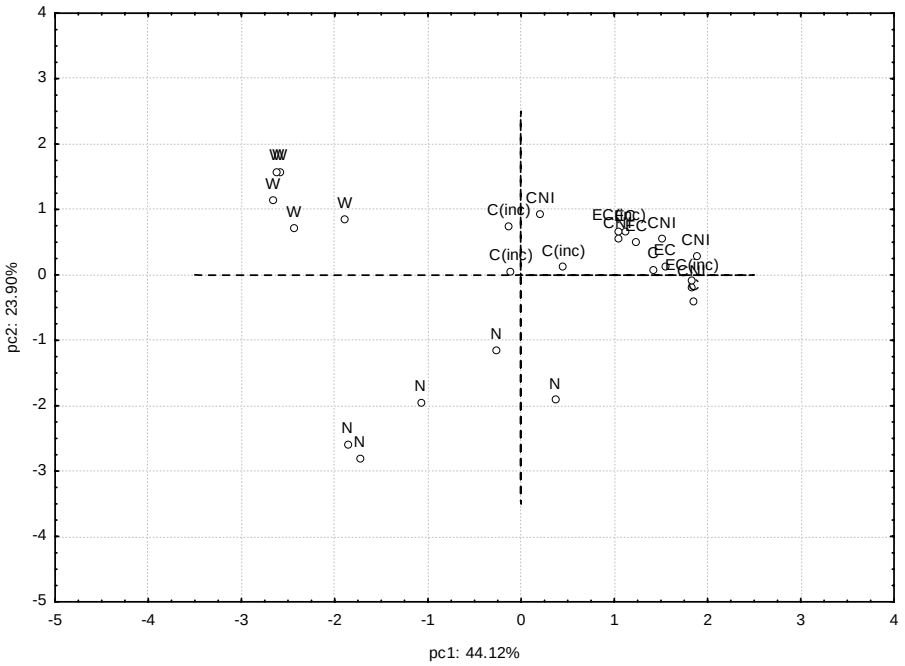

Figure 10.1 Principal component analysis of essential oil groups utilised for *Leptospermum scoparium* in this study N, Northland; W, Waikato; C(inc), Coromandel *L. scoparium* var. *incanum*; C, Coromandel other varieties; EC(inc), East Coast *L. scoparium* var. *incanum*; EC, East Coast other varieties; CNI, Central North Island.

A cluster analysis dendrogram prepared using the leaf essential oil profile data based on the 6 chemical groupings A-F from the Northland, Waikato, East Coast and Central North Island regions demonstrated the existence of three principal chemotypes (Types 1, 2, and 3) (Figure 10.2). The Coromandel locations represented an amalgam of these chemotypes and were excluded from this initial core population analysis.

Populations from localities showing Type 1 (eudesmols-enriched) essential oil profiles initially branch from the other populations and later divide into Waikato and Northland. The other major branch divided into Type 2 (triketones-enriched) present in the East Coast, and the Type 3 (nor-triketones-enriched) found in the Central North Island.

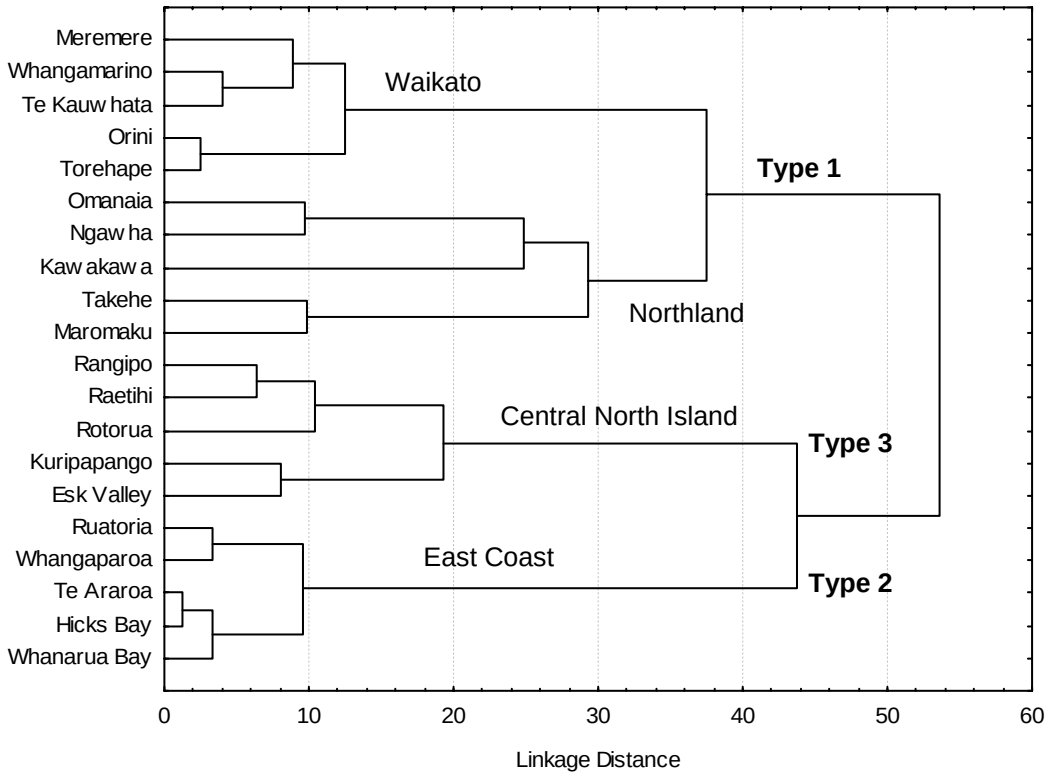

Figure 10.2 Dendrogram of cluster analysis of Northland, Waikato, East Coast, and Central North Island *Leptospermum scoparium* populations using essential oil profiles.

Evidence of inter-variety hybrids is plentiful. Geographic proximity can explain most of the hybrid types. The most eastern Northland location sampled, Kawakawa, displayed a low level of nor-triketones found in the Coromandel, indicating gene flow across the Hauraki Gulf, however the nor-triketones type has not spread throughout Northland.

The eudesmols are present in slightly enhanced proportions in the more eastern locations around the core of the Central North Island, evidence the Waikato population has been spreading around the volcanic plateau, as the Rotorua, Kuripapango and Esk Valley populations contain this oil, but not the montane country at Rangipo and Raetihi. Likewise the most western East Coast location, Whanarua Bay, shared a trace of Central North Island population nor-triketones character.

The essential oil profiles from the Coromandel show more variability. A dendrogram (Figure 10.3) of this region analysed without data from the other regions revealed a division into two branches.

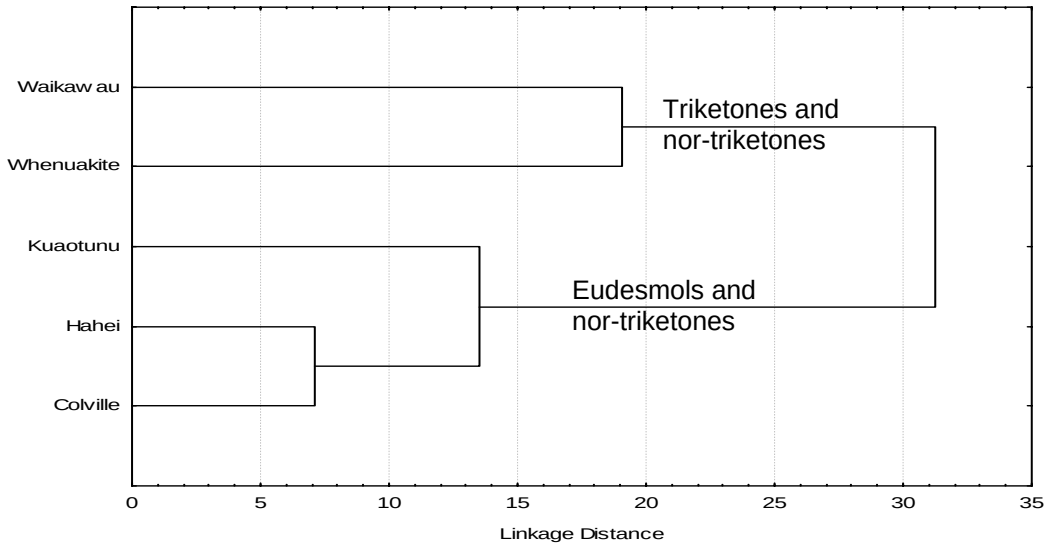

Figure 10.3 Dendrogram of cluster analysis of Coromandel *Leptospermum scoparium* populations using essential oil profiles.

Plants from the two southern localities containing greater levels of triketones and nor-triketones oils, whereas the three northern locations contained a blend of eudesmols and nor-triketones.This indicates a degree of gene exchange between the three essential oil types in the Coromandel; eudesmols from Northland, triketones from the East Coast, and nor-triketones from the Central North Island.

Cluster analysis of the five regions demonstrates the grouping of these *L. scoparium* populations. The dendrogram (Figure 10.4) of the cluster analysis confirms the two major divisions, eudesmols-enriched Northland and Waikato populations, and the triketones/nor-triketones-enriched East Coast and Central North Island. The Coromandel populations group with Type 2 and 3 divisions due to the levels of these essential oils components despite the enhanced eudesmols recorded in plants from the Colville, Kuaotunu and Hahei localities.

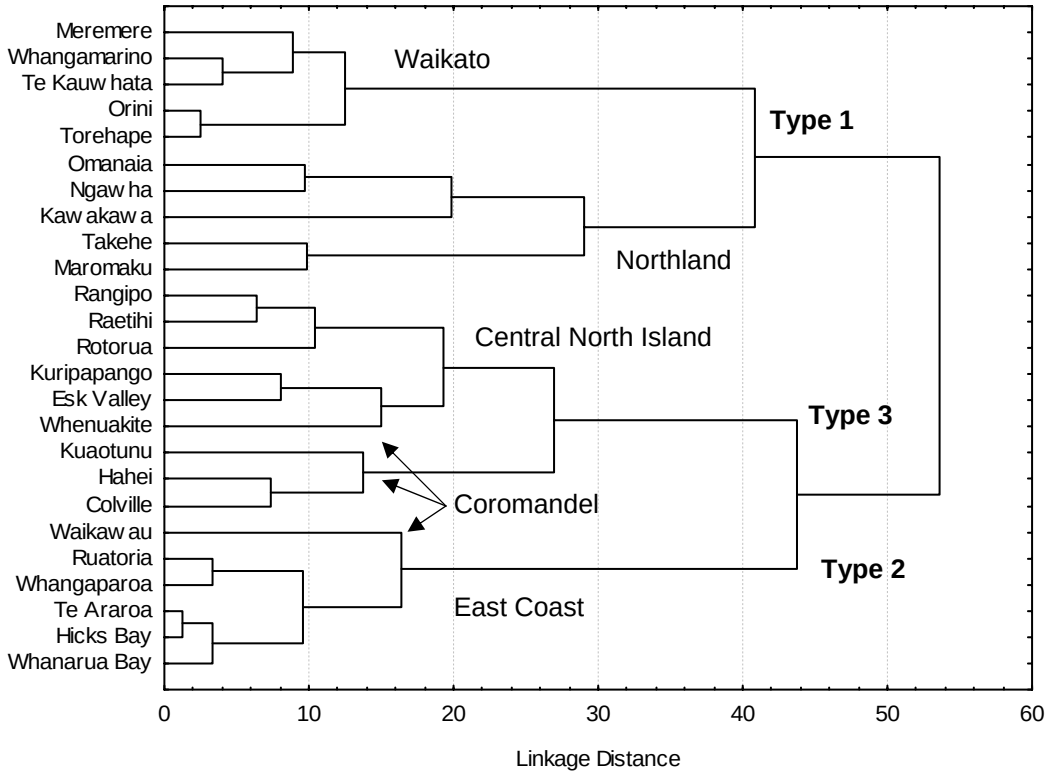

Figure 10.4 Dendrogram of cluster analysis of Northland, Waikato, Coromandel, East Coast and Central North Island *Leptospermum scoparium* populations using essential oil profiles.

10.6 Chemotaxonomic varieties and UMF® yield

The *Leptospermum scoparium* chemotaxonomic variety distribution in the northern half of the North Island and the UMF® yield described in Chapter Six within those localities was compared.

The eudesmols-enriched populations of Northland and Waikato yielded mānuka honey containing a high level of non-peroxide antibacterial activity, mean values of 14.8 and 15.3 UMF® respectively.

The *L. scoparium* in the Coromandel localities utilised in the chemotaxonomic analysis yielded different levels of UMF® activity. *L. scoparium* in the three north-eastern locations, Colville, Kuaotunu and Hahei, contained an enhanced level of eudesmols, similar to *L. scoparium* var. *incanum*. These areas were included in the Coromandel high UMF® division, yielding a mean activity of 14.9 UMF®. In the other two Coromandel locations, where *L. scoparium* contained an enhanced level of either triketones or nor-triketones, the UMF® yield was in the low activity Coromandel division, mean activity 10.5 UMF®.

Mānuka honey was received from two of the Central North Island localities discussed in this chapter. Both of these populations were nor-triketone-enriched varieties. The Raetihi and Kuripapango locations represented the low activity Taranaki sub-region and the Hawkes Bay region respectively, and yielded a low mean activity of 8.9 and 8.4 UMF®.

The East Coast localities fell within the two UMF® subdivisions of that region. The north-eastern coastal locations Hicks Bay and Whangaparoa represented the higher activity found in this region, mean activity 12.5 UMF®. The other three localities sampled for chemotaxonomic analysis, Whanarua Bay, Te Araroa, and Ruatoria yielded a low mean activity mānuka honey, 10.9 UMF®. Eudesmols were not prevalent in this region, and little difference was detected between the oil

profiles afforded by *L. scoparium* in the East Coast region. This is contrary to the morphological analysis, where *L. scoparium* var. *incanum* traits were clearly evident along the northern coast of the East Coast.

The distribution of these *L. scoparium* varieties throughout the balance of New Zealand cannot be shown as leaf samples for oil extraction were not obtained, and a nor-triketones extraction is required. However, of interest to this study is the presence of a *L. scoparium* variety growing abundantly on the pakihi soils of the northern West Coast about Westport. The leaf material from this variety contains an enhanced level of monoterpenes (Douglas et al. 2004), a similar profile to *L. scoparium* var. *incanum* growing on the infertile gumlands of Northland. The West Coast high activity sub-region is located where this unnamed variety is present, and yields a mean activity of 14.4 UMF®.

10.7 Morphological and chemotaxonomic analyses

The divisions deduced from the chemotaxonomic analysis broadly follow those from the morphological analysis described in Chapter Nine.

Leptospermum scoparium var. *incanum*, predominantly present in Northland, would appear to have spread into sections of the northern Coromandel peninsula.

The Coromandel region essential oil profiles also provided evidence of long distance dispersal and gene flow, as characteristic oil types of both the East Coast and Central North Island were encountered in this region.

This is contrary to the other *L. scoparium* populations with distinct oil profiles; shared essential oil constituents exist in the margins of the ranges of these populations and can be explained by short-distance dispersal and interbreeding. A trace of nor-triketones is found on the eastern side of Northland adjacent to the Coromandel, and the eudesmols typical of the Northland population are present in

the northern Coromandel. Eudesmols are recorded around core populations of the Central North Island, yet this oil type has not penetrated the high altitude locations. The most western location on the East Coast shares a trace of nor-triketones with the Central North Island.

Essential oil profiles confirm the Waikato *L. scoparium* var. *linifolium* is related to *L. scoparium* var. *incanum*, in accordance with the morphological analysis.

L. scoparium var. *myrtifolium* is widespread throughout the centre of the island, and is closely related to the unnamed variety found on the East Coast. Two distinct oil profiles are present, the East Coast variety is triketones-enriched whereas the Central North Island is nor-triketones-enriched. It is likely this is a localised change; the additional methylation to produce triketones is not recorded in the core nor-triketones populations of the Central North Island. The paucity of eudesmol or monoterpenes on the northern coast of the East Coast is unusual, as plant material collected at Hicks Bay and Whangaparoa exhibited a degree of the morphological characteristics typical of the Northland *L. scoparium* var. *incanum*.

The Australian *L. scoparium* varieties' essential oils have not been recorded to produce triketones or nor-triketones, perhaps indicating the Central North Island and East Coast populations are endemic to New Zealand. Nevertheless the New Zealand *L. scoparium* var. *incanum* shares an enhanced monoterpenes oil profile with the Australian south-eastern mainland variety *L. scoparium* var. *scoparium*.

Unfortunately the distillation method used in previous New Zealand publications, contrary to the solvent extraction method utilised in this study, does not reveal the presence of nor-triketones in *L. scoparium* essential oil profiles (Perry et al. 1997b; Porter et al. 1998; Porter & Wilkins 1999; Douglas et al. 2004). Therefore no comment on the New Zealand distribution of nor-triketone oil components can be made.

However Douglas et al. (2004) reported *L. scoparium* growing in the northern region of the West Coast that yielded an essential oil that had an enriched monoterpenes content similar to the Northland population, and noted an enhanced level of triketones localised in the Marlborough Sounds. The West Coast pakihi areas in the north of the West Coast and the north South Island yielded high and low UMF® in mānuka honey respectively, parallel to the UMF® reported from varieties with these oil types in the North Island.

In conclusion to this section, the essential oil and morphological analyses described the same core *L. scoparium* varieties with similar distribution. The distribution of the monoterpenes/eudesmols-enriched *L. scoparium* var. *incanum* and the closely related *L. scoparium* var. *linifolium* explains much the reported UMF® variability in the northern half of the North Island. The regions/sub-regions yielding high activity UMF® honey appear to carry these varieties, whereas the regions-sub-regions carrying the triketones/nor-triketones enriched essential oil profiles yield low activity UMF® honey. The following chapter re-evaluates the *Leptospermum scoparium* variety status within the study area of the North Island using population genetics.

Chapter 11

Population genetics of *Leptospermum scoparium* in the North Island

This chapter describes the population genetics analysis performed on *Leptospermum scoparium* populations from the North Island of New Zealand.

Similar Myrtaceae analyses are considered, particularly for Australasian taxa, and the RAPD technique is introduced. Analysis of the North Island *L. scoparium* populations studied is discussed. Four genetically distinct varieties are revealed, and the varieties are concurrent with those reported from the morphological and chemotaxonomic analyses.

The varieties distribution is considered, and a relationship with the distribution of UMF® is shown.

The material in this chapter has not been submitted for publication, as a thesis embargo on material detailing the reasons for field variation of UMF® has been arranged with the commercial sponsors of this study.

11.1 Hypothesis

The hypothesis that the variability of UMF® in mānuka honey arises from different *Leptospermum scoparium* varieties is further explored. This chapter describes the population genetics analysis utilising material collected from the same plants as those studied in the previous two chapters. Agreement between the varieties proposed by the morphological and chemotaxonomic studies and this method is explored to corroborate the variety status of the species.

11.2 Population genetics

The variety status of *Leptospermum scoparium* in New Zealand or Australia has not been examined by a population genetics study. Two New Zealand Myrtaceae taxa have been studied by these methods to determine genetic variability in small or isolated populations for conservation purposes; both studies investigated *Metrosideros* species (Drummond et al. 2000; Young et al. 2001).

Genetic diversity resulting from microevolutionary processes may be divided into variation between populations or within populations (Wallis 1994). Several methods have been described to measure genetic variability between plant populations; the general intention is to characterise a genetic marker that illustrates the diversity whilst testing a hypothesis. The marker must have the correct sensitivity, therefore a technique to determine population subdivision was employed. Whilst the Random Polymorphic DNA (RAPD) method has restrictions, particularly marker dominance and analytical difficulties, the method is widely used, technically convenient and inexpensive compared to the other multi-locus technique, Amplified Fragment Length Polymorphic DNA (AFLP), or the microsatellite single-locus technique (Sunnucks 2000).

Interest in the essential oils derived from the Australian tea-tree, *Melaleuca alternifolia*, has led to population genetics analyses in association with

chemotaxonomic and morphological treatments to study the possibility of enhancing oil yield. The genetic study utilised microsatellite variation and revealed two main genetotypes (Rossetto et al. 1999), yet monoterpenes chemotype control remained undetected by isozyme and microsatellite analysis (Shelton et al. 2002), an example of a relatively sensitive genetic analysis that did not illustrate the genetic expression of different monoterpene essential oils. The present study of *L. scoparium* showed different enriched classes of essential oils in the species' varieties, a broader distinction than different compounds within the same essential oil type studied in *Melaleuca alternifolia*.

In contrast, analysis of varieties of the morphologically variable *Metrosideros polymorpha*, an abundant endemic tree in Hawai'i, showed a correlation between morphological characteristics and genetic diversity determined by RAPD analysis, and illustrated the overlap between the previously described taxonomic varieties (James et al. 2004). Similarly studies of *Eucalyptus* species have successfully employed RAPD analysis techniques to establish genetic difference in taxonomic divisions, both between species (Kiel & Griffin 1994) and within species (Nesbitt et al. 1995; Li 2000).

The purpose of this examination of *L. scoparium* was to determine whether the morphological and chemotaxonomic variability described, and therefore the variety status of the species, was supported by genetic variation.

11.3 Sites, materials and methods

The sites from which leaf material was taken are those described in Section 9.2. Clean juvenile leaf material from each plant was washed, blotted dry, and stored separately mixed with activated silica gel granules. The dried material was stored at 4 °C.

11.3.1 DNA extraction

Total genomic DNA was extracted from 300 mg of plant material using a CTAB protocol modified from Doyle and Doyle (1987) and Martin et al. (1999), described in Table 11.1.

Table 11.1 CTAB protocol used for *Leptospermum scoparium* genomic DNA extraction.

Cetyltrimethyl ammonium bromide (CTAB) buffer, 5 ml CTAB was made, 0.3 g polyvinyl pyerolidine (PVP-40), and 50 µl β-mercaptoethanol per sample. CTAB buffer and PVP-40 was mixed on magnetic stir plate until dissolved, and β-mercaptoethanol added in fumehood. The solution was incubated at 60ºC in a waterbath for 45 minutes, shaken occasionally.
Four 15 ml polypropylene conical tubes were labelled per sample. 0.3 g of silica-dried plant material was ground with liquid nitrogen. To each sample 5 ml of CTAB solution was added and 50 µl of 10mg/ml Proteinase K. Solution incubated at 65ºC for 1 hr, shaken every 10 minutes.
The tubes were centrifuged, 2,400 rpm for 10 minutes at room temperature. The supernatant was poured into new polypropylene tube and volume estimated. Chloroform:isoamyl alcohol (24:1 v/v) was added, 2/3 of supernatant volume. The tube was inverted until a good immersion obtained, and gas pressure released from tube.
The tubes were centrifuged, 2,400 rpm for 10 minutes at room temperature. The upper aqueous phase was transferred to a new polypropylene tube using wide bore pipette tip and volume estimated. Chloroform:isoamyl alcohol (24:1 v/v) was added, 2/3 of retained aqueous solution volume. The tube was inverted until a good immersion obtained, and release gas pressure from tube.
The tubes were centrifuged, 2,400 rpm for 10 minutes at room temperature. The upper aqueous phase was transferred to a new polypropylene tube using wide bore pipette tip, filled with -20ºC 95% ethanol, and stored in -20ºC for at least 1 hr.
The tubes were centrifuged, 4,000 rpm for 10 minutes at 4ºC. The supernatant was decanted away and tubes allowed to air-dry.
The pellet was resuspended in 200 µl of sterile water, using 37ºC waterbath to assist dissolving. The sample was transferred to 1.5 ml Eppendorf tube. 700 µl of -20ºC 7.5M ammonium acetate 95% ethanol solution (6:1 v/v) was added, tube inverted until mixed and stored in -20ºC freezer for at least 1 hr.
Eppendorf tubes were centrifuged, 12,000 rpm for 5 minutes at room temperature. The supernatant was discarded, and Eppendorf tubes filled with -20ºC 70% ethanol and inverted gently.
Eppendorf tubes were centrifuged, 12,000 rpm for 5 minutes at room temperature. The supernatant was discarded, and air-dried in speed vac for 10 minutes at ≈40ºC, until no ethanol remained.
The pellet was resuspended in 100 µl of sterile water, using 37ºC waterbath to assist dissolving. 1 µl of RNAse was added, and sample incubated in 37ºC waterbath for 1 hr; then stored at 4ºC.

To quantify the success of the extraction, 5 µl of the DNA solution was mixed with 3 µl of loading buffer and electrophoresed for 1 hr on a 1% agarose gel containing ethidium bromide, as described in Section 11.3.3. The gels were photographed under UV light by Alphamager (Alpha Innotech) and DNA concentration was estimated visually. Extracted DNA of similar quantity for three plants from each site was selected for further analysis.

11.3.2 RAPD PCR reaction

The polymerase chain reactions (PCR) were performed in an Eppendorf Mastercycler Gradient Thermal cycler (Eppendorf). The DNA amplification cycle was an initial 2 min denaturation period at 94 °C for strand separation, then 40 cycles consisting of strand separation for 30 s at 94 °C, primer annealing for 1 min at 36 °C, extension for 2 min at 72 °C, and a final extension for 5 min at 72 °C. A 25 µl reaction mixture was prepared in a sterile laminar flow unit to prevent contamination, each mixture contained 11.25 µl sterile double distilled H_20, 2.5 µl dNTPs, 2.5 µl 10x buffer (Roche), 4 µl $MgCl_2$ (Roche), 1.25 µl primer, 1 µl Taq polymerase (Roche EC 2.7.7.7), and 2.5 µl genomic DNA extract.

Initial tests showed the level of contaminants derived from the plant extract, probably water-soluble phenolic compounds that had not been removed during the chloroform/isoamyl alcohol extraction, was high and inhibiting the PCR reaction. Dilutions of the DNA solutions were performed, and the reactions were consistent with a 1:100 dilution of the extracted DNA.

With each reaction round a positive and negative control was performed, the former providing a known banding pattern and the latter the reaction mix without DNA, conditions necessary for reproducible banding patterns (Parker et al. 1998).

11.3.3 Electrophoresis

Electrophoresis gels were 1.5% agarose in tris-borate EDTA (TBE) buffer solution with 1 µl of 1 $mg.ml^{-1}$ ethidium bromide added for detection. Electrophoresis (90 v) was performed for 3 hours. 15 µl of reaction mix and 5 µl of loading buffer were analysed for each sample, all taken from one round of PCR amplification, along with the negative and positive control. 15 µl 100bp ladder (New Zealand Biolabs, NS231S) was loaded to each gel, 1:10 dilution in tris/EDTA (TE) buffer with 5 µl loading buffer. Visualisation was performed under UV conditions by Alphamager (Alpha Innotech).

11.3.4 RAPD primer selection

Twenty 10-mer primers were trialled, derived from previous RAPD studies of Myrtaceae. The sequences of these are provided in Table 11.2. The primers were manufactured by Invitrogen Life Technologies. Trial evaluations revealed six of the primer sequences provided repeatable markers on electrophoresis gels, and therefore were selected for full analysis.

Table 11.2 Primer sequences trialled (5'–3'). Those primers selected are shown in bold.

RAPD01	ACCTGAACGG	RAPD11	CTGATGCGTG
RAPD02	**AGCCGTGGAA**	**RAPD12**	**GAAACGGGTG**
RAPD03	**AGCGCCATTG**	RAPD13	GCATGGAGCT
RAPD04	ATCGGAAGGT	**RAPD14**	**GCGTTGGATG**
RAPD05	ATCTGCGAGC	RAPD15	GGATCTCGAC
RAPD06	**CAAGCCAGGA**	RAPD16	GGGTAACGCC
RAPD07	**CAGACAAGCC**	RAPD17	GGGTGGTTGA
RAPD08	CAGGCGCACA	RAPD18	GTGACGTAGG
RAPD09	CATCCCCCTG	RAPD19	TCACCGAACG
RAPD10	CCCTACCGAC	RAPD20	TGAGCGGACA

11.3.5 Gel band scoring

Photographs were taken of the gel under UV, and were assessed for the presence/absence of bands manually. The 100bp ladder and positive control were employed to align the different photographs. Bands were scored as present or absent. Only markers that were unambiguous, well amplified, and reproducible in replicate tests were scored.

11.3.6 Analysis of data

Each marker in a RAPD primer profile was treated as an independent locus with two alleles, presence or absence of the band. PCR amplification products were scored manually, 1 for presence and 0 for absence, and a binary matrix was created. Nei's genetic distance (Nei 1972) was analysed by unweighted pair-group using arithmetic averages (UPGMA), generating a hierarchical cluster analysis dendrogram that assesses true tree values (Nei 1987). Hardy-Weinberg equilibrium was assumed.

11.4 Results and discussion

Six primers produced clear reproducible markers in bands and were therefore scored. A total of 54 bands were recognised, and of those, 33 bands were polymorphic and used in the analysis.

11.4.1 Core *Leptospermum scoparium* populations

Analysis of the core *Leptospermum scoparium* populations deduced from the previous two chapters was completed, and the areas where there was significant indication of variety hybridisation were excluded. Northland and Waikato were represented by all sites in those regions, the Central North Island region by the Rangipo and Raetihi sites, and the East Coast region the Ruatoria and Te Araroa sites. Northland material corresponded to *Leptospermum scoparium* var. *incanum* with lanceolate foliage and enhanced monoterpenes and eudesmols essential oil profile, the Waikato region *Leptospermum scoparium* var. *linifolium* with linear foliage and enhanced eudesmols essential oil profile, the Central North Island region *Leptospermum scoparium* var. *myrtifolium* with ovate foliage and nor-triketones essential oil profile, and the East Coast an unnamed *Leptospermum scoparium* variety with linear-lanceolate leaves and triketones essential oil profile.

The UPMGA dendrogram (Figure 11.1) illustrated the division of the *L. scoparium* core populations in two major groups, the northern and southern branches. The morphological and chemotaxonomic analyses would appear to be corroborated; the northern branch contains *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium*, and the southern branch contains *L. scoparium* var. *myrtifolium* and the unnamed variety carrying triketones in the essential oils. The southern populations were more closely related than the northern counterparts. The primer set did not reveal genetic diversity within the populations on the East Coast, Central North Island, Waikato, or west and east Northland. This may be interpreted in two ways, either these populations do not contain genetic diversity and therefore there is no evidence of out-breeding or gene flow, or the RAPD primer set did not depict diversity within the populations. The latter explanation is more likely: these populations are not separated by large species-inhospitable distances.

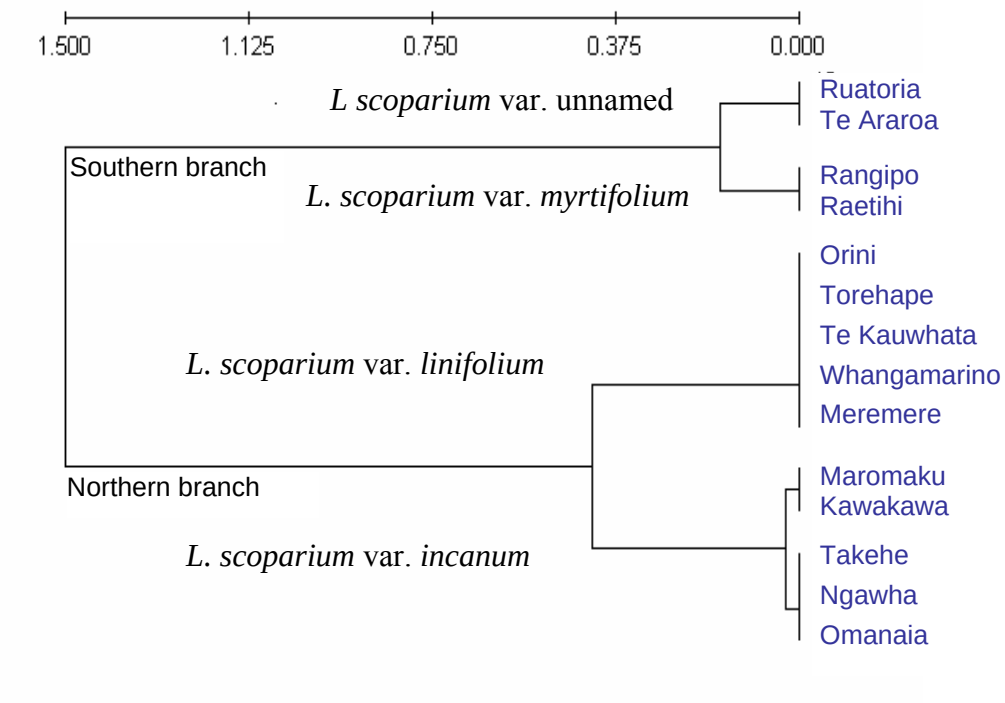

Figure 11.1 UPMGA dendrogram of core *Leptospermum scoparium* populations in the study area.

11.4.2 *Leptospermum scoparium* population analysis

Analysis of the studied *Leptospermum scoparium* populations discussed in the previous two chapters was also completed.

The UPMGA dendrogram (Figure 11.2) again illustrated the division of the *L. scoparium* populations into two major groups, the northern and southern branches. However this is complicated by the arrangement of the Coromandel region populations.

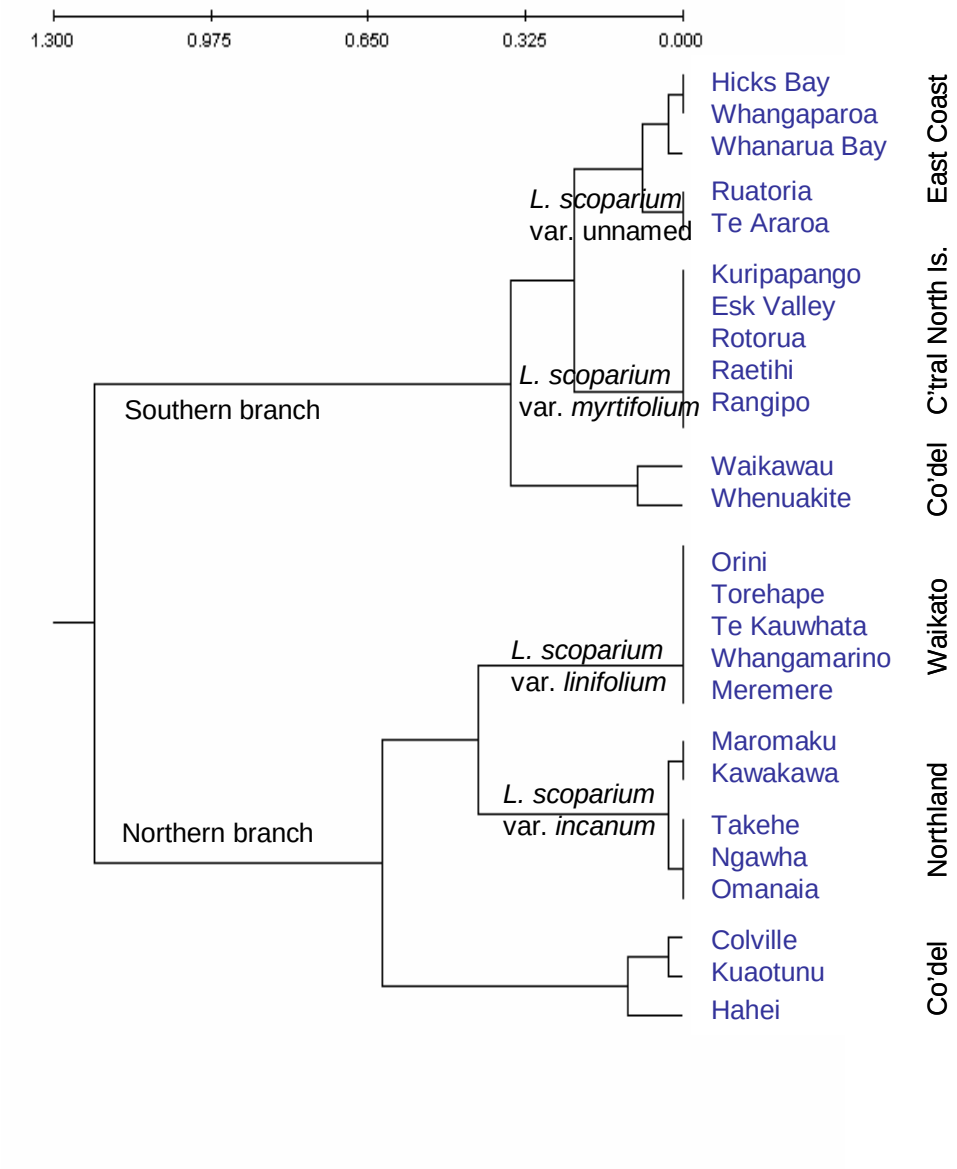

Figure 11.2 UPMGA dendrogram of *Leptospermum scoparium* populations in the study area.

The northern branch initially divided into three Coromandel populations and a combination of the core Northland and Waikato populations. Whilst these Coromandel populations, Colville, Kuaotunu, and Hahei, contained significant morphological elements that indicated an *L. scoparium* var. *incanum* genotype, the plants also contained either relatively enhanced nor-triketones or triketones in their essential oils. Therefore whilst the overall characteristics of these

populations indicated an alliance with *L. scoparium* var. *incanum* that is depicted in the dendrogram, the genetic difference is properly represented.

The dendrogram also indicates the Waikato and Northland populations, both carrying enhanced monoterpenes and eudesmols essential oils, are more closely related to each other than the three Coromandel populations discussed. The Northland and Waikato populations, representing *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* respectively, do not display the triketones or nor-triketones essential oils except for in the east of Northland where gene flow with the Coromandel region has probably occurred.

The southern branch contains the Central North Island and East Coast populations, however the two remaining Coromandel populations, Waikawau and Whenuakite, also cluster with this division. These Coromandel populations divide from the main southern branch before any other divisions. Waikawau *L. scoparium* leaf material contained enhanced triketones and less nor-triketones essential oils, whereas the Whenuakite population yielded the reverse. Both of these populations share characteristics with the southern varieties, however the essential oils with relatively enhanced monoterpenes are similar to those of the Waikato populations and the morphological grouping with *L. scoparium* var. *linifolium* reinforces this placement with, but initially divided from, the main southern branch.

Subsequently the main southern branch divides into the Central North Island *L. scoparium* var. *myrtifolium* and the unnamed variety present on the East Coast. This is in agreement with the essential oil profiles: essential oils with enriched nor-triketones were present in the former and enriched triketones in the latter populations. The Central North Island populations do not reveal any further genetic diversity, again probably due to the in sensitivity of the RAPD primers.

However the East Coast populations are genetically different, Ruatoria and Te Araroa remain grouped together, divided from Whanarua Bay, Hicks Bay, and

Whangaparoa. The final division of the East Coast further separated, *L. scoparium* collected from Hicks Bay and Whangaparoa clustered separately from the Whanarua Bay population. Therefore the morphological analysis that divided these populations is probably appropriate, East Coast *L. scoparium* derived from the Hicks Bay and Whangaparoa populations being genetically different from the other populations found in this region, yet this genetic analysis does not show the Hicks Bay and Whangaparoa populations, which displayed *L. scoparium* var. *incanum* morphological traits, to be aligned with the northern branch.

11.5 Genetic analysis, concurrence, and UMF® yield

The conclusions from the comparison between the distribution of the *Leptospermum scoparium* varieties and the UMF® activity deduced from the genetic analysis are similar to those in the previous two chapters.

Genetic analysis shows that *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* are closely related, and Northland and the Waikato wetlands that carry these varieties yield high UMF® activity mānuka honey. The genetic analysis also shows that the populations situated in the Coromandel region that also yield high UMF® activity honeys are related to major northern branch that includes *L. scoparium* var. *incanum*. Morphologically these Coromandel populations, Colville, Kuaotunu, and Hahei, are very similar to the Northland population yet the enhanced level of triketones and nor-triketones essential oils found in these locations indicates a degree of relatedness to the major southern branch.

Therefore the geographic UMF® variability in the Coromandel can be explained as gene flow in the high activity UMF® areas with *L. scoparium* var. *incanum*, whereas Coromandel areas yielding low activity UMF® honey retain more of the characteristics of *L. scoparium* var. *myrtifolium* located in the Central North Island or the unnamed triketones-producing variety present on the East Coast.

The East Coast *Leptospermum scoparium* populations that yield a mid-activity UMF® mānuka honey are genetically distinct from the other East Coast populations, and therefore the morphological analysis that suggested a degree of relatedness to *L. scoparium* var. *incanum* may be appropriate, however neither the genetic nor the chemotaxonomic analyses aligned the Hicks Bay and Whangaparoa populations with *L. scoparium* var. *incanum*. Migration of *L. scoparium* var. *incanum* into this region must be insignificant, as the *in situ* triketones-producing varieties' characteristics continue to dominate the local population.

The distinct *L. scoparium* var. *myrtifolium* in the Central North Island is recognised by the three methods, and is closely related to the unnamed triketone-producing variety present on the East Coast. Both of these varieties share characteristics with the Coromandel populations, and are found in regions where low UMF® activity mānuka honey is yielded.

Regrettably this portion of the study did not encompass all of New Zealand, therefore comment cannot be made regarding other *L. scoparium* populations, particularly the variety found in the West Coast on the South Island that has been reported to share morphological and chemotaxonomic characteristics with *L. scoparium* var. *incanum*.

For the most part the morphological and chemotaxonomic analyses would appear to be confirmed. Two major subdivisions and four varieties of *L. scoparium* are present in the study area.

The northern branch contains the closely related *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium*, both varieties producing essential oils with enhanced eudesmols whilst the former also has enriched monoterpenes essential oils. *L scoparium var. incanum* has lanceolate foliage, retains mostly unsplit capsules, often has pink-tinged petals, and the core population would appear to be in

Northland from which migration into other regions has occurred. The migration would appear to be principally coastal about the North Island. *L. scoparium* var. *linifolium* inhabits the Waikato lowland wetlands, has linear foliage and retains half the capsules unsplit. Both varieties yield high activity UMF® mānuka honey, and consequently where *L. scoparium* var. i*ncanum* has replaced or partially replaced other *L. scoparium* populations in other regions mid/high-activity UMF® mānuka honey is harvested.

The southern branch contains *L. scoparium* var. *myrtifolium* and the unnamed variety producing essential oils with triketones. *L. scoparium* var. *myrtifolium* carries enriched nor-triketones essential oils, has ovate foliage, most capsules are split, and inhabits the high altitude Central North Island. Genetic analysis shows the unnamed variety from the East Coast is closely related to *L. scoparium* var. *myrtifolium*. Foliage is linear-lanceolate and a quarter of capsules remain unsplit, and the triketones essential oils probably represent an additional biosynthetic methylation of the nor-triketones reported in the Central North Island. Both of these varieties yield low activity UMF® mānuka honey.

These three analyses show relatedness between geographically separated populations. The varieties' method of range extension and reasons for the replacement of *in situ L. scoparium* populations are discussed in Chapter Twelve.

Chapter 12

Summary, discussion and recommendations arising from the *Leptospermum scoparium* (mānuka) honey study

This chapter summarises and discusses the findings of the study, and provides an explanation for the current distribution of *Leptospermum scoparium* varieties throughout New Zealand.

The conclusions that mānuka honey containing UMF® activity is derived from *L. scoparium* nectar, the collection of other nectar sources with *L. scoparium* nectar proportionally dilutes the UMF® activity of mānuka honey, the dilution of the UMF® activity can be accounted for, and the adjusted UMF® activity illustrates a geographically-determined pattern that is not significantly influenced by the environmental factors are discussed. The conclusion that *L. scoparium* varieties determine the UMF® activity in the geographical regions is considered. The distribution of the *L. scoparium* varieties are discussed, in particular serotinous characteristics, and a transport method is proposed.

Finally the findings of the study are applied to the management of *L. scoparium* and the mānuka honey resource.

12.1 Source of UMF® in mānuka honey

From consideration of published data it was reasoned that the non-peroxide antibacterial activity (UMF®) present in honey harvested in New Zealand is derived from the nectar of *Leptospermum scoparium*.

UMF® is not recorded to occur in honeys harvested elsewhere in the world, apart from a honey derived from a *Leptospermum* species in south-east Australia. Therefore the introduced plant species in New Zealand that provide surplus nectar for collection by honeybees are not the source of UMF®. The other indigenous plant species that provide surplus nectar are also not the source of UMF®, as these indigenous plant species do not have a range throughout New Zealand that is the same as the range from which honey containing UMF® is harvested. Furthermore indigenous plants that are visited by honeybees yet do not provide surplus nectar are also rejected, as again the range of these species does not encompass the range of in which UMF® is harvested.

Whilst honey-bees will harvest honeydew, the excretory waste products of insects, an insect species does not have the appropriate range throughout New Zealand and there is no evidence of honeydew in mānuka honey containing UMF®.

Accordingly only *L. scoparium* has a distribution that is comparable to honey harvested that contains UMF®, and is therefore the source UMF®.

12.2 Dilution of UMF® by other honey types

The study presents the first New Zealand-wide appraisal of mānuka honeys that contain UMF® activity. UMF® activity was recorded throughout the country, and found to range between 4 and 16 activity units. UMF® activity below 4 units was less than the minimum level of measurement of the agar diffusion assay. Amalgamation of the honey samples supplied by the commercial apiarists into

discrete geographical entities with similar UMF® activity produced 77 areas within 11 regions. The field UMF® activity of the areas can be divided into bands of activity (Figure 12.1); sixteen areas contained 4–5.9 UMF® units, thirteen areas contained 6–7.9 UMF® units, eight areas contained 8–9.9 UMF® units, fourteen areas contained 10–11.9 UMF® units, six areas contained 12–13.9 UMF® units, and fourteen areas contained 14–15.9 UMF® units. Six areas without measurable activity (<4 UMF® points) were also noted. Disregarding the six areas that did not contain measurable UMF®, approximately half of the areas contained an activity of less than 10 UMF® units.

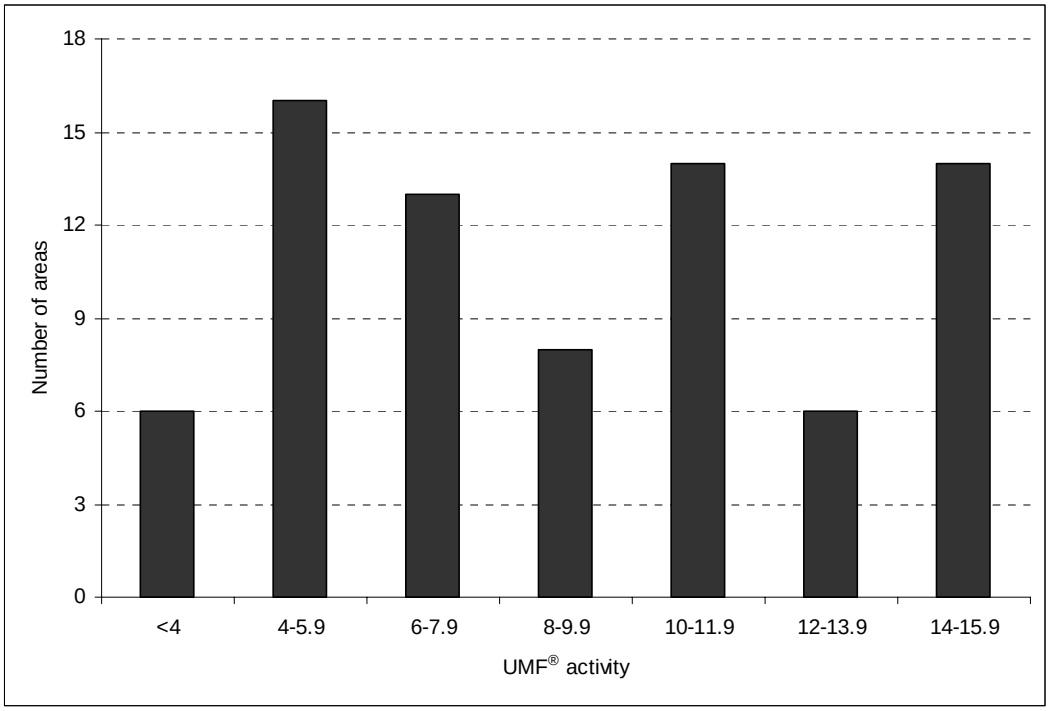

Figure 12.1 The unadjusted UMF® activity in areas of New Zealand from which mānuka honey is harvested. The UMF® activity is divided into bands.

The UMF® activity in mānuka honey reduces in a proportional manner when the *L. scoparium* nectar is diluted with nectar collected from other plant species. This occurs when a blended honey is created by the honeybees, or by apiarists combining the contents of a hive or hives that contain honeys sourced from different plant species.

A method was established, based on the thixotropic properties of mānuka honey, to determine the mānuka content of a honey. All of the other honey types harvested during the same season as mānuka honey illustrated similar physical behaviour to each other, having the Newtonian characteristics of a concentrated sugar solution. However, mānuka honey was highly viscous, approximately ten-times more viscous than the other honey types. The creation of mānuka honey blends in known proportions with the other honey types allowed a standard curve to be drawn; increasing concentrations of mānuka honey brought about a logarithmic increase in viscosity. Mānuka honey was shown to be a thixotropic fluid, and the thixotropic agent can be precipitated so it is probably a protein or colloid. Mānuka honey behaved as a Newtonian sugar solution when the thixotropic substance was removed, identical to the other honey types.

Following adjustment of the UMF® activity recorded in the areas to account for the dilution of the mānuka honey by other honey types and the effect of this dilution on the UMF® activity, the range of activities reported in the geographic areas was substantially altered (Figure 12.2).

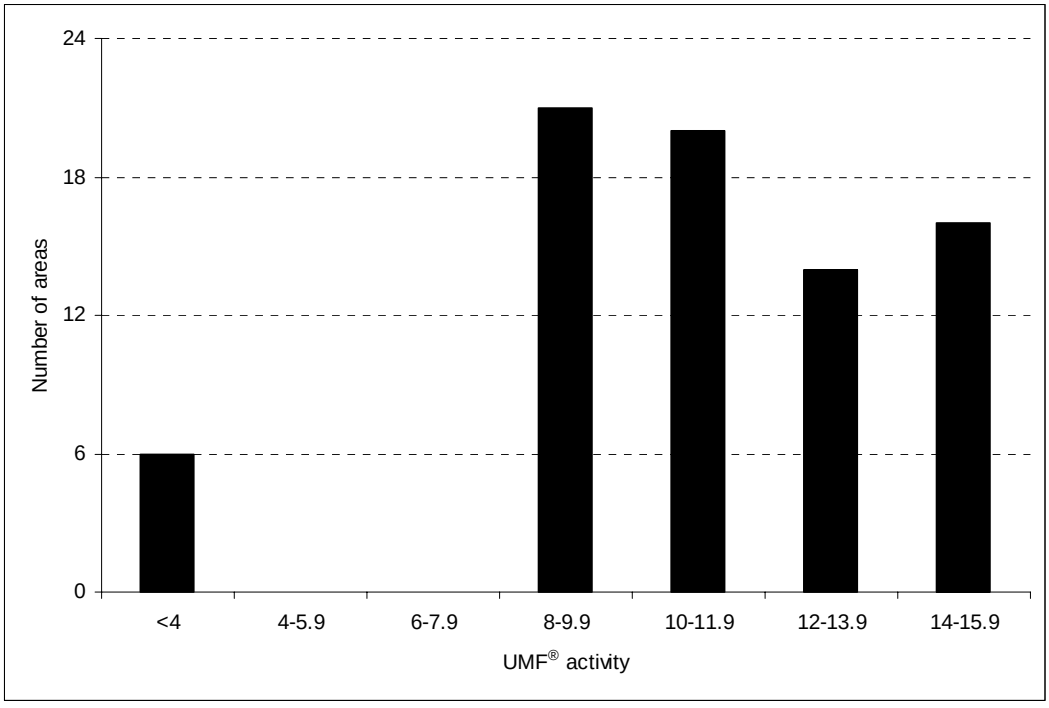

Figure 12.2 The adjusted UMF® activity in areas of New Zealand from which mānuka honey is harvested, accounting for the dilution of mānuka honey with other honey types. The UMF® activity is divided into bands.

There were no areas with activity of 4–5.9 UMF® or 6–7.9 UMF® units, twenty-one areas contained 8–9.9 UMF® units, twenty areas contained 10–11.9 UMF® units, fourteen areas contained 12–13.9 UMF® units, and sixteen areas contained 14–15.9 UMF® units. Disregarding the six areas that did not contain measurable UMF®, approximately one-third of the areas contained an activity of >10 UMF® units. In effect all the areas that yielded mānuka honey also yielded measurable UMF® with an activity greater than 8 units.

The reliability of the viscosity method can be considered in terms of the unique thixotropic characteristic of mānuka honey. Honeys produced from the other floral sources harvested with *L. scoparium* nectar, most commonly *Kunzea ericoides* and *Trifolium* spp., are Newtonian fluids and considerably less viscous than mānuka honey. One plant species introduced to New Zealand, *Calluna vulgaris* from which ling honey is derived, has been reported to yield a thixotropic honey (White 1975b). However the flowering season does not coincide with *L. scoparium*, and the range of *C. vulgaris* is montane. These honey types are not considered to be harvested together (K. Clements pers. comm.).

A number of mānuka honey samples were received from six discrete geographical areas that did not contain measurable UMF® activity. These samples contained some mānuka honey; the dominant aroma and flavour of honey derived from *Leptospermum scoparium* nectar masked the other honey types contained in the honey blends. However the viscosity analysis revealed these samples contained less than 30% mānuka honey. An argument could be made for the inclusion of these non-detectable samples with a nominal value of 2 UMF® units, however the UMF® may have ranged between 0–4 units making UMF® adjustment to account for the mānuka content unreliable. As these honeys were principally derived from another floral source, they were excluded from the study.

For the first time it was shown that all mānuka honey, that is predominantly from *L. scoparium* nectar, contains UMF® activity. The dominant aroma and flavour of mānuka honey will disguise the other honey types that include a small proportion

of mānuka honey. Therefore honey that contains mānuka honey should also contain UMF® activity. If the mānuka honey does not contain UMF® activity, it is highly probable that that honey contains less than 30% mānuka honey. A blended honey should not be presented as mānuka honey, despite the aroma and flavour.

The adjustment of the UMF® activity in the mānuka honey samples to account for the dilution by other honey types revealed geographically different levels of UMF® activity in monofloral mānuka honey. Instead of all the areas adjusting to the maximum UMF® activity recorded, 14–15.9 UMF® units, the areas still contained considerable variability, ranging between approximately 8–16 UMF® units. Thirteen areas yielded mānuka honey that the viscosity method determined to be 100% monofloral, and therefore the UMF® activity of these areas was not adjusted. These areas had an UMF® activity range of 10–15.6 units, agreeing with the range of UMF® activity reported for the adjusted activity measurements.

This indicated another factor or factors, additional to the dilution of the UMF® activity in mānuka honey by other honey types, was influencing the UMF® activity in monofloral mānuka honey.

12.3 Geographical divisions of UMF® activity

The adjustment of the UMF® activity within the 71 areas to account for the dilution of the mānuka honey by other honey types allowed the comparison of UMF® activity in monofloral mānuka honey between areas, both within and between regions throughout New Zealand.

The difference in the adjusted UMF® activity within seven of the regions considered in isolation was not statistically significant. The Northland, Waikato, Gisborne, Hawkes Bay, Wairarapa, northern South Island, and eastern South Island regions each yielded homogenous levels of UMF® activity. However four regions contained sub-regions that yielded different levels of UMF® activity that

was statistically significant. The Coromandel and West Coast regions each yielded three levels of activity, and the East Coast and Taranaki regions two levels each.

Statistically insignificant differences were observed between the regions and sub-regions, and the final amalgamations and analysis revealed five divisions (Figure 12.3) that were significantly different (p <0.001).

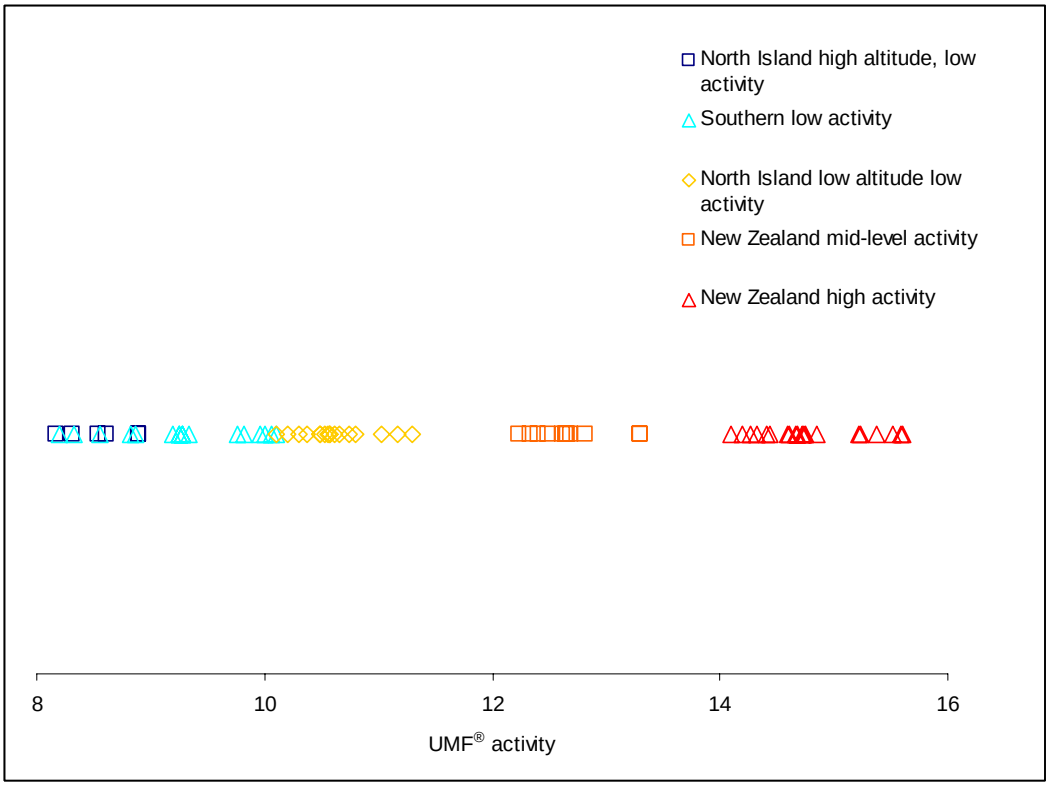

Figure 12.3 Mean adjusted UMF® activity of mānuka honey from areas within the five activity divisions in New Zealand.

The lowest mean UMF® activity of 8.6 units from these divisions was present in mānuka honey yielded in the high altitude districts of the North Island, namely the hill country towards the Central Plateau in the Hawkes Bay and the western Central Plateau inland of Taranaki. The southern low activity division was a little higher, with a mean value of 9.2 UMF® units, and encompassed the Wairarapa, northern and eastern South Island, and the more inland sections of the West Coast. The third low activity division was comprised of most of the North Island north of

the Wairarapa, including the low activity sections of the Coromandel and East Coast, the lower altitude Taranaki areas and the Gisborne region, and yielded a mean value of 10.6 UMF® units.

The medium activity division was present in three regions in both islands, and yielded a mean value of 12.7 UMF® units. The medium activity districts were present in conjunction with sub-regions representing the high activity division in both the Coromandel and West Coast regions, yet the East Coast was surrounded by a low activity sub-region. The high activity division was also present in both islands, particularly in the north of the North Island, Northland, Waikato and sections of Coromandel, but also found in an area of the West Coast. The value of mean activity yielded in this division was 14.8 UMF® units.

Considerably more similarity of the data was apparent between the low activity divisions than the separation between the medium and high UMF® activity divisions. To some extent a continuum would appear to exist between the divisions, and this is more pronounced in the low UMF® activity divisions.

Therefore UMF® activity in mānuka honey differed through New Zealand, and whilst the adjustment for the dilution of UMF® by other honey types accounted for much of the variability between and within regions, another factor was responsible for the differences reported.

12.4 Variability in true (adjusted) UMF®

Three avenues were investigated to establish an explanation for the variability of UMF® activity in mānuka honey that had been adjusted to account for the dilution by other honey types. The impact of another species, an environmental influence, and the genetic varieties of *Leptospermum scoparium*, were considered.

Two explanations for the variability in UMF® activity within and between regions were dismissed. Another species was not found that would influence *L. scoparium* throughout its entire range in a manner that would explain the variability of UMF® activity. To study the influence of the environment, a generalised additive model was created utilising the environmental factors reported to affect plant nectar production. A significant correlation was not drawn until a region/sub-region coding was included in the analysis, indicating a regional factor other than environmental parameters was responsible for the variability of UMF® activity.

Three studies were completed to establish whether the distribution of varieties of *L. scoparium* explained the variability if UMF® activity. The *L. scoparium* plant material used for morphological, chemotaxonomic, and genetic analyses was collected from five regions of the North Island, and represented the range of UMF® activity variability in mānuka honey. The three methods employed to study the varieties provided for the most part the same illustration of relatedness between the *L. scoparium* varieties. The varieties may be split into the northern and southern branches.

Two *L. scoparium* varieties were present in the northern branch. In Northland, and in the sub-region of the Coromandel that yields honey with high UMF® activity, *L. scoparium* var. *incanum* is widespread, carrying lanceolate foliage with enhanced monoterpenes and eudesmols essential oils, and retains most seed capsules unsplit. There are also morphological traces of this variety on the East Coast. These geographical regions/sub-regions yielded high/medium activity honey, with mean values of 14.8, 14.9, and 12.5 UMF® units respectively. *L. scoparium* var. *linifolium* is closely allied to *L. scoparium* var. *incanum*. *L. scoparium* var. *linifolium* is found in the Waikato, has linear leaves with enhanced eudesmols essential oils, and dominates the lowland peat wetlands in the north Waikato. The mean UMF® yield from this region is 15.3 units. Despite the leaf morphology of these varieties appearing different, the three methods of study aligned these two varieties.

The southern branch also contained two varieties, which were aligned by the three methods of study. In the Central North Island *L. scoparium* var. *myrtifolium* is present, defined by ovate leaves and containing enhanced nor-triketones essential oils. This region produced a low mean UMF® activity of 8.6 units. An unnamed variety is associated with *L. scoparium* var. *myrtifolium*, and is principally present on the East Coast. This variety has linear-lanceolate leaves that contain the triketone essential oils. The sub-region of the East Coast where this variety is located yielded a mean UMF® activity of 10.9 units. A blend of these characteristics are also present in the southern Coromandel indicating hybridisation, a sub-region that yielded low mean UMF® activity of 10.5 units.

Other studies of *L. scoparium* have revealed another unnamed variety present on the West Coast pakihi soils, the sub-regions of that region that yielded high and medium mean UMF® activity of 14.4 and 13.1 units respectively. The reported characteristics of this variety are shared with *L. scoparium* var. *incanum*: enhanced monoterpenes essential oils and the retention of unsplit seed capsules. These characteristics are not present in the other varieties of *L. scoparium* in New Zealand, and strongly suggest a degree of relatedness. Likewise essential oil analysis has also reported triketones in the essential oil present in *L. scoparium* in the Marlborough Sounds, a district encompassed in this study by the northern South Island region yielding a low mean UMF® activity of 9.1 units

Accordingly *L. scoparium* in the study area may be divided into two branches. The northern branch contains *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium*, and most probably the variety present on the West Coast pakihi soils is closely related. This branch yields mānuka honey with high UMF® activity. These varieties are found in Northland, a section of the Coromandel, Waikato, traces on the East Coast, and most probably sections of the West Coast. The southern branch contains *L. scoparium* var. *myrtifolium* and an unnamed variety from the East Coast with triketones essential oil. This branch yields mānuka honey with low UMF® activity, and is present in the southern Coromandel, East

Coast, Central Plateau, and northern South Island. The hybridisation of the varieties yielding high UMF® activity mānuka honey with the low UMF® activity varieties would appear to explain the areas of New Zealand which yield a medium level of UMF® activity. The areas of Coromandel and the West Coast yielding medium-level UMF® activity are positioned between high and low UMF® activity yielding areas, and areas of the East Coast that yielded medium UMF® activity mānuka honey exhibits *L. scoparium var. incanum* morphological characteristics.

12.5 Distribution of *Leptospermum scoparium* varieties

The current distribution of these *Leptospermum scoparium* varieties appears fragmented. However, consideration of the biogeography of New Zealand, the impact of humankind on the environment, a proposed distribution mechanism, and the selection advantages of the *L. scoparium* varieties provides an explanation of the current distribution.

The origins of *L. scoparium* in New Zealand have not been established, and the earliest finds of *Leptospermum* pollen in upper Cretaceous and older Tertiary beds are interpreted as generic rather than species representatives (Couper 1953, 1960). It has been suggested a more recent dispersal from Australia following the Miocene is most likely (Thompson 1989). New Zealand and Australia shared a similar climate from the late Oligocene to the late Miocene, and this is considered to be the principal period of long-distance dispersal of plant species to New Zealand from Australia (McGlone et al. 2001). During the late Pliocene and Pleistocene the New Zealand climate became cooler, driven by tectonic southward movement and orogenic processes, and the Pleistocene glacial and inter-glacial cycles fragmented the environment further (McGlone et al. 2001). However the North Island climate was moderated by oceanic influences and cold-temperate refuges remained north of the Waikato and around the East Coast, with temperate rain forests continuing to exist in the far north of Northland (Stevens 1974; Wardle 1991). As forest cover is thought to have dominated in the interglacial

periods the opportunity for establishment by *L. scoparium* would have been limited to coastal scrub and disturbed sites (McGlone et al. 2001). However, recent widespread land clearance by human inhabitants has greatly extended the range available to *L. scoparium*.

Thompson (1989) has suggested a stepwise dispersal from the Australian evolutionary centre since the Miocene. *L. scoparium* seed is non-persistent once shed from the capsules (Mohan et al. 1984) and dispersion is rapid from established populations, particularly in open, low-fertility or disturbed environments. The cold-tolerant varieties of *L. scoparium* would certainly have survived in glacial refugia, and probably a greater range was inhabited (Burrows et al. 1979). However, a genotypic range of cold tolerance has been recorded, indicating a level of environmental adaptation (Decourtye & Harris 1992). The seral characteristics of *L. scoparium* and its role in heathland (Burrows et al. 1979) makes the species an ideal candidate to have survived glacial cycles, despite the difficulty of maintaining adaptation to the environmental extremes through climate fluctuations (McGlone et al. 2001).

A hypothesis explaining the derivation of the *L. scoparium* populations in the studied area of the North Island may be formed from the data. In the study area the Central North Island population displays the greatest adaptation to cold. This population may represent an ancient extant population, a cold-tolerant ovate-leaved variety with a biosynthetic pathway allowing C-methylation of acyl-phloroglucinol producing nor-triketones. The range of this variety could be expected to have increased during interglacial periods, in particular the exploitation of cold and infertile heathland found in the Central North Island as the climate warmed. Traces of this population are now isolated. Evidence of the nor-triketone is present in the Coromandel, which cannot be explained by geographic proximity as the intervening Waikato population does not carry nor-triketones oil. Therefore this variety must have inhabited the Coromandel in the past, perhaps during the last glacial period. The Australian *L. scoparium* oil profiles do not contain nor-triketones (Brophy et al. 1999). Accordingly the

Central North Island population may represent a variety that established in New Zealand and evolved this biosynthetic pathway independently, or dispersed to New Zealand when the Australian varieties carried the genome for this type of essential oil. However, it may be that nor-triketones have not been reported in Australian studies because the steam distillation method was employed rather than solvent extraction as used in the present study; steam distillation would not be expected to extract the marginally volatile nor-triketones.

Associated with the Central North Island is the East Coast population. Leaf morphology is different, and the triketones essential oils found in the East Coast represents a further methylation step; the addition of another methyl group to the nor-triketones. This group probably represents a genetic change that has become established in the East Coast region, and is not represented in the Central North Island. Triketones-containing oil is present in the Coromandel, however the transport of seed may provide an explanation. Endemism of New Zealand wetland plants is markedly less than forest species, and this is thought to correlate with migratory wetland bird species (McGlone et al. 2001). Therefore the coastal migration of bird species could explain the movement of East Coast *L. scoparium* variety containing triketones to the Coromandel. Douglas et al. (2004) report triketones oils in the Marlborough Sounds and this may be another example of coastal dispersion. The Australian *L. scoparium* varieties do not carry the steam distillable triketone oils either, which suggests the Central North Island and East Coast populations are unique, having a biochemical pathway which ultimately leads to the synthesis of triketones essential oils.

Other varieties have appeared, and these are more likely to have been dispersed from Australia than to have evolved in New Zealand. Establishment would have occurred in a disturbed coastal environment, a habitat suitable for *L. scoparium*. The Northland population has a similar oil profile to that of *L. scoparium* var. *scoparium* from Victoria and Tasmania, and *L. scoparium* var. *eximium* from Tasmania, exhibiting enriched levels of α-pinene and eudesmols (Brophy et al. 1999). *L. scoparium* var. *incanum* is highly serotinous (Harris 2002), similar to

the Australian varieties (Bond et al. 2004); however the lack of lignotubers suggests a derivation from the Australian *L. scoparium* var. *scoparium* rather than from *L. scoparium* var. *eximium*. It may be that the adaptation to fire of this variety has allowed dominance in environments that have been regularly burnt, particularly the Northland gumlands and to a lesser extent in the northern Coromandel and East Coast. As the Northland variety has spread along the coasts, again probably assisted by migratory wetland birds, *in situ* populations of *L. scoparium* have been replaced where serotiny is a selective advantage. Consequently the East Coast does carry the Northland pink-flowered *L. scoparium* var. *incanum*, confirming gene flow between Northland/Coromandel, yet the region continues to maintain mostly the triketones-rich population. This process of replacement has also not been completed in the north Coromandel, where a mix of Northland leaf morphology and Northland, Central North Island, and East Coast oil profiles are found.

However another example of complete population replacement may be provided by the fire-prone Westland pahiki, again with a coastal link with Northland. Bond et al. (2004) have reported a highly serotinous *L. scoparium* population present in this area, from which Douglas et al. (2004) classified an α-pinene monoterpenes-enriched variety that is similar to the Northland *L. scoparium* var. *incanum*.

The original Waikato population has probably been displaced by a linear-leaved variety, which would appear to be spreading into the hill country of the Central North Island and the southern Coromandel. Initial colonisation would appear to have occurred on the low-fertility Waikato wetlands, where *L. scoparium* root aerenchymous tissue (Cook et al.1980) allows survival of extended periods of waterlogging. Following volcanic tephra disturbance of this environment the initial woody pioneer would have been the most proximate *L. scoparium* variety. Again the oil profile is similar to the Australian *L. scoparium* var. *scoparium*. The Waikato population does not carry nor-triketones or triketones essential oils, and is separate from the major southern branch, the Central North Island/East Coast cluster. The oil profile of this population is similar to the Northland

population and these populations were shown to be related, yet the leaf morphology significantly differs. Whilst there may be an evolutionary link in New Zealand, the number of long-distance migratory birds in the Firth of Thames probably provides a more likely explanation.

This study, using leaf morphology, essential oil profiles and genetic analysis, indicates two major divisions, each divided into two sub-divisions, of *L. scoparium* exist in the study area of the North Island. *L. scoparium* var. *incanum* describes the Northland variety, *L. scoparium* var. *linifolium* the Waikato variety, and *L. scoparium* var. *myrtifolium* the Central North Island, and the East Coast variety is unnamed. The distribution of *L. scoparium* var. *myrtifolium* would appear to indicate a currently reduced range, and this variety may have survived in northern New Zealand glacial refugia. This variety carries a unique biosynthetic pathway for nor-triketones that has not been reported in Australia, and likewise the closely related population of the East Coast has an additional biosynthetic methylation of nor-triketones producing triketones essential oils. Establishment of *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* has occurred, the former displacing populations where serotiny is an advantage particularly in Northland, the latter initially inhabiting the Waikato wetlands. *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium* are closely related, indicating a relatively recent common ancestral species.

12.6 Recommendations arising from the study

The subsidiary intention of the thesis was to determine whether the management of the resource could provide a more assured supply of mānuka honey with high UMF® activity.

In the short-term, the improvement of the supply of mānuka honey with high UMF® activity will rely on harvesting techniques. Commercial apiarists should follow a number of guidelines to minimise blending floral sources. Hives that are

positioned where a number of floral sources are available to the honeybees throughout the season should have the supers separated before honey extraction to ensure the mānuka honey is as pure as possible; the common honey blend derived from *L. scoparium* and the later flowering *Kunzea ericoides* is an obvious example.

The dominant aroma and flavour of mānuka honey results in honey blends with minimal mānuka honey retaining to some degree the qualities of a monofloral mānuka honey. Therefore it has been difficult to accurately establish the purity of mānuka honey, and these somewhat dubious blends find a premium market in the culinary trade. This study has produced a method that allows rapid and economical analysis of the purity of mānuka honey. Whilst a large databank of honey viscosities, particularly those commonly harvested with mānuka honey, would have to be created prior to commercial application, the use of the viscosity method would ensure apiarists were rewarded for the purity of the mānuka honey rather than the quantity supplied. The honey manufacturing and packing companies could then blend mānuka honey with other honey types for the culinary market, and confidently certify the product as containing a certain percentage of mānuka honey.

The other recommendations are of a long-term nature. The varieties of *L. scoparium* in New Zealand require conservation. The increased seral range of the species, brought about by human impact, will allow more rapid hybridisation of *L. scoparium* varieties, and environmental disturbance will continue to erode the unique status of the varieties unless conservation steps are taken, recognised as a risk to indigenous flora by Simpson (1992). Therefore a study of the genetic diversity of *L. scoparium* needs to be completed for the whole of New Zealand so a representative common garden and seed-bank resource can be established before the unique characteristics of varieties are lost. The taxonomic status of *L. scoparium* in the New Zealand would be established, and despite the non-recognition of the varieties in recent New Zealand Flora, a taxonomic review of the species aggregate is required.

After the genetic diversity is established in New Zealand a meaningful comparison with the south-eastern Australian persistent-capsule group of *Leptospermum* (Thompson 1989) could be made using the same criteria as the New Zealand study. This should promote a considerable advance in the understanding of the relationship between the New Zealand and Australian species and varieties contained within this *Leptospermum* group.

The development of plantations of *L. scoparium* should be pursued. The varieties of the species that yield mānuka honey with high activity UMF® are relatively restricted in geographic distribution. Much of the *L. scoparium* scrub which established after initial land clearances has been removed in New Zealand, and therefore the total source is now limited. Any area of *L. scoparium* where forest development can occur will eventually return to primary vegetation unless the environment is managed to arrest forest succession. It should be noted the seral populations of *L. scoparium* that exist today, and are harvested for mānuka honey, represent the first stage of the return of large areas of difficult and poorly managed agricultural land to primary forest. This includes all the seral populations of *L. scoparium*, and only excludes the populations found on Northland gumlands, the Waikato wetlands, the West Coast pakihi soils and exposed high altitude situations where a hospitality barrier exists. These unfavourable areas have not been developed and are unlikely to be economically viable for agriculture in the near future.

Therefore the promotion of plantations for marginal farming environments may prove to be a reasonable suggestion, particularly for areas of New Zealand that are subject to wide-scale erosion, as the stabilising effects of *L. scoparium* are well documented. Any areas set aside for plantation development would need to be managed in the long-term to prevent the establishment of forest species. Furthermore, careful consideration of the variety and environment of the proposed plantation would need to be made.

In association with the *L. scoparium* plantation development, cultivar development could be considered. Ornamental improvements of *L. scoparium* hybrids have continued for more than half a century and these cultivars have been shown to reproduce true to type. Therefore the development of a cultivar that yields mānuka honey with high activity UMF® and is well suited to plantation management could be arranged.

It is hoped that this study has been of some use to the New Zealand honey industry, and that some of these recommendations are implemented to improve the supply of an increasingly valuable resource, mānuka honey with high activity UMF®.

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