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ESSENTIAL OILS FROM NEW ZEALAND MANUKA AND KANUKA: CHEMOTAXONOMY OF *LEPTOSPERMUM*

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Key Word Index—*Leptospermum*; *Kunzea*; Myrtaceae; manuka; chemotaxonomy; chemotypes; leptospermone.

Abstract—Standardized steam distillation and GC analytical methods for oils from manuka, *Leptospermum scoparium*, are described. These methods were used to analyse two oils from each of 15 *L. scoparium* populations derived from all around New Zealand, seven Australian *Leptospermum* populations and one population of *Kunzea sinclairii*. These populations were all grown from seed at a single site. Principal component analyses of the levels of 50 GC peaks in these 46 oils revealed compositional patterns. *Kunzea sinclairii* oils were distinguished from *Leptospermum* oils by higher α-pinene levels (mean 76%). Australian *Leptospermum* oils had significantly higher 1,8-cineole (mean 20%) and total monoterpene levels (mean 51%) than New Zealand *L. scoparium* oils (1,8-cineole mean 0.9%, total monoterpene mean 14%). This indicates the need for further taxonomic study of plants currently included in *L. scoparium* in Australia and New Zealand. There is evidence for three chemotypes of *L. scoparium* in New Zealand, conforming in part to morphological types: a high-pinene chemotype in the far north, a high-triketone (especially leptospermone) chemotype on the East Cape, and a type containing a complex of sesquiterpenes found over the rest of the country. An oil from the East Cape chemotype showed the strongest antimicrobial activity. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

We are investigating a range of native and introduced plants that could form the basis of an essential oil and extract industry in New Zealand [1]. Two native oil-producing species with great potential are manuka, *Leptospermum scoparium* J. R. et G. Forst, and kanuka, *Kunzea ericoides* (A. Rich.) J. Thompson. These myrtaceous species grow as shrubs or small trees throughout New Zealand in habitats ranging from lowland to subalpine areas [2]. Indeed, manuka has been described as '...probably the most widely distributed, abundant and environmentally-tolerant member of the New Zealand woody flora' [3]. These plants are used in traditional Maori remedies [4, 5]. Manuka oil from a selected plant line is currently being developed as an antimicrobial product.

Leptospermum scoparium is the only one of the 79 *Leptospermum* species that is native to New Zealand [6], and *K. ericoides* and *K. sinclarii* (Kirk) W. Harris are the only two of the *ca* 30 *Kunzea* species native to New Zealand [7]. Taxonomists differ as to whether *L. scoparium* and *K. ericoides* are endemic to New Zealand or occur in Australia as well [8]. Morphological variants of manuka are found in different geographical regions of New Zealand [3]. Analyses of seedling leaf size and shape indicated three broad groupings (Harris, W., unpublished results, 1995): a northern group (N of *ca* 38° S) with larger linear-lanceolate leaves, a southern group with more ovate leaves, and a distinctive group with small narrow leaves centred on the East Cape of the North Island. It is hoped that chemotaxonomy, using essential oil compositions, may contribute to the debate on *L. scoparium* in Australia and also help to clarify the status of the morphological variants in New Zealand.

There is little recent published information on manuka oils or on any other *Leptospermum* oils. Gardner identified 'terpene' (1%), a sesquiterpene (70.5%), citronellyl cinnamate (6%), esters of a sesquiterpene alcohol (14.5%) and 'leptospermol' (3%) in a southern manuka oil [9]. A northern collection of manuka yielded α-pinene (12%); eudesmene and a second sesquiterpene (60%); citronellol and geraniol, both free and as their isovaleric, cinnamic and acetic

esters (6%); citronellal and citral (1%); cineole (1%); 'leptospermol' (8%); azulene (traces); and isovaleric, cinnamic and acetic acids (4%) [10]. The 'leptospermol' of the early researchers, renamed leptospermone, has been shown to be an acylated β-triketone (**1**) [11, 12]. A survey using GC found leptospermone in the Australian species *L. flavescens*, *L. lanigerum*, and *L. flavescens* var. *grandiflora*, as well as other triketones including flavesone (**2**) [13]. Fertilizer had some effect on the composition of essential oils of *L. petersonii* (mostly geranial and citronella), *L. flavescens* (mostly α-pinene and cineole) and *L. luehmannii* (mostly α-pinene) [14]. The leaf oils of *L. sphaerocarpum*, *L. lanigerum* var. *macrocarpum* and *L. scoparium* var. *rotundifolium* contained mostly monoterpenes, especially α-pinene and 1,8-cineole, which was taken as supporting '...their close botanical relationship' [15]. This conclusion was based on only one oil from each species, thus ignoring the possibility of different chemical types (chemotypes) within a single species. Sampling different populations and within populations is desirable whenever analysing a plant species for its secondary chemistry: '...at the very least, some 10 or 20 samples should be analysed' [16].

We now report analyses of 46 oils distilled from foliage samples from one *Kunzea* population and 22 *Leptospermum* populations, derived from around New Zealand and from Australia.

RESULTS AND DISCUSSION

This study required a standardized method for steam distillation of small foliage samples from individual manuka shrubs. Laboratory scale stills were used with a 2 hr distillation time, since GC analyses showed that oil composition changed only slightly for longer distillations. Repeat distillations of a bulk sample of manuka foliage showed that the mean oil yield (0.25 ml g^{-1} dry matter) had a coefficient of variation of 8%, and mean levels of GC peaks had coefficients of variation ranging from 25% for the main monoterpene to *ca* 10% for the sesquiterpenes. Foliage samples contained leaves and stems. Distillations of partially separated leaf and stem samples showed that most of the oil came from the leaves, and that oil compositions were similar for stem and leaf oils.

The standardized distillation method was used to prepare oils from 23 different plant populations, growing at a single site, but derived from seed collected from different sites around New Zealand and Australia (Table 1). The 15 New Zealand *L. scoparium* populations were selected to cover a wide latitudinal range and to include regional morphotypes (Fig. 1). However, populations from southern regions and subalpine scrub lands were not available, as they were killed by manuka blight. Seven Australia-derived *Leptospermum* populations were sampled. In addition to putative Australian *L. scoparium*, populations of *L. continentale* J. Thompson and *L. juniperum* Smith were sampled, because Thompson has commented on the difficulty of differentiating these three species [6]. A population of *K. sinclairii* was included to check the previously reported difference in essential oil composition between *Leptospermum* and *Kunzea* [8]. Sampling plants grown at the same site eliminated possible environmental effects on essential oil contents, and sampling at a single time eliminated possible seasonal differences. As the plants were raised from seed, the two samples taken from each population represented two genotypes.

Steam distillation of these foliage samples gave a wide range of oil yields (Table 1). All 46 of these oils were analysed by GC on a relatively short (10 m) column in order to minimize analysis times. A longer column did not resolve any more major peaks, even in the complex sesquiterpene region. Fifty GC peaks, which reached at least 0.5% in more than one of the 46 oils, were selected as chemotaxonomic characters. These peaks were characterized by their retention indices (RIs), and some were identified with chemical components (see Table 2 and below). Identifications of other oil constituents, particularly the complexity of sesquiterpenes, will be published elsewhere.

The GC analyses showed a very wide range of oil compositions, so the peak level results were subjected to principal components analysis (PCA) [17]. PCA is a method of expressing a complex, multivariate data set in terms of a small number of principal components (PCs). Each successive PC accounts for the maximum amount of variance in the data not already accounted for by the previous PCs. Clusters of samples with similar compositions can be searched for in plots of sample scores against PCs. PCA on the full GC data set (not presented) showed the two *K. sinclairii* samples clustered separately from all the *Leptospermum* samples, mainly on their high content of peak 2, α-pinene. The mean level of α-pinene in the *K. sinclairii* oils (76%) was significantly ($P < 0.01$) higher than in the Australian (17%) or New Zealand (6%) *Leptospermum* oils, so this seems to be a useful taxonomic character for separating these taxa. Our chemotaxonomic study on oils of various *Kunzea* species will be described in a subsequent paper.

PCA on the GC data for the 44 *Leptospermum* oils showed that the first two PCs accounted for 63% of the total variance of the data set, with the first PC accounting for 47%. Figure 2 shows the 44 *Leptospermum* oils plotted in terms of these two PCs. The main contributor to the first PC was peak 7 (1,8-cineole, eigenvector −0.70), with peak 2 (α-pinene) also making a contribution (eigenvector −0.47). The main contributors to the second PC were peaks 7 and 24 (eigenvectors 0.50 and −0.47, respectively). The Australian oils were generally separated from the New Zealand oils, scoring lower on the first PC due to the higher levels of 1,8-cineole in the Australian oils. The only overlap involved the high-pinene New Zealand oils from populations 1 and 4 (see below). Statistical

Table 1. Details of *Leptospermum* and *K. sinclairii* samples, oil yields and total monoterpene levels

Popn	Species	CHR No.	Site of origin	Lat. S	Oil yield*	Monoterpenes†
1	*L. scoparium*	496691	Lake Waipareheka, Ngawha, N. Auckland, NZ	35°24′	0.22	40
2	*L. scoparium*	496690	Mt. Hobson, Great Barrier Island, NZ	36°11′	0.26	10
3	*L. scoparium*	496704	Whakaparapara Rd., Great Barrier Island, NZ	36°15′	0.55	11
4	*L. scoparium*	496692	Okahukura Peninsula, Kaipara Harbour, N. Auckland, NZ	36°21′	0.8	30‡
5	*L. scoparium*	496702	Cape Colville, Coromandel Peninsula, S. Auckland, NZ	36°28′	0.57	6
6	*L. scoparium*	496703	Karakatuwhero Beach, East Cape, NZ	37°36′	0.36	3
7	*L. scoparium*	496700	Kuirau Park, Rotorua, S. Auckland, NZ	38°03′	0.08	14
8	*L. scoparium*	496701	Mt. Tarawera, S. Auckland, NZ	38°14′	0.41	3
9	*L. scoparium*	496693	Puatai Road, Gisborne, NZ	38°31′	0.14§	6
10	*L. scoparium*	496696	Pupu Springs, Nelson, NZ	40°51′	0.33	19
11	*L. scoparium*	496699	Akatarawa, Wellington, NZ	41°05′	0.25§	5
12	*L. scoparium*	496697	Waima/Ure River, Marlborough, NZ	41°43′	0.24	18
13	*L. scoparium*	496694	Okiwi Bay, Marlborough, NZ	42°13′	0.17	10
14	*L. scoparium*	496698	Whalesback, N. Canterbury, NZ	42°28′	—‖	8
15	*L. scoparium*	496695	Lawrence-Waipori, Otago, NZ	45°53′	0.29¶	9¶
16	*L. scoparium***	496705	Mt. Wellington, Tasmania, Aust.	42°54′	0.26	56
17	*L. scoparium* var. *eximium***	496706	Port Arthur, Tasmania, Aust.	43°09′	0.02§	34
18	*L. scoparium***	496710	Gembrook Forest, Victoria, Aust.	37°57′	0.14	53
19	*L. scoparium***	496707	East Gippsland, S.E. Victoria, Aust.	37°47′	0.76	86
20	*L. juniperinum***	496708	East Gippsland, S.E. Victoria, Aust.	37°47′	0.02§	35
21	*L. continentale***	496711	Booroopki-Edenhope, Victoria, Aust.	36°49′	0.03§	19
22	*L. scoparium***	496709	Mt. Imlay, New South Wales, Aust.	37°11′	0.47	73
23	*K. sinclairii*	496712	Mt. Hobson, Great Barrier Island, NZ	36°11′	1.11	85

*In ml/100g dry matter, mean of two values unless noted otherwise.
†Peaks 1–11, as a % of total of peak areas, mean of two values unless noted otherwise.
‡Three samples analysed by GC.
§One value only, oil yield of other sample too low to measure.
‖Oil yields too low to measure.
¶Only one sample.
**Name on seed sample received.

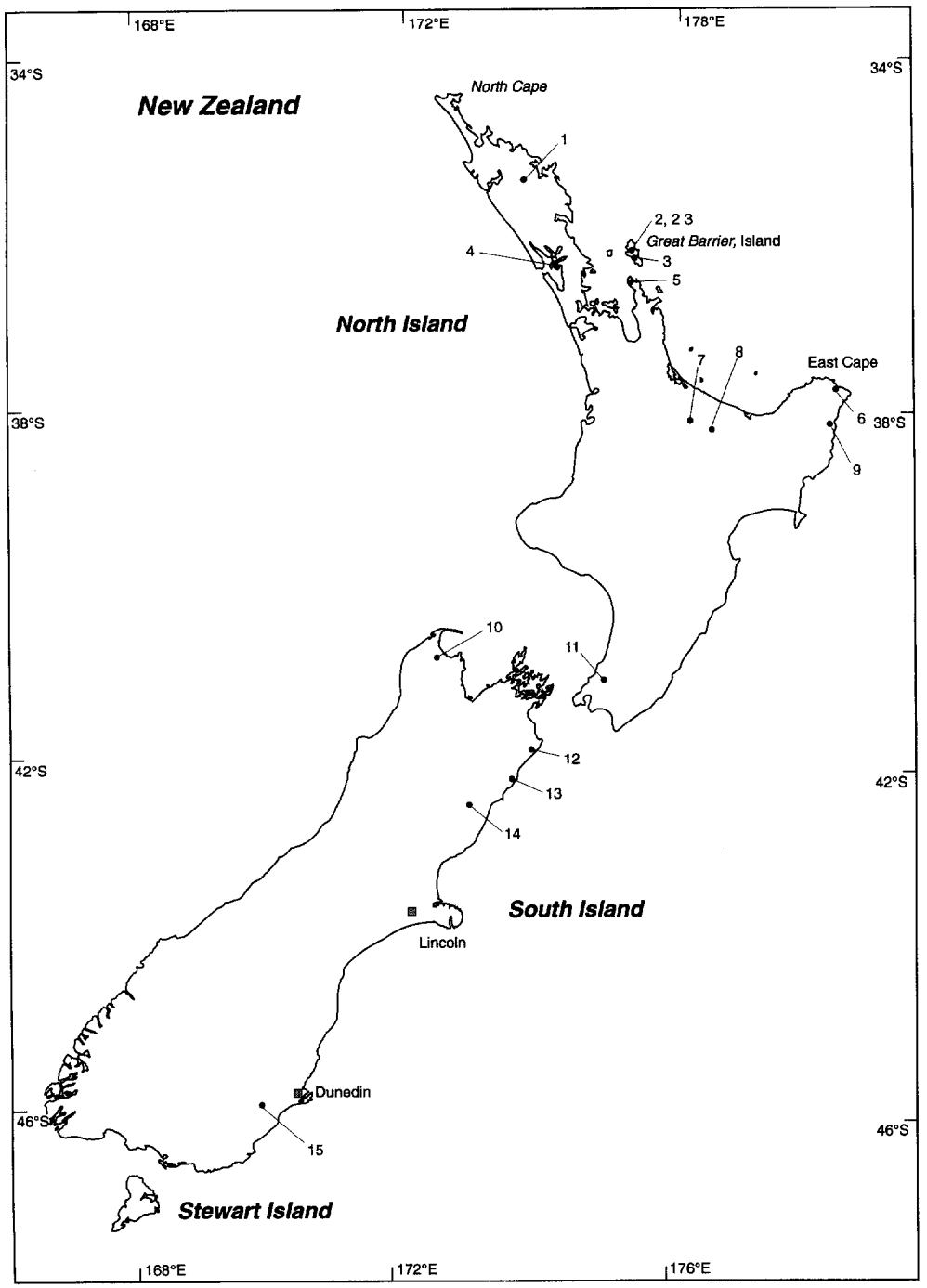


Fig. 1. Sites of origin of New Zealand *L. scoparium* samples.

analyses showed that both 1,8-cineole (mean 20%) and total monoterpene levels (mean 51%; Table 2) were significantly ($P < 0.01$) higher in the Australian *Leptospermum* oils than in those from New Zealand (1,8-cineole mean 0.9%, total monoterpene mean 14%). In particular, populations 19 and 22 were distinguished by their high levels of 1,8-cineole (34–48%) and α-pinene (17–24%) and also by their leaf morphology. These two populations, which were described as *L. scoparium*, seem to belong to a distinctly different taxon from the New Zealand representatives of this species. The Australian populations 18, 20 and 21 conform most closely to the description of *L. continentale* J. Thompson [6]. The Tasmanian populations 16 and 17, also received as *L. scoparium*, are morphologically different from the other Australian populations and the New Zealand *L. scoparium*. Further investigation is needed to determine the taxonomic status of these chemically different entities.

Table 2. Identifications, retention indices and levels* of GC peaks in *Leptospermum* foliage oils

Peak no.	Identification	RI	Australian‡	Northern§	East Cape‖	Southern¶
1		920	0.2 (0.1)	0.7 (0.8)	0.0 (0.0)	0.1 (0.2)
2	α-Pinene	927	17.3 (7.3)	22.5 (3.7)	0.7 (0.9)	2.9 (3.8)
3		961	0.3 (0.4)	0.3 (0.4)	0.0 (0.0)	0.1 (0.3)
4	β-Pinene	967	2.5 (2.7)	6.0 (0.5)	0.1 (0.1)	0.5 (0.6)
5	Myrcene	977	0.8 (0.4)	4.7 (4.8)	1.1 (0.0)	3.8 (5.9)
6	*p*-Cymene	1009	4.2 (3.6)	0.8 (0.8)	0.2 (0.0)	0.6 (0.7)
7	1,8-Cineole	1017	19.9 (16.6)	1.6 (0.3)	0.3 (0.1)	0.7 (1.0)
8		1042	1.0 (1.1)	1.2 (1.4)	0.2 (0.0)	0.5 (0.9)
9	Linalol	1079	1.0 (0.6)	1.5 (1.4)	0.2 (0.0)	2.4 (2.7)
10		1152	1.4 (1.4)	0.2 (0.2)	0.1 (0.0)	0.1 (0.1)
11		1163	2.0 (3.0)	0.2 (0.2)	0.0 (0.1)	0.1 (0.1)
12	*n*-Dodecane†	1200	—	—	—	—
13		1210	0.6 (0.6)	0.0 (0.0)	0.1 (0.0)	0.7 (1.5)
14		1243	0.0 (0.0)	0.1 (0.1)	0.0 (0.1)	0.5 (0.5)
15		1288	1.2 (1.3)	0.5 (0.1)	0.0 (0.0)	0.8 (1.1)
16		1324	2.7 (5.1)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)
17		1331	0.0 (0.1)	1.5 (1.0)	4.2 (0.9)	1.9 (1.7)
18	Methylcinnamate	1334	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)	3.3 (3.4)
19		1349	0.3 (0.5)	0.2 (0.2)	0.1 (0.0)	0.4 (0.6)
20		1354	0.7 (0.7)	0.9 (0.7)	5.4 (1.9)	4.7 (5.8)
21		1362	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.3 (0.3)
22		1370	0.1 (0.1)	1.2 (1.3)	1.2 (1.1)	2.4 (3.6)
23		1396	0.2 (0.2)	0.5 (0.2)	0.8 (0.4)	1.0 (0.4)
24		1398	12.4 (10.7)	11.8 (11.5)	2.2 (1.2)	5.3 (3.9)
25		1408	0.2 (0.2)	0.1 (0.0)	0.1 (0.2)	0.1 (0.1)
26		1416	0.7 (0.6)	0.8 (0.4)	1.2 (0.6)	2.6 (2.4)
27		1422	0.4 (0.3)	0.5 (0.1)	0.0 (0.0)	1.1 (1.2)
28		1428	1.7 (1.7)	1.9 (0.9)	6.4 (1.6)	4.4 (4.5)
29		1434	1.2 (0.9)	0.5 (0.2)	0.7 (0.2)	0.8 (0.4)
30		1451	0.0 (0.1)	2.1 (2.1)	3.2 (2.1)	3.5 (3.3)
31		1453	0.2 (0.3)	0.5 (0.8)	0.6 (0.9)	2.0 (1.6)
32		1460	0.5 (0.5)	4.6 (1.1)	4.0 (3.8)	8.4 (6.6)
33		1462	0.1 (0.1)	0.9 (0.1)	1.7 (0.3)	1.3 (0.4)
34		1469	5.1 (5.1)	5.2 (1.1)	4.7 (3.5)	10.4 (5.4)
35		1476	0.1 (0.1)	0.8 (0.5)	1.0 (0.2)	1.5 (1.4)
36		1482	0.0 (0.0)	0.5 (0.5)	0.1 (0.1)	0.5 (0.6)
37		1491	0.3 (0.4)	4.6 (1.6)	11.7 (0.8)	6.9 (2.4)
38		1498	0.2 (0.3)	2.4 (1.1)	3.9 (1.2)	4.6 (2.7)
39		1506	0.0 (0.1)	2.1 (1.4)	4.7 (0.3)	2.6 (1.3)
40	Flavesone	1510	0.1 (0.2)	0.1 (0.1)	8.3 (0.3)	0.1 (0.2)
41		1516	0.2 (0.3)	0.1 (0.1)	0.0 (0.0)	0.3 (0.5)
42		1524	0.1 (0.1)	0.2 (0.2)	0.1 (0.0)	0.2 (0.2)
43		1538	2.5 (2.1)	0.2 (0.3)	1.0 (0.4)	0.5 (0.5)
44		1545	2.1 (1.2)	1.2 (0.8)	0.5 (0.5)	1.0 (0.8)
45		1557	1.2 (1.0)	0.2 (0.0)	0.2 (0.2)	0.3 (0.3)
46		1564	0.8 (0.6)	0.5 (0.5)	0.3 (0.1)	0.6 (1.0)
47	Isoleptospermone	1585	0.4 (0.6)	0.0 (0.1)	5.3 (0.9)	0.5 (0.6)
48		1587	0.3 (0.5)	0.1 (0.1)	0.0 (0.0)	0.1 (0.2)
49	Leptospermone	1592	0.6 (0.7)	0.8 (0.5)	18.9 (0.7)	1.7 (1.1)
50		1608	1.2 (1.7)	3.3 (4.2)	1.1 (0.0)	1.1 (1.3)
51		1610	1.0 (1.4)	3.8 (4.4)	0.2 (0.3)	1.1 (1.2)
52	*n*-Octadecane†	1800	—	—	—	—
1 to 11	Monoterpenes	—	50.6 (24.4)	39.9 (4.6)	3.0 (1.1)	11.8 (9.1)
19 to 39	Sesquiterpenes	—	24.7 (16.0)	42.1 (6.0)	53.7 (3.0)	64.7 (10.3)
40, 47, 49	Triketones	—	1.2 (0.9)	1.0 (0.5)	32.5 (1.9)	2.4 (1.6)

*Mean peak area, expressed as % of total GC peak area (standard deviation).
†Internal standard.
‡Populations 16–22.
§Population 1.
‖Population 6.
¶Populations 10–15.

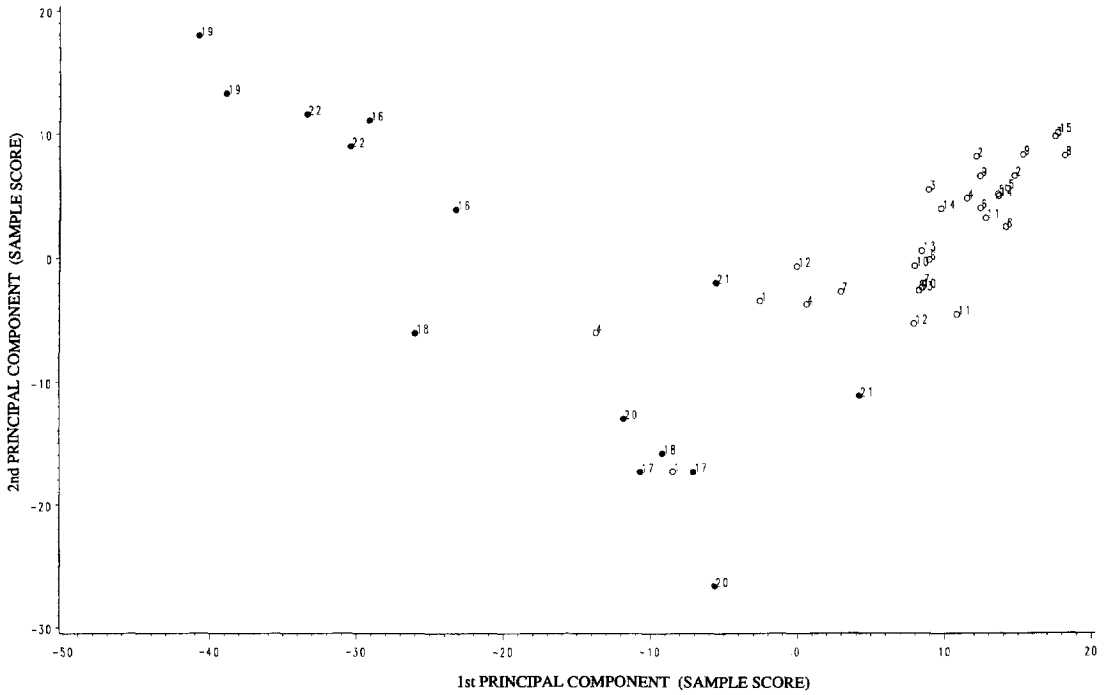


Fig. 2. Compositions of Australian (●) and New Zealand (○) *Leptospermum* oils in terms of the first and second PCs, labelled with population numbers (Table 1).

The GC data set was further reduced to focus on the 30 New Zealand *L. scoparium* oils. This data set was subjected to PCA to search for any compositional patterns that might align with the three morphological and geographical groupings described above (see Introduction). The first two PCs accounted for 58% of the total variance of the data set, with the first PC accounting for 35%. Figure 3 shows the 30 *L. scoparium* oils plotted in terms of these two PCs. The main contributors to the first PC were peak 2 (α-pinene, eigenvector +0.54), peak 32 (eigenvector −0.58) and peak 34 (eigenvector −0.52). The main contributors to the second PC were peaks 2, 32, 34 and 49 (eigenvectors −0.67, −0.35, −0.27 and +0.28, respectively). Three of the oils, from populations 1 and 4, stand out as high on the first PC and low on the second PC (Fig. 3), because these oils had the highest levels of α-pinene (20–36%). The highest α-pinene oil also had the highest level (12%) of peak 4, identified as β-pinene (α- and β-pinene levels were strongly correlated over the 30 oils; $R^2 = 0.77$). Populations 1 and 4 had two of the most northern sites of origin (Table 1), so we could hypothesize a high-pinene chemotype of *L. scoparium* in northern New Zealand. This hypothesis was supported by analyses of two oils from a wild *L. scoparium* population growing at the North Cape of New Zealand (34° 26′ S). These oils had α-pinene contents of 45 and 47% and β-pinene contents of 7 and 10%. However, this high-pinene chemotype does not correspond exactly to the large-leaved northern group. Two other oils from population 4 had relatively low α-pinene levels (4 and 8%) and oils from large-leaved populations 2, 3 and 5 also had lower pinene levels, as can be seen in a scatter diagram (Fig. 4). Further sampling will be needed to define the geographic boundaries of the proposed high-pinene chemotype of *L. scoparium* and to explore the relationship between leaf size and pinene content. The high monoterpene contents of Australian and northern New Zealand *Leptospermum* populations may have some function with respect to fire ecology, since monoterpenes are volatile and highly flammable. The manuka heaths of the gumlands of Northland and the Coromandel Peninsula have a history of burning that pre-dates the human occupation of New Zealand [18].

The PCA plot separates both oils from population 6 as highest on the second PC (Fig. 3). These oils have the highest levels of peak 49 (mean 19%), one of the most retained peaks in the GC analyses (Table 2). The ^{1}H NMR spectrum of one of these oils was dominated by singlets at 1.35 and 1.44 ppm, as described for the ring methyls of leptospermone (**1**) and flavesone (**2**) [19]. Extraction of this oil with aqueous base, the established method for isolating these triketones [19], gave a fraction containing mostly peak 49 plus lower levels of peaks 40 and 47. The ^{1}H NMR spectrum of this fraction showed all the signals expected for **1** as the major component, with signals for **2** at a lower level [19]. GC–mass spectral analysis supported the identification of peak 49 as **1** and peak 40 as **2** (mass spectra do not seem to have been reported previously). Peak 47 was isomeric with **1**, and we have tentatively identified it as isoleptospermone (**3**), a compound that has only been reported once as a natural product [12]. Further work on the chemistry of these triketones will

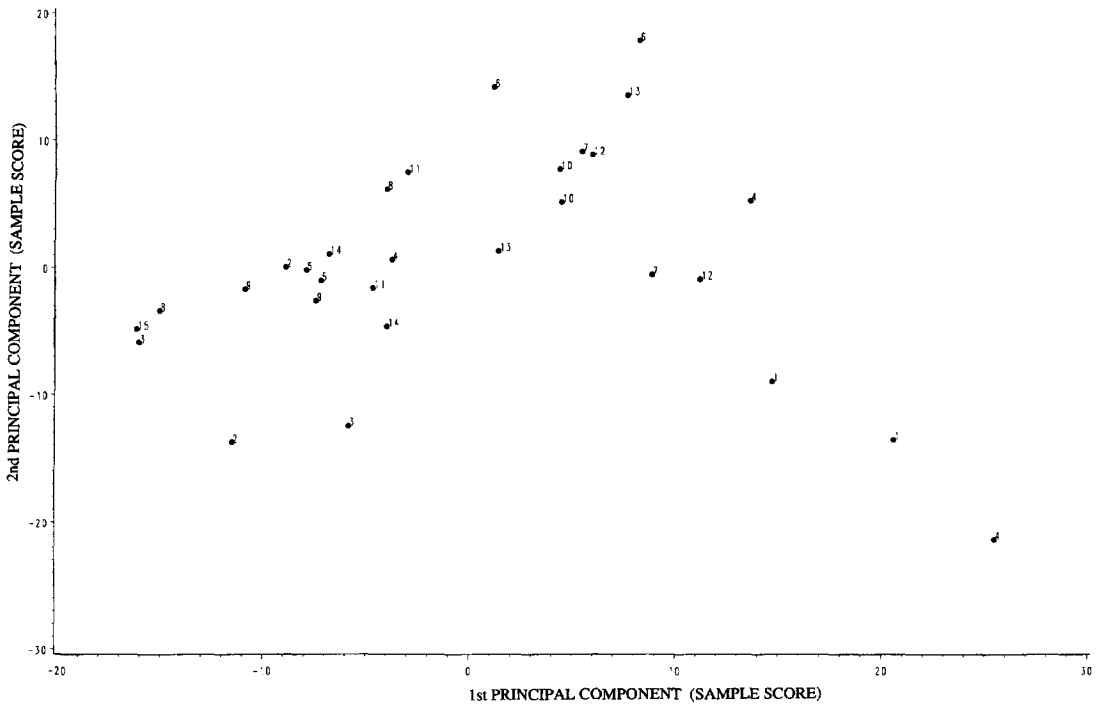


Fig. 3. Compositions of New Zealand *L. scoparium* oils in terms of the first and second PCs, labelled with population numbers (Table 1).

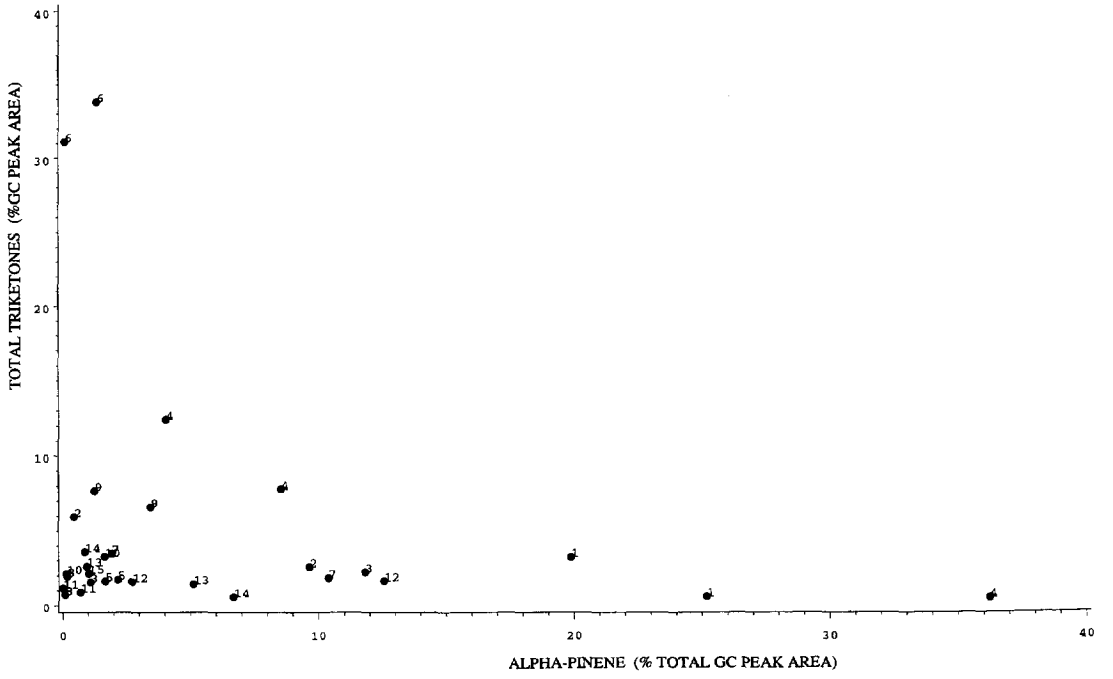
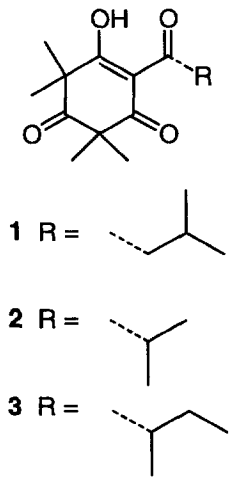


Fig. 4. Compositions of New Zealand *L. scoparium* oils in terms of the α-pinene (peak 2) and total triketone (peaks 40, 47 and 49) contents, labelled with population numbers (Table 1).

be published elsewhere. The levels of **1**–**3** were strongly correlated in the 30 *L. scoparium* oils (**1** and **2**, $R^2 = 0.82$; **1** and **3**, $R^2 = 0.75$). The highest total triketone levels, >30%, were in the East Cape population 6 (Fig. 4), so there is some evidence for a high-triketone chemotype corresponding to the morphologically defined East Cape group (see Introduction). This was supported by analyses of five oils from a wild *L. scoparium* population growing near the East Cape. These oils had a mean total triketone level of 27% (s.d. 4%). On the other hand, two of the oils from North Auckland population 4 also had relatively high triketone levels (12 and 8%, Fig. 4). Further sampling will be needed to define the geographic



boundaries of this proposed high-triketone chemotype of *L. scoparium* and how this relates to the leaf morphology.

The *L. scoparium* populations from further south in New Zealand yielded oils containing a complex mixture of sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Table 2). The levels of peaks 32 and 34, two of the main sesquiterpene hydrocarbons, were strongly correlated ($R^2 = 0.95$) in the 30 New Zealand oils, so these components are probably biosynthetically related. Some oils contained relatively high levels of other components, allowing these to be identified by ^{1}H NMR spectroscopy. Myrcene reached 21% in one oil from population 10, and the other oil from this population contained 9% linalol. Both oils from population 13 contained relatively high levels (11 and 6%) of peak 18, which was identified as *trans*-methylcinnamate. Further oil analyses, combined with morphological studies, would be needed before proposing any further chemotypes of *L. scoparium*.

In view of the interest in the antimicrobial properties of manuka oil, we tested oils from the three New Zealand chemotypes identified above against the Gram-positive bacterium *Bacillus subtilis* and the dermatophyte *Trichophyton mentagrophytes* (Table 3). A sample of Australian tea tree oil, from the terpinen-4-ol chemotype of *Melaleuca alternifolia* Cheel (Myrtaceae), was also tested. The oil from the East Cape chemotype was most active against *B. subtilis*, both in terms of inhibition zones and minimum inhibitory dose. The East Cape chemotype also gave larger inhibition zones against *T. mentagrophytes*, but the southern chemotype was active at a lower dose. The Australian tea tree oil was surprisingly inactive in these assays, but this may have been due to loss of the volatile active component, terpinen-4-ol [20], from the filter paper discs used to apply the diluted oils. The components responsible for the antimicrobial activities of the *L. scoparium* oils will be described elsewhere.

The results described above are important both for the taxonomy of *L. scoparium* in New Zealand and Australia, and for the development of manuka oil production as an industry. We have shown that essential oil composition is a potentially useful tool in describing the systematics of *Leptospermum*, as it has proved in another genus of the Myrtaceae, *Eucalyptus* (see, e.g. Doran *et al.* [21]). The above results show that plants currently included with *L. scoparium* in Australia are different in chemotype from those in New Zealand (Table 2). We propose that there are at least three chemotypes of *L. scoparium* in New Zealand: high pinenes, high triketones and high sesquiterpenes (Table 2). These chemotypes conform in part with morphological and geographical groups, so further study may lead to the definition of subspecies. The different chemotypes of New Zealand *L. scoparium* seem to have different biological activities, so it is vital for the development of manuka oils for medicinal use that oil compositions should be standardized.

EXPERIMENTAL

Plant materials. The populations studied (Table 1) were all grown from seed at Landcare Research, Lincoln, New Zealand (43° 38′ S, 172° 30′ E) from 4 plantings: populations 2, 5–8, 11, 12, 14 and 23 sown September 1983; populations 1, 3, 4, 9, 10, 13, 15 and 17–20 sown November 1984; population 16 sown November 1986; populations 21 and 22 sown July 1989. Foliage was harvested from 2 plants of each population on 15 March 1994, and voucher specimens were deposited in the Landcare Research Herbarium (CHR), Lincoln (voucher nos in Table 1). Samples were sorted to remove dead material and woody stems (diameter >5 mm) and stored at 3° and r.h. 18%. Plant material was chopped into nominal 1 cm lengths immediately before steam distillations, which were performed between 19 March and 5 April. Subsamples were taken immediately before steam distillation for determination of dry matter content, by drying to constant wt at 80°. *Leptospermum scoparium* foliage for testing distillation parameters was harvested from a wild population growing at an altitude of 365 m at Waiora, near Dunedin (45° 50′ S, 170° 15′ E). Foliage was collected from multiple plants, treated as above, and distilled at the following times: for distillation time trial, March 1994; for repeatability study, July 1994; for leaf and stem study, February 1995. Voucher specimens are deposited at Invermay (IY95/036 and IY95/039). Foliage from 2 individuals in a wild population of *L. scoparium* growing at Tom Bowling Bay, near North Cape, North Auckland (34° 26′ S, 172° 58′ E) was collected in March 1995, treated and distilled as above. A voucher specimen has been retained (CHR 485681). Foliage from 5 individuals in a wild population of *L. scoparium* growing at Kururangi Hilltop, near East Cape (37° 33′S, 178° 10′E) was collected in December 1955, treated and distilled as above. A

Table 3. Antimicrobial assays* on *L. scoparium* chemotype oils and Australian tea-tree oil

	Bacillus subtilis†				*Trichophyton mentagrophytes*‡			
Dose§	North‖	East¶	South**	Tea-tree††	North‖	East¶	South**	Tea-tree††
1200	2	6	0	1	3	8	3	0
600	1	4	0	1	3	7	3	0
300	0	1	0	0	0	3	3	0
150	0	1	0	0	0	3	1	0
75	0	0	0	0	0	0	1	0
37.5							0	

*Results are width of inhibition zone, in mm.
†American Type Culture Collection no. 19659.
‡American Type Culture Collection no. 28185.
§Dose of oil in μg per disc.
‖Northern *L. scoparium* oil, population 1.
¶East Cape *L. scoparium* oil, population 6.
**Southern *L. scoparium* oil, population 15.
††Melaleuca alternifolia oil.

voucher specimen has been retained at Redbank Research Station (RB 96/34).

Steam distillations. Four different laboratory scale stills were used. Two of the stills were glass, (600 × 100 mm i.d. and 450 × 102 mm i.d.). The other 2 stills were stainless steel (500 × 100 mm i.d.). Plant material (150–750 g) was packed to a uniform density (0.14 g ml^{-1}) to the top of the column, with chopped barley straw used to pack the base of the column for small samples. Steam at 65 kPa (±5 kPa) was passed through the samples for 2 hr, to give total condensate vols of 790–1060 ml. Oil yields were measured with a graduated pipette, before drying over Na_2SO_4 and storage at −18° until GC analysis. For oil yields <0.1 ml, hexane (1 ml) was added to recover the oil for GC analysis.

GC analyses and component identifications. Oils were analysed as 1% sols in hexane containing 2 ref. compounds (0.5% *n*-dodecane and 0.5% *n*-octadecane). Eight oils recovered in hexane were analysed by diluting the soln 1:1 with hexane containing ref. compounds. Samples were analysed using a 10 m WCOT DB-1 column (0.25 mm i.d., 0.25 μm film thickness) with H_2 as carrier gas (55 cm s^{-1}) and a temp. programme of 80–155° at 5° min^{-1}. Injections (0.5 μl) were made into a split (100:1) injector set at 260°. The FID was set at 350°. GC software was set up to recognize 50 peaks, after using the ref. compounds to correct for R_t variations between runs. Peak areas of ref. compounds were excluded, and areas of remaining peaks were normalized. GC data on the 46 oils in this study are available as Supplementary Material. ASCII files of these GC results were subjected to statistical processing using SAS software. PCAs were run on unscaled peak data, and significance of differences was tested by analysis of variance (GLM procedure). DB-1 Kovats RIs of the 50 peaks were measured by co-injection of a manuka oil with *n*-alkanes (C8–12, and 14–18) using the standard GC method above. RIs were calcd by linear interpolation between uncorr. R_ts of the *n*-alkanes bracketing the peak in question (Table 2).

Major components in selected oils were identified by comparing their 200 MHz ^1H NMR spectra (in $CDCl_3$) with spectra of ref. compounds and by co-injection with ref. compounds. One oil (0.5 ml LCN-20A, population 6) was dissolved in Et_2O (5 ml) and extracted with 5% aq. Na_2CO_3 (3 × 5 ml). The combined carbonate frs were acidified (pH *ca* 2) with conc. HCl and extracted with Et_2O (3 × 5 ml). The combined Et_2O frs were dried, filtered and evapd to yield triketones (290 mg). GC-MS analysis: 30-m HP-1 WCOT column with He as carrier gas, and a temp. programme of 50–150° at 5° min^{-1}. Injector temp. 210°; mass selective detector 180°. Leptospermone (**1**, Registry No. 567-75-9) GC-MS *m/z* (relative intensity): 266 $[M]^+$ (40), 251 (37), 223 (18), 196 (84), 163 (36), 126 (46), 113 (39), 112 (39), 111 (46), 96 (100), 95 (33), 85 (51), 83 (43), 81 (70), 70 (89). Flavesone (**2**, RN 22595-45-5) GC-MS *m/z* (rel. int.): 252 $[M]^+$ (85), 237 (35), 209 (35), 182 (78), 181 (37), 164 (93), 139 (100), 119 (63), 113 (47), 105 (58), 96 (92), 91 (40), 81 (67), 71 (70), 70 (90). Isoleptospermone (**3**, RN 7375-66-8) GC-MS *m/z* (rel. int.): 266 $[M]^+$ (64), 251 (37), 209 (34), 196 (66), 178 (51), 163 (36), 140 (38), 113 (33), 96 (100), 95 (37), 85 (34), 83 (37), 81 (66), 70 (73).

Antimicrobial assays. Leptospermum scoparium oils used are identified in Table 3. Commercial *M. alternifolia* oil contained 34% terpinen-4-ol by GC, identified by co-injection with authentic terpinen-4-ol. Oils were diluted with rectified spirits (EtOH–H_2O, 19:1) to give a series of samples for antimicrobial disc assays. Samples (30 μl) were dried on to 6 mm filter paper discs, which were placed on to seeded agar Petri dishes and incubated for 24–48 hr at 30°. Activity showed as a zone of growth inhibition around the disc, with its width recorded from the edge of the disc in mm. Standard discs with chloramphenicol (30 μg)

gave 12–13 mm inhibition zones against *B. subtilis*, and discs with nystatin (100 units) gave 7–8 mm zones against *T. mentagrophytes*.

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