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Defining North Island manuka chemotype resources

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1 Executive summary

A survey of the essential oil content and chemical composition of North Island manuka (*Leptospermum scoparium*) was conducted to determine the range and variability of essential oil compounds. The purpose of the survey was to complete the resource knowledge base for New Zealand manuka oil and provide a basis for the expansion of the manuka oil industry. Foliage from 44 sites and 132 individual plants was collected during February–March 2001. The drying, distillation and chemical analysis of the 132 foliage samples was completed in April–June 2001.

The average yield of steam distilled manuka oil, based on a standard dried sample, was 0.3% but ranged from <0.1 to 1.1%.

The levels of 48 chemical compounds were measured in each oil sample by gas chromatography. Broad plant to plant and/or site to site variations were found. Four major chemical types (chemotypes) and one minor chemotype can be identified:

1. **monoterpene rich oils** (i.e. C10 oils) are regionally predominant in Northland, and are dominated by the compounds α- and β-pinene, myrcene, 1,8-cineole and linalool. An unusual oil, with geranyl acetate as the predominant compound, was identified at one site in the lower North Island. The mean total monoterpene level in the North Island manuka oils was 17.1%, the minimum 2.1% and the maximum 66.3%,
2. **sesquiterpene rich oils** (i.e. C15 oils) are the common chemotype in the North Island. The predominant compounds are γ-ylangene + α-copaene, β-caryophyllene, α-humulene and α-muurolene. The mean total sesquiterpene level in the North Island manuka oils was 56.8%, the minimum 16.2% and the maximum 77.4%,
3. **triketone enriched oils** within the sesquiterpene rich oils are localised to the East Cape region. This chemotype contains elevated levels of the biologically active triketones flavesone, isoleptospermone and leptospermone. The mean total triketone level in the North Island manuka oils was 5.8%, the minimum 0.1% and the maximum 33.3%,
4. **monoterpene – sesquiterpene mixed oils** are more common north of Hamilton,
5. **methyl cinnamate enriched oils** predominate at two sites in the western North Island. The mean cinnamate level in the North Island oils was 6.0 %, the minimum 0.1% and the maximum 23.9%.

Four major oil chemotypes were identified throughout the North Island, but the triketone rich oils only occur in the East Cape region.

The reasons for the occurrence of these regional chemotypes are not known. The triketone enriched oils are commercially valuable because of their antibiotic activity but they represent only a minor portion of the manuka resource. The geranyl acetate oil and methyl cinnamate oils have commercial potential as they are used in the flavour industry and natural "organic" sources are keenly sought. The commercial potential for each chemotype or specific oil needs further assessment, and the opportunities for commercial development (e.g. teas, aroma oils or fragrance compounds) requires investigation.

2 Introduction

Manuka (*Leptospermum scoparium*) is present throughout New Zealand, in many diverse ecosystems from the lowland to sub-alpine areas. This genus belongs to the family Myrtaceae, a family rich in leaf oils. The essential oil can be extracted from manuka leaves by steam distillation. The chemical make-up of manuka oils varies between different New Zealand locations, and our previous survey of the South Island determined that there were four predominant chemotypes: monoterpene rich; sesquiterpene rich; enhanced triketones in sesquiterpene rich oils and mixed oils with a balance of monoterpenes and sesquiterpenes (Crop & Food Research unpublished results). This survey, to determine the range and variability in North Island manuka essential oil compounds, will complete the resource knowledge base for New Zealand manuka oil, and provide a basis for the expansion of the manuka oil industry.

Manuka is the most abundant New Zealand shrub or small tree. In favourable open conditions it is a fast growing, conical-shaped bush to about 4 m high, but local varieties vary in leaf form, flower size and colour, and can be creeping matted plants or small trees reaching 8 m high.

Oil sacs, known as schizogenous secretory cavities, are present in the leaves of manuka and appear as transparent trans-lucid points on the leaf surface.

The oil in the glands can be removed from the leaves by heating to vaporise the oil, and cooling at the end of the distillation process allows the collection of the oil.[1] Previous work shows that manuka oil yield changes throughout the year, but its composition is relatively stable (Crop & Food Research unpublished results).

Manuka oil, particularly the triketone rich chemotype, has activity against pathological bacteria, e.g. *Staphylococcus, Listeria, Enterococcus*[2] and some fungi, e.g. *Trichophyton, Microsporum,* as well as anthelmintic and insecticidal activities. The unique activity of the East Cape manuka oils against Gram positive bacteria, e.g. *Staphylococcus aureus* and its antibiotic resistant strain MRSA, has been conclusively proven to be due to the presence of triketones.[3,4] This biologically active chemotype is now widely used as a topical oil or ointment, or in personal hygiene products. Non triketone essential oils from manuka are also used in the toiletries, hygiene, aromatherapy and herbal medicinal markets.

Manuka foliage is collected from the sample stem

3 *Method*

The North Island land mass was sub-divided into 43 blocks on a 1:100 000 scale map by taking the latitude and longitude grid squares and dividing each square in half horizontally. A grid pattern of sampling locations was selected to give an orderly coverage over the land mass, but individual sites were chosen within each grid based on the presence of manuka and following landowner contact. Sampling was undertaken during February-March 2001, with three individual plants at 44 sites harvested for foliage (Fig. 1). The plants at each site were generally within 50 m of each other, although at three sites (Cape Reinga, Kennedy Bay, Waimarino) a single plant was at a greater distance. The survey provided 132 oil samples for analysis and allowed the variability in each population to be assessed. Where practical, each site and plant was photographed and the site recorded by GPS for future reference and location. At most sites, the basal circumference, height, canopy width, sooty mould severity, presence of borer, and occasionally flower colour were recorded for each sample plant. The cut foliage sample (3-5 kg) was bagged, numbered, and sent by courier post to the Invermay Research Centre, Mosgiel.

Each bag was sub-sampled to retain a botanical voucher of the sample bush (for future reference), while the main portion was slowly dried at 3°C and 17% RH for 20 days prior to distillation. Each dried sample was chopped into nominal 2.5 cm lengths and distilled under standardised conditions for 2 hours in laboratory designed stills.[3] The volume of collected oil was measured and retained in freezer storage in individual glass vials. The oil samples were analysed at the Plant Extracts Research Unit, Chemistry Department, University of Otago, by gas chromatographic (GC) analysis and the 48 major oil components identified.[3] Subsequent to the chemical oil

analysis, 24 sites were chosen for macro-micro element analysis of the 72 leaf samples to determine the variability in chemical content.

Figure 1: Location of sampling sites.

On-site transport at Waimiha

4 Results

Forty-four sites and 132 plants were sampled throughout the North Island (Fig. 1; Table 1), of which 26 were from road reserves, 13 from farmed land, and 5 from production forests. The mean height of the plants sampled was 4.2 m (range 1.2–8.1 m); the mean canopy width was 2.6 m (1–6.5 m); the mean basal circumference was 468 mm (180–2800 mm); and the mean age of the cut stem was 13 years (5–41 years).

Manuka stem borer was present in 48% of the plants sampled. Sooty mould was present in 85% of the population with 30% of the population having an infection judged to be moderate to severe. Flowers were present on 13% of the plants and, apart from pink-flowering plants (*Leptospermum scoparium* var. *incanum*) sampled at the Spirits Bay – Te Hapua Road sites, the plants were white-flowered.

The harvested foliage and small stems received at the laboratory had an average dry matter of 53.5% (the range was 33.1-68.6%) while the plant material slowly dried in a controlled atmosphere seed store. When distilled it had an average dry matter of 90.2% (85.8–93.9%). The percentage oil yield from each bush calculated from the laboratory oil yield was corrected for individual dry matter variability. The average oil yield was 0.3% (where % equates to ml/100 g dry matter). The range was 0.03–1.09% (Fig. 2). Some Northland plants had oil yields close to or above 1%. The yields from plants 1, 27, and 108 at sites 8, 9, and 19 were affected by oil losses during distillation.

The GC analyses measured levels of 48 compounds in each oil and this data is summarised to show the mean, minimum and maximum in Table 2. The survey confirmed that the manuka oils fit into four major chemical types

(chemotypes) and one minor chemotype. These were: monoterpene rich oils; sesquiterpene rich oils; triketone enriched oils within the sesquiterpene rich oils; monoterpene – sesquiterpene mixed oils; and oils with elevated methyl cinnamate. Monoterpenes, sesquiterpenes, triketones and methyl cinnamate are different classes of compounds as they are products of four separate biochemical pathways.

Four sites in Northland gave manuka oils dominated by monoterpene chemistry (Table 1). The average level at these sites varied between 42.1 and 58.9% (Table 1; Fig. 3). The compounds most common were α-pinene, myrcene,1,8-cineole and linalool. There were elevated monoterpene levels (>25%) in plants at further sites, which had high plant to plant variation. These sites were all north of Hamilton. The highest monoterpene level was 66.3%, the North Island average 17.1% and the minimum 2.1% (Fig. 3). Of special interest is the high level of geranyl acetate (54%) found in the manuka oil from Makara. This can be defined as a new chemotype in the monoterpene oils, with potential use in the fragrance industry.

Table 1: North Island manuka survey – site location, and compound class proportion (%).

Site	Location (Three plants/site)	Monoterpenes (Mean %)	Sesquiterpenes (Mean %)	Triketones (Mean %)	Cinnamate (Mean %)
1	Te Hapua/ Cape Reinga	29.7	60.4	4.4	1.4
2	Spirits Bay Road	57.8	22.0	0.3	0.6
3	Karatia	58.9	18.8	1.2	1.3
4	Karikari	34.8	42.5	3.3	4.3
5	Broadwood	57.4	25.1	1.9	2.4
6	Ruapekapeka	42.1	34.1	3.9	3.1
7	Twin Bridges	14.6	61.9	9.4	4.3
8	Tutukaka	40.6	43.2	3.6	2.2
9	Maitahi Road	36.9	44.7	5.3	3.6
10	Warkworth	31.5	48.0	5.1	2.6
11	Ararimu Forest	19.3	52.2	8.2	6.0
12	Kennedy Bay Road	8.7	63.1	9.9	6.2
13	Hikuai	14.3	62.7	5.3	6.4
14	Waikato Heads	13.4	65.9	5.8	8.2
15	Hauraki Plains	27.3	46.8	4.7	6.7
16	Te Akau	17.6	61.2	5.3	7.9
17	Karioi	11.0	65.5	5.9	6.0
18	Te Kaha	4.2	65.7	20.2	6.8
19	Te Araroa	2.7	66.4	25.6	5.1
20	Moanatuatua	12.3	58.7	4.7	8.3
21	Tauranga Road	4.8	70.3	5.5	6.4
22	Kawhia	5.7	72.7	4.8	4.7
23	Pueora	5.8	74.0	5.9	5.8
24	Waiotapu	4.9	70.6	10.0	5.3
25	Takapau Inland	9.5	71.2	6.9	2.3
26	Upper Motu	4.2	74.4	4.8	5.4
27	Waimiha	5.6	67.8	3.6	10.6
28	Waimata Road	8.3	67.4	8.6	2.6
29	Awakino Gorge	6.7	65.8	3.3	15.2
30	Waitahanui	3.8	78.7	5.1	4.4
31	Te Reinga Road	8.6	72.3	4.7	3.5
32	Tarawera	5.0	74.0	4.4	4.3
33	Tangerekau	7.6	58.3	4.3	17.7
34	Waimarino	4.6	73.7	5.4	7.4
35	Kaweka	5.9	64.1	3.7	6.8
36	Waverley	10.6	66.1	3.9	4.5
37	Taihape	14.5	60.5	3.4	6.5
38	Guavas Forest	6.6	70.3	4.3	5.4
39	Kiwitoa	7.4	63.9	3.7	9.3
40	Te Uri	7.3	71.3	4.1	6.8
41	Alfredton	6.9	67.5	4.3	9.8
42	Otaki Forks	10.5	65.7	3.9	11.9
43	Makara	53.9	30.8	1.6	4.2
44	Pahaoa River	10.0	59.1	11.2	10.9

The remaining 35 North Island sites gave sesquiterpene rich oils. These occur almost exclusively south of Hamilton, and are the most widespread chemotype in the North Island (Table 1). The predominant compounds are γ-ylangene + α-copaene, β-carophyllene, α-humulene and α-muurolene. The highest sesquiterpene level was 77.4%, the North Island average was 56.8% and the minimum was 16.2% (Table 2; Fig. 4).

A special interest in this survey was to ascertain if there were triketone rich oils in areas outside the East Cape. Apart from the two East Cape sites where mean triketone levels were above 20%, there were only two plants with levels above 15% (Ararimu, Waiotapu) in the rest of the survey (Fig. 5). The highest triketone level was 33.3%, the North Island average was 5.8%, and the minimum 0.1% (Table 2).

Methyl cinnamate enriched oils occurred at three locations. At Tangerekau and Awakino Gorge sites the mean levels were greater than 15% of the oil. The highest cinnamate level was 23.9%, the North Island average was 6.0%, and the minimum 0.1% (Table 2; Fig. 6). This is of interest as methyl cinnamate is used in the fragrance industry.

Waiotapu site (No. 24). Triketone oils are rare outside the East Cape region.

Oil glands on the under surface of the leaf

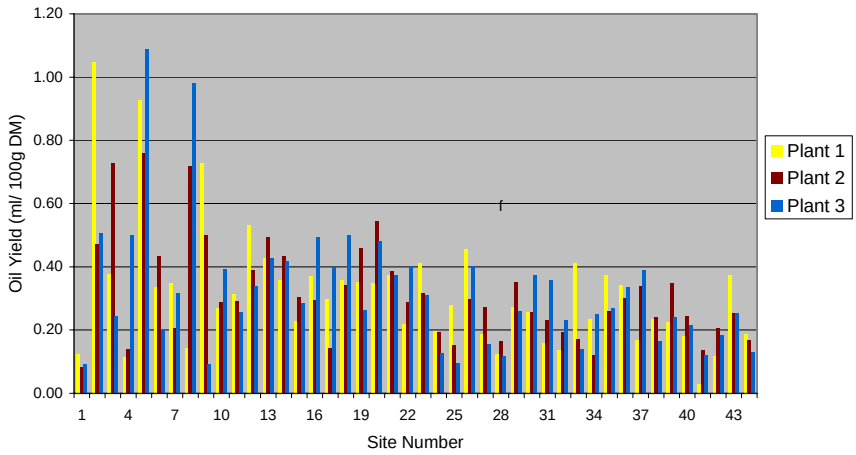

Figure 2: Percentage oil yield for each plant (ml/100 g dried herb).

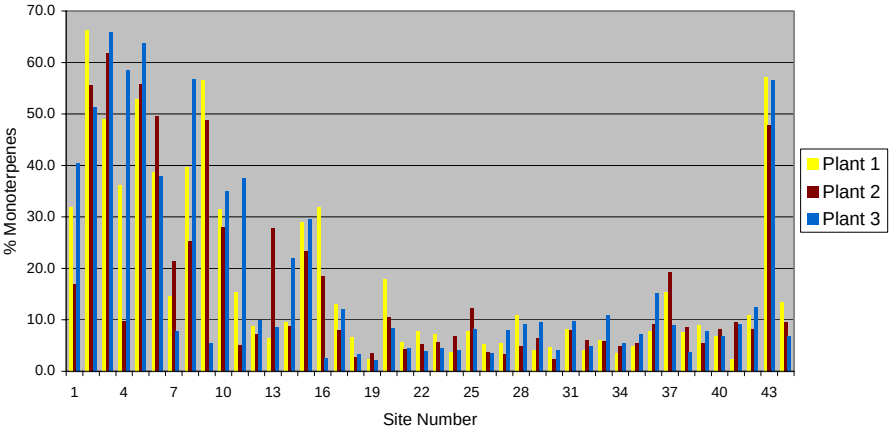

Figure 3: Percentage monoterpene in the essential oil.

*Table 2: North Island manuka (*L.scoparium*) compounds (summary of 132 plants)*

Peak	Compound name	Class	Mean	Minimum	Maximum
1	α-pinene	Monoterpene	4.9	0.1	37.4
2	β-pinene	Monoterpene	1.6	0.0	17.7
3	Myrcene	Monoterpene	2.1	0.0	25.7
4	p-cymene	Monoterpene	0.5	0.0	2.8
5	1,8-cineole	Monoterpene	2.1	0.0	25.3
6	β-Ocimene	Monoterpene	0.0	0.0	0.2
7	γ-Terpinene	Monoterpene	0.7	0.0	3.3
8	α-Terpinolene	Monoterpene	0.0	0.0	0.3
9	Linalool	Monoterpene	3.1	0.1	27.0
10	Terpinen-4-ol	Monoterpene	0.4	0.0	2.8
11	α-Terpineol	Monoterpene	0.5	0.0	5.7
12	n-Dodecane	Reference peak	-	-	-
13	Citronellol	Monoterpene	0.0	0.0	0.7
14	Citronellyl formate	Monoterpene	0.2	0.0	1.2
15	Methyl citronellate	Monoterpene	0.1	0.0	1.8
16	*cis*-Methyl cinnamate	Cinnamate	1	0.0	4.6
17	Methyl geranate	Monoterpene	0.9	0.0	24.1
18	Citronellyl acetate	Monoterpene	0.0	0.0	0.4
19	*trans* Methyl cinnamate	Cinnamate	5	0.1	22.2
20+21	γ-Ylangene + α-copaene	Sesquiterpene	2.6	0.0	27.3
22	Geranyl acetate	Monoterpene	1.4	0.0	54.1
23	β-Elemene	Sesquiterpene	2.9	0.1	16.1
24	α-Gurjunene	Sesquiterpene	0.7	0.0	2
25	β-Caryophyllene	Sesquiterpene	5.7	0.1	32.5
26	$C_{15}H_{24}$	Sesquiterpene	0.5	0.0	11.2
27	Aromadendrene	Sesquiterpene	0.8	0.0	4.2
28	$C_{15}H_{24}$	Sesquiterpene	1.4	0.0	5.8
29	α-Humulene	Sesquiterpene	5.6	0.0	19.0
30	$C_{15}H_{24}$	Sesquiterpene	0.8	0.1	9.8
31	α-Amorphene	Sesquiterpene	2.7	0.1	8.8
32	β-Selinene	Sesquiterpene	4.4	0.0	15.0
33	$C_{15}H_{24}$	Sesquiterpene	4.4	0.1	22.2
34	α-Selinene/viridiflorene	Sesquiterpene	7.7	0.2	18.0
35	α-Muurolene	Sesquiterpene	2	0.0	21.5
36	Cadinene	Sesquiterpene	1.2	0.0	7.3
37	Trans-calamenene	Sesquiterpene	5.8	0.0	13.5
38	γ-Cadinene	Sesquiterpene	3.4	0.0	13.1
39	Flavesone	Triketone	2.7	0.0	5.7
40	Cadina-1,4-diene	Sesquiterpene	0.6	0.0	7.0
41	Calacorene	Sesquiterpene	0.2	0.0	0.9
42	?	Sesquiterpene	0.0	0.0	0.4
43	Caryophyllene epoxide	Sesquiterpene	0.4	0.0	2.4
44	?	Sesquiterpene	0.4	0.0	2.7
45	Isoleptospermone	Triketone	0.8	0.0	9.2
46	Leptospermone	Triketone	1.8	0.0	19.8
47	β-Eudesmol	Sesquiterpene	1.7	0.0	11.1
48	α-Eudesmol	Sesquiterpene	1.2	0.0	9.6
49	n-Octadecane	Reference peak	-	-	-
50	grandiflorone	Triketone	0.0	0.0	0.3
	Total monoterpenes		17.1	2.1	66.3
	Total sesquiterpenes		56.8	16.2	77.4
	Total triketones		5.8	0.1	33.3
	Total cinnamate		6.0	0.1	23.9

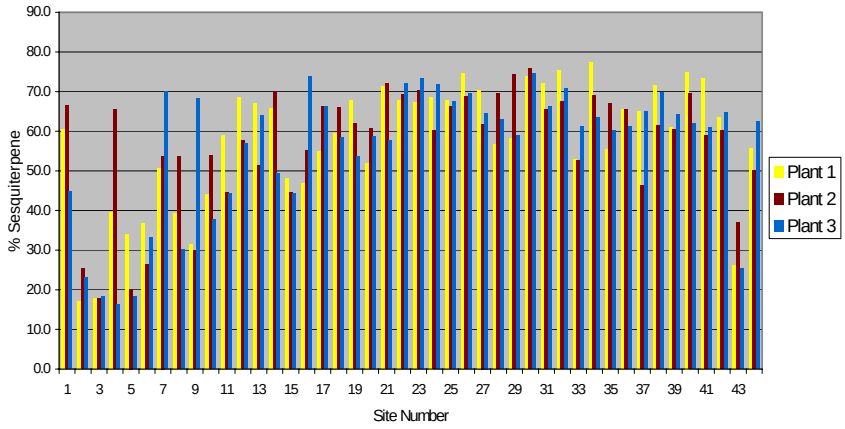

Figure 4: Percentage sesquiterpene in the essential oil.

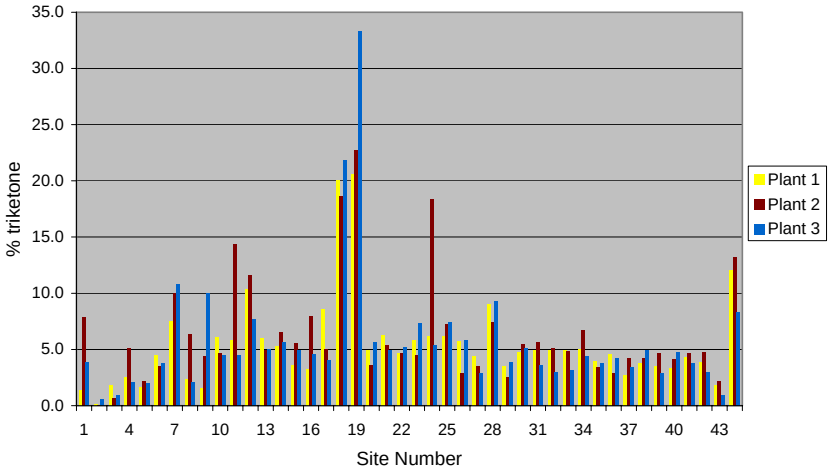

Figure 5: Percentage triketone in the essential oil.

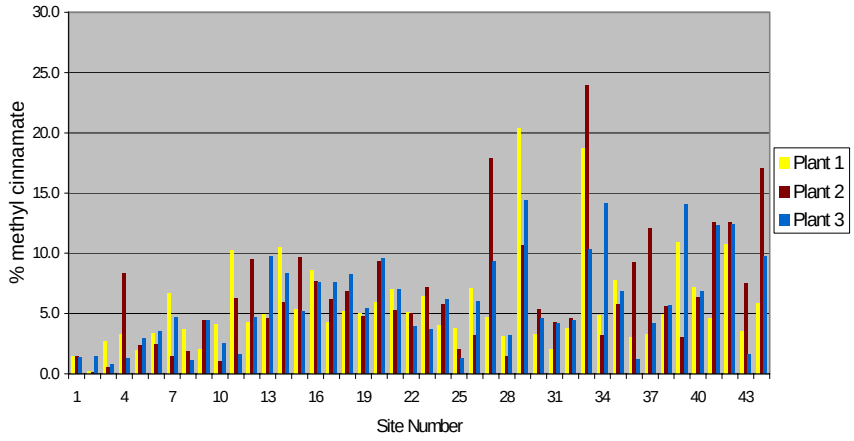

Figure 6: Percentage methyl cinnamate in the essential oil.

5 Potential opportunities for production

At present, commercial interest is focused on the triketone rich oil, which is active against gram positive bacteria. This chemotype only dominates in the East Cape manuka stands. Tairawhiti Pharmaceuticals, Te Araroa, have been harvesting the wild manuka stands since 1990 to produce this oil. In the long term, plantation development using selected high quality plants and mechanical harvesting would be an additional option to wild harvesting. This will require the identification and multiplication of improved lines with superior habit, foliage production, chemistry and resistance to the scale insect *Eriococcus* and manuka giant scale *Coelostomidia* species responsible for the black sooty mould.

The survey has identified two unusual chemotypes, high in either geranyl acetate or in methyl cinnamate. This is of interest as both these compounds are used in the flavour/fragrance industry and are sought from organic sources. A commercial evaluation is required particularly to determine if elite plants are present in the chemotype.

This survey has identified the chemotypes of manuka present in the North Island. Apart from the triketone rich oils, there is a need to ascertain the commercial potential of oils with limited biological activity. They may have potential productive uses as teas or fragrance or aroma extracts and compounds.

6 Technology transfer

All participants in the survey and the rural press will be provided with a summary of the results. A research paper on the manuka chemotypes in New Zealand will be reported in the international science literature and the results will also be reported to the NZ Ecological Society conference 2001.

7 Acknowledgements

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