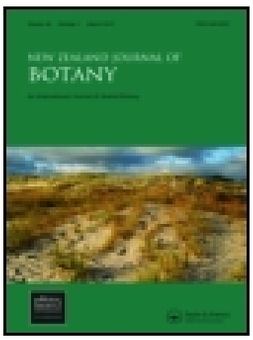

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Variability in essential oil chemistry and plant morphology within a *Leptospermum scoparium* population

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Abstract Essential oil composition and plant morphology were observed over four years in individual plants raised from seed of a wild population of *Leptospermum scoparium* (Myrtaceae) collected at a single site in New Zealand. Principal component analyses of data from young and mature plants showed no significant grouping of plants on the basis of oil composition, but identified differences between the essential oil components contributing most to variation in oil composition in both young and mature plants. The dominant variables were six sesquiterpene components in young plants, and three monoterpenes and two sesquiterpenes in mature plants. Levels of these components differed significantly at the population level between young and mature plants and also within and between seasons. Levels of all these components varied markedly within and between individual plants at all sample times. The habit, leaf size and density, and stem and foliage colour also varied markedly between individual plants. The variation observed indicates the need for more extensive sampling and statistical analysis over more than one growing season if sufficiently reliable data on essential oil compositions in individual plants or populations are to be obtained for chemotaxonomic or plant selection purposes.

Keywords *Leptospermum scoparium*; manuka; essential oil; monoterpenes; sesquiterpenes; morphology; variability

INTRODUCTION

Leptospermum scoparium (Myrtaceae), commonly known as manuka, is a small tree or shrub found throughout New Zealand (Allan 1961). It occurs in scattered or dense populations in widely differing climatic and altitudinal zones throughout the country (Yin et al. 1984), in wet sites (Robertson et al. 1991), and as a pioneer of cleared or disturbed ground (Mark et al. 1989).

Early New Zealand records show that the bark, leaves, sap, and seed capsules of manuka were used in beverages or medicinal preparations (Best 1905;

Brooker et al. 1981). Isolation from the leaves of leptospermol (Gardner 1924, 1925; Short 1926), which was later re-named leptospermone (Briggs et al. 1938, 1945), reports of its anthelmintic and insecticidal properties (Briggs et al. 1945; Briggs 1947), and demonstration of activity of the oil against *Staphylococcus aureus* (Atkinson & Brice 1955) suggested potential in the plant for pharmaceutical use. Non-commercial medicinal use of manuka from wild populations has persisted but significant areas continue to be cleared for cultivation. The potential for the loss of important resources of chemotypes and genotypes with biological activity and potential pharmaceutical use is significant. Over the last 10 years, interest in this essential oil has developed into commercial production in New Zealand of a range of oils from different geographical populations and with different properties for the hygiene and aromatherapy markets.

Significant variability in an increasing number of characteristics of this species is being reported. Variation in leaf shape and size, and plant size, shape, and form have been used to define communities (Yin et al. 1984) or provide new selections for development as ornamental cultivars (Harris 1994). Variants in flower colour, size, and form have been selected and, by hybridisation with other species, have been incorporated into new ornamental and cut flower cultivars (Hobbs 1989; Bicknell 1995). Variations in cold tolerance related to geographical or altitudinal sites (Greer et al. 1991; Harris & Decourtye 1991) and in tolerance to soil pH (Berninger 1992) have also been described. The composition of essential oils extracted from plants from different sites also varies widely (Porter 1991; Perry et al. 1997), and this is reflected in variation in biological activity of the oil (Porter 1991).

Other research on the chemistry of manuka oils has covered different classes of chemical compounds (Murin et al. 1959; Tan et al. 1988; Mayer 1990; Haberlain & Tschiersch 1994). The oil chemistry has also been used in chemotaxonomic studies of manuka (Harris et al. 1992; Perry et al. 1997). The research applied to commercial development has concentrated on the essential oil from natural stands of manuka from East Cape, North Island, now recognised as a chemotype because of the distinctive chemical composition of its oil (Porter 1991; Perry et al. 1997). The antiseptic activity of the oil has been identified with a fraction containing three main compounds (Porter 1991), now known from GCMS studies to be the three ß-triketones flavesone, leptospermone, and iso-leptospermone (Joulain 1996; Perry et al. 1997). Because oil composition varies greatly between populations at different sites, similar variation in biological activity must be expected.

Current New Zealand commercial essential oil production is based on harvesting wild stands of manuka, but it is important that sustainable, managed areas are established for the expected increased levels of future oil production. This will require the identification and multiplication of improved lines with superior habit, foliage production, chemistry, and resistance to the insect *Ericoccus* and *Coelostomidia* species responsible for black sooty mould (Hoy 1961). There is experience with the genetics of the genus in developing ornamental cultivars (Bicknell 1995), and there is some information on the variability of foliage and plant characteristics in single plants (Yin et al. 1984). However, it is obvious from field observations that there is marked variability at any single site in both the aroma, and therefore chemistry, of the foliage, and the physical characteristics of the foliage and whole plant which are relevant to oil production. Harris (1994) has described inter- and intra-population variation relating to selection of ornamental lines, but there is a lack of detailed numerical data defining variability in oil chemistry at the individual plant level at which plant improvement programmes must operate.

The aim of this study is to present data representing individual plants on the range and type of variability of oil chemistry and foliage characteristics within a small seedling population from a single site, and to assess the adequacy of oil composition data obtained from single samplings for use in plant selection and chemotaxonomic studies.

MATERIALS AND METHODS

Seedling establishment

In autumn 1990, seed capsules were collected from five plants in a 5 m^2 area of manuka plants growing from a natural population on the side of Canaan Road, Takaka Hill, Nelson Province, New Zealand (40°58′S, 172°52′E). Seed was released from all capsules over five days of air drying in a greenhouse, mixed and cleaned by sieving, sown immediately into trays, and covered only with a minimal layer of crushed chip. Seed germinated within 10 days (22–24°C with daily misting). Seedlings were transferred into 5 cm diam. × 10 cm deep plastic tubes, and hardened off beneath shade cover before growing on outside.

Experimental plot

In spring 1992, 232 15-cm-high individual plants with visible differences in leaf size and colour of stems were selected to illustrate the available extremes of morpohological variability. They were planted into a trial plot (1.5 m row × 0.5 m plant spacing) at Nelson Research Centre, Riwaka. In 1993, 16 plants with distinctive foliage or form were individually identified and numbered 1–16. Fifteen further distinctive plants were added in 1995 and numbered 17–31. The original 16 young plants are included in the mature group and retain the same numbers in both groups. Two plants (4 and 16) died during this experiment. Some young plants were attacked by scale insects (*Coccoidea*), but later attacks established sooty mould on the mature wood of all plants. High volume sprays of all season oil in autumn or winter protected the young foliage used for oil extraction in the following season. The block was irrigated as necessary and fertilised once per year in spring with Nitrophoska (N:P:K 12:5:14).

Oil extraction and analysis

It is desirable in plant improvement programmes to assess plant variability and make selection decisions as early as possible. In chemotaxonomic studies also, it may be desirable to make observations on both young and mature plants. Earlier observations on natural populations in Canterbury and Nelson indicated that some plant, foliage, and oil characters may differ in young (1–2 years) and mature plants, and that oil composition differed within and between seasons (N. G. Porter unpubl. data). Foliage of the current season's growth was harvested from all sides of individual plants in the seedling population in each of four years (1993–96) and in summer and autumn within one growing season (1995/96) to separate and confirm differences in oil composition related to genotype from developmental or seasonal differences. At each harvest time, foliage of the current season's growth was chopped into sections 2–4 cm long and thoroughly mixed before 50 g samples were immersed in 200 ml of new hexane (Shell X4 solvent) and held at ambient temperature for 4–7 days, with periodic gentle stirring. The hexane extracts were then thoroughly stirred and sub-sampled for gas chromatographic (GC) analysis of oil composition on a HP5840 or HP6890. The following operating conditions were used on both machines:

Column:	non-polar BP1 (Alltech) or HP1 (Hewlett-Packard) 30 m, 0.32 mm id, 0.25 μm film thickness fused silica capillary column;
Carrier gas:	hydrogen at 54 kPa inlet pressure;
Temperature (°C):	injector 240, detector 300, column 80 to 135 at 2 min^{-1}, 135 to 250 at 10 min^{-1}, purge at 270 for 3 min;
Injection:	splitless mode, 1 μl of the X4 extracts after five-fold dilution with hexane;
Detection:	by flame ionisation detector.

Levels of individual components are expressed as peak area percentages (uncorrected for detector response). These results refer only to the volatile essential oil components. Non-volatile wax components extracted by the solvent (after 33 minutes retention time in Fig. 1) were eliminated during the integration of peak areas. Component identities were assigned by comparison of retention times and indices with standard monoterpene compounds and with sesquiterpene components identified by GCMS in other manuka oils (Porter et al. 1996).

Oil yields were not measured because sufficient foliage was not available on the individual plants for reliable values.

To provide data on the oil composition in the natural population, a large sample of current season's foliage was collected in March 1993 along several hundred metres of roadside from the same area and population as the sample of seed. The oil was extracted from duplicate 80 kg samples of chopped foliage by steam distillation in a mobile trailer-mounted extraction unit using 1 kg steam min^{-1} for 2.5 hours in an unpressurised stillpot. Accumulated oil was run off from the separator, filtered, dried, and stored at 4°C until dilution to 0.5% in dichloromethane prior to GC analysis as for the hexane extracts. GC analysis of oil extracted from this foliage by solvent (as above) gave comparable oil profiles and levels of major components.

Plant characteristics

Following Wilson et al. (1991), observations on young bushes in 1993 included measurements of plant height and width as well as measurements of leaf dimensions and subjective comment on plant habit (growth density, erectness), leaf density, and stem and leaf colour. Dimensions of the mature leaf subtending the uppermost young shoot on the main stem were measured by hand micrometer gauge. The height and width of individual bushes were affected by pruning during the course of the experiment and

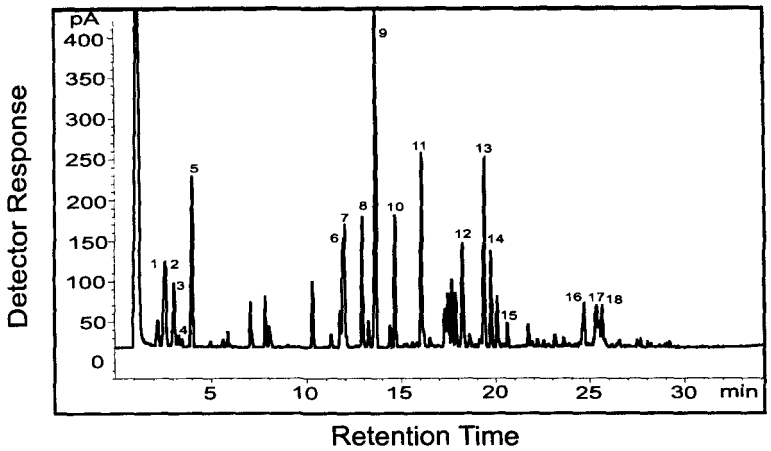

Fig. 1 GC profile of the essential oil obtained from wide sampling of the natural Canaan Road population of *L. scoparium*. Peak numbers relate to components identified in Table 1.

so were not included on otherwise similar observations of mature bushes in 1996.

Data analysis

Data from the March 1993 harvest describe young plants while those from the January and December 1995 and April 1996 harvests describe mature plants, and provide comparisons between and within growing seasons. Principal component analysis (SAS Institute version 6.08 software) was used to examine variation in oil composition data. Analysis of variance (Minitab release 11) was used to assess differences in levels of major components at different harvest times.

RESULTS

Oil composition

An oil sample obtained from a single extensive sampling of a natural population as described above for the Canaan Road population has normally been accepted as an adequate representation of the "typical" oil of a sampling site. The GC profile of such a steam-distilled oil sample from the Canaan Road population is shown in Fig. 1. The levels of the major components in this oil are summarised in Table 1, where peak numbers relate to those in Fig. 1. The profile shows close similarities with profiles of solvent-extracted oils from some individual plants, e.g., plant 13 in the 4/96 harvest.

In the oils from the 16 young plants (March 1993 and including the natural population oil), the first three principal components accounted for 69% of the variation. Six peaks were identified as major contributors to these principal components: α-Cubebene, ß-Elemene, ß-Selinene, α-Selinene, Calamenene, and Elemol. In the oils from the larger group of mature plants (January and December 1995 and April 1996), the first three principal components accounted for between 69 and 88% of the variation. Five peaks were identified as major contributors to these principal components: α-Pinene, β-Pinene, Myrcene, β-Elemene, and Elemol. Sesquiterpenes

Table 1 Levels (peak area percent) of major components of the natural Canaan Road population oil of *L. scoparium*. Peak numbers relate to the GC profile in Fig. 1. CAS number is the Chemical Abstract Services Registry Number, uniquely identifying each component.

Peak no.	Component	CAS number	Peak area percent
1	α-Thujene	2867–05–2	1.5
2	α-Pinene	80–56–8	3.4
3	ß-Pinene	127–91–3	2.1
4	Myrcene	123–35–3	0.2
5	Linalool	78–70–6	5.2
6	Unknown		2.9
7	α-Cubebene	17699–14–8	3.9
8	α-Copaene	3856–25–5	4.2
9	ß-Elemene	33880–83–0	11.1
10	ß-Caryophyllene	87–44–5	3.8
11	Unknown		5.9
12	α-Selinene	473–13–2	4.3
13	Calamenene	485–77–2	6.3
14	δ-Cadinene	483–76–1	2.8
15	Elemol	639–99–6	1.2
16	γ-Eudesmol		2.5
17	ß-Eudesmol	473–15–4	3.2
18	α-Eudesmol	51317–08–9	2.9

were identified as the most important variables in the young plants while monoterpenes were more important in the mature plants.

The levels of some oil components varied across a very wide range (Table 2), and some individual plant oils could be distinguished by extreme levels of one or more oil components. However, none of these analyses revealed any significant, distinct clustering of plants based on chemical composition of the oil. It must be concluded that this seedling population exhibits a continuum of variation of oil composition in individual plants rather than groups of plants based on distinctive oil compositions. The continuum of oil variation seen in the young plants appears to persist as the plants mature.

The oil components identified as the major contributors to the first three principal components in the 12/95 data set (α-Pinene, β-Pinene, Myrcene, β-Elemene, and Elemol) are used as the common basis for the comparisons between seasons (1/95 and 12/95), within a season (12/95 and 4/96), and between young and mature (3/93 and 12/95). Table 2 compares means and ranges of these components in oil samples from the three other sample dates to illustrate the range of variation in oil composition at the population level at the different sample times. Total monoterpene levels are also included.

Table 3 summarises significant differences at the population level among the six components shown in Table 2.

All of the oil components except β-Elemene differ significantly between the young and mature plants. The pinenes and β-Elemene also differ significantly within a single season. Because all components except Myrcene differ significantly between seasons, single samples, even if replicated within the population, will not adequately describe the possible variation. Sampling protocols should therefore allow for replication between and within seasons and stages of plant maturity to provide reliable data on oil composition at the population level.

Table 4 presents the levels of the same oil components for selected individual plants to illustrate the type and range of variation in monoterpene and sesquiterpene levels at the individual plant level.

Table 4 also illustrates the extent of the variation between and within seasons, and with plant maturity. Variation of oil component levels between

Table 2 Means and ranges of levels of α-Pinene, β-Pinene, Myrcene, β-Elemene, and Elemol (peak area percent) at different sample dates in oil samples from 16 individual plants in the seedling population of *L. scoparium*. Values for the natural population are from one oil sample only. Total peak area percentages of monoterpenes include those components having GC retention times between 2.3 and 7.0 in Fig. 1.

	Natural population		Seedling population			
Sample date	3/93		3/93	1/95	12/95	4/96
α-Pinene	3.4	Mean	0.8	7.6	20.9	15.8
		Minimum	0.0	3.9	12.3	6.9
		Maximum	3.4	9.9	38.6	34.3
β-Pinene	2.1	Mean	0.6	2.7	12.4	9.0
		Minimum	0.0	1.2	6.8	3.9
		Maximum	1.2	4.5	20.2	18.5
Myrcene	0.3	Mean	0.3	8.1	7.7	6.7
		Minimum	0.0	4.0	4.2	2.8
		Maximum	0.8	12.6	13.2	12.4
β-Elemene	11.1	Mean	11.7	5.9	20.3	15.2
		Minimum	0.1	1.4	8.1	7.0
		Maximum	25.8	14.9	35.5	26.0
Elemol	1.2	Mean	2.3	1.3	5.6	4.3
		Minimum	0.0	0.0	0.0	0.0
		Maximum	9.0	7.7	23.5	19.9
Total Mono-terpenes	10.6	Mean	11.1	67.6	45.6	38.9
		Minimum	5.5	35.8	26.0	22.6
		Maximum	27.7	82.9	75.1	82.9

Table 3 Analysis of variance *F* ratios and *P* values from comparisons of young and mature plants (3/93 and 4/96), mature plants within a single growing season (12/95 and 4/96), and between growing seasons (1/95 and 12/95). ns = not significant, $P > 0.05$.

Comparison		α-Pinene	β-Pinene	Myrcene	β-Elemene	Elemol	Total mono-terpenes
3/93:4/96	*F*	265.0	214.4	125.3	2.0	51.2	4.8
	P	<<0.01	<<0.01	<<0.01	ns	<<0.01	0.04
12/95:4/96	*F*	13.4	12.4	2.6	11.1	1.12	4.7
	P	<0.01	<0.01	ns	<0.01	ns	0.03
1/95:12/95	*F*	241.0	129.4	2.4	118.5	9.4	80.1
	P	<<0.01	<<0.01	ns	<<0.01	<0.01	<<0.01

Table 4 Levels of α-Pinene, β-Pinene, Myrcene, β-Elemene, Elemol, and total monoterpenes (peak area percent) of individual plant oils. nd = not detected at 0.1% level. ns = plants not selected for sampling until after first two sampling dates.

Plant number	Date	α-Pinene	β-Pinene	Myrcene	β-Elemene	Elemol	Total monoterpenes
			Natural population				
	3/93	3.40	2.09	0.34	11.13	1.18	10.64
			Seedling population				
1	3/93	nd	0.96	0.33	4.49	1.48	8.86
	1/95	7.63	2.59	8.42	3.34	nd	64.08
	12/95	12.28	6.79	4.15	24.64	8.24	26.01
	4/96	18.30	9.96	6.09	9.17	3.72	37.84
2	3/93	0.18	0.77	0.12	12.57	6.32	5.46
	1/95	8.58	2.90	8.43	5.31	2.04	68.80
	12/95	18.29	9.06	6.27	24.82	13.70	36.68
	4/96	20.71	11.16	7.23	15.48	6.36	42.50
3	3/93	0.50	0.52	0.07	23.95	3.03	7.17
	1/95	6.24	2.29	3.98	14.90	1.54	48.91
	12/95	19.51	11.12	6.73	29.64	3.29	41.03
	4/96	15.53	9.00	5.43	24.61	2.51	33.71
5	3/93	0.43	0.92	nd	11.63	1.18	9.60
	1/95	7.65	2.58	7.43	4.31	nd	66.44
	12/95	20.63	10.00	6.76	26.83	3.47	42.36
	4/96	16.77	8.87	5.59	8.04	1.03	37.54
6	3/93	2.04	0.65	0.53	5.76	0.75	27.70
	1/95	5.70	1.90	8.74	5.20	7.68	59.66
	12/95	20.30	17.96	6.68	17.27	1.38	51.37
	4/96	13.60	11.62	4.87	7.00	0.47	36.90
8	3/93	0.13	1.17	0.13	6.19	1.21	9.78
	1/95	6.79	3.30	8.30	1.99	nd	69.14
	12/95	25.41	13.85	9.84	8.05	23.47	53.27
	4/96	16.93	8.79	7.00	12.07	19.88	35.75
10	3/93	1.33	0.89	0.47	15.65	9.01	14.53
	1/95	6.30	2.28	9.98	7.45	2.95	68.28
	12/95	23.59	13.07	11.60	18.22	13.07	52.26
	4/96	14.33	8.57	10.40	16.73	6.72	38.41
11	3/93	0.25	0.34	0.55	23.49	1.71	7.60
	1/95	5.37	1.85	6.95	11.72	0.81	52.78
	12/95	13.56	7.47	4.51	35.52	1.58	30.30
	4/96	10.85	6.00	3.68	20.39	1.40	24.18
12	3/93	1.23	0.37	0.28	1.27	0.10	6.11
	1/95	4.05	1.30	5.01	4.41	nd	35.75
	12/95	18.30	10.35	7.85	24.42	nd	39.24
	4/96	12.39	7.32	6.85	19.00	1.41	29.91
15	3/93	0.30	0.56	0.64	25.76	nd	9.74
	1/95	9.27	3.11	7.65	9.69	nd	72.71
	12/95	18.47	8.57	5.60	29.97	nd	35.88
	4/96	11.90	6.09	3.84	26.00	nd	27.94
29	3/93	ns	ns	ns	ns	ns	ns
	1/95	ns	ns	ns	ns	ns	ns
	12/95	38.58	20.15	13.24	9.57	6.50	75.12
	4/96	34.31	15.11	12.37	10.00	2.86	68.50
30	3/93	ns	ns	ns	ns	ns	ns
	1/95	ns	ns	ns	ns	ns	ns
	12/95	27.53	12.86	8.99	11.32	9.91	57.03
	4/96	8.28	4.32	2.76	16.22	11.18	23.80

individual plants is extensive at all sample dates with high coefficients of variation – from 15.9 to 146.4%. Tables 2 and 4 also illustrate the dominant role of monoterpene components identified by the principal component analyses. At the population level, the mean values of total monoterpenes levels fall from 45.6% in midsummer (12/95) to 38.9% in autumn (4/96). Differences in levels in mature plants between years (67.6% in 1/95, and 45.6% in 12/95) are even more marked as are differences between young and mature plants (11.2% in 3/93, and 38.9% in 4/96). One example of the extremes of variation is the failure to detect Elemol at >0.1% in plant 15 at any time.

These results confirm early observations (N. G. Porter unpubl. data) suggesting that pinene levels are highest during the main spring/summer period of foliage growth, and drop as the foliage hardens off during autumn. Recent analyses of oils obtained in a single sampling of individual plants in an East Cape population also found that monoterpene levels varied extensively, e.g., α-Pinene mean 3.5%, minimum 0.1%, maximum 16.2% (N. B. Perry & N. J. Brennan pers. comm. 1996). The mean levels of major monoterpenes in those samples were lower than the mean levels in the mature plants of the Canaan Road population.

Plant and foliage characteristics

Variation between individual plants in foliage characteristics were obvious in preliminary examination of the natural Canaan Road population. Table 5 summarises observations and measurements on plant and foliage characteristics in selected young and mature plants showing the full range of that variation.

Significant variation in plant and leaf characters are reported by Yin et al. (1984) and Wilson et al. (1991). All the characters observed in this trial exhibit obvious variation between individual plants. Plant and leaf size ratios varied more than twofold between both young and mature individual plants, reflecting similar ranges of variation in width and height or length. During spring and early summer extension growth, leaves and young stems showed variable and sometimes striking red or purple coloration. While this coloration provides another dimension of variation, it is seasonal and temporary, and reflects the stage of development of the foliage and the season's climate. The stem and leaf colour of mature foliage in the autumn is less striking, but it is less variable with time and climate and so is more suitable for making regular comparisons between seasons. The leaf colour appeared to be largely consistent between young and mature plants. Similarly, the habit of most young plants was a useful indicator of mature plant habit, but there are sufficient exceptions, four of the 16 young plants, to require confirmation of second year observations in subsequent years.

DISCUSSION

The striking variations in chemistry and morphology found in this seedling population come not from plants from diverse populations and sites, but from seedlings derived from a few plants of a single site in one natural population and subsequently grown at a single site. This trial, therefore, illustrates the genotypic potential for variation in both the oil

Table 5 Observations of individual plant morphology: plant and leaf ratios, plant height or leaf length divided by width; leaf density, density of leaves on young stems; plant habit, branching pattern and overall bush shape. Plant ratios determined only while plants were young; nd = not determined because plants were selected when partly mature and plant height and shape were affected by removal of foliage for oil analyses.

Plant	Plant ratio	Leaf ratio	Leaf density	Leaf colour	Stem colour	Plant habit
3	2.0	3.3	Sparse	Olive green, copper tinge	Grey	Open, upright
5	2.7	3.2	Medium–dense	Clear green	Grey, copper tinge	Compact, upright
7	2.1	2.8	Dense	Clear bright green	Grey, copper tinge	Loose, very upright
10	2.6	3.6	Sparse–medium	Dull green	Grey	Open, upright
19	nd	4.3	Sparse–medium	Clear green, copper tinge	Grey	Medium, upright
20	nd	2.2	Dense	Clear green	Purple brown	Compact, upright
22	nd	2.4	Dense	Dull green	Grey	Compact, upright
23	nd	4.0	Medium	Clear bright green	Grey, purple tinge	Compact, very upright
26	nd	3.2	Sparse–medium	Clear green, yellow tinge	Grey, dark brown tinge	Open, spreading
30	nd	2.8	Sparse	Dull green, yellow tinge	Grey	Open, spreading

chemistry and physical structure of the plant that can be seen at any one time. Genotypic control of oil composition has not given rise to distinct groups within this population, but has established obvious differences in composition between other populations (Perry et al. 1997). Superimposed on this genotypic variation is continuing change within and between seasons, which is particularly obvious and significant in the oil composition as the plant chemistry reflects seasonal, climatic, and plant effects.

The mean data derived from an oil obtained by a single wide sampling of this natural population obscure extensive variation between individual plants. Although standardisation of plant tissues or parts taken in samples (position, physiological state, maturity etc.) and replication within and between populations at any given sampling time can improve the reliability of the data and conclusions, data from a single sampling time cannot indicate how oil compositions may change with time at the plant or population level. It is therefore essential that a detailed understanding of the dynamics of oil composition and the plant habit be obtained by repeated observations between and within seasons to ensure that plant selection or chemotaxonomic decisions relating to perennial plants are based on true genotypic expression rather than seasonal fluctuations. Although this study is based on single plant data, the variation observed indicates the need for more extensive sampling and statistical analysis over several seasons if sufficiently reliable data on oil compositions in individual plants or populations are to be obtained for chemotaxonomic or plant selection purposes.

The extent and continuing nature of the variation seen in this population have practical consequences for commercial oil production and associated plant selection programmes. Both commercial production and selection may depend on achieving target yields of specific oil components, which in turn are dependent on the combination of oil composition and foliage production. Selection and crop management decisions must, therefore, integrate both foliage and oil variation, which apparently proceed on different time-scales within and between seasons. It has not been possible from these data to identify any relationship between plant or foliage characteristics and oil composition. In this trial, numerical data on some plant habit characters used by Yin et al. (1984) and Wilson et al. (1991) (erectness, apical dominance, plant shape, and branch angle) were not measured because of the effect of foliage pruning and minimal trial plot spacing. These characters, and others such as foliage and leaf density, regeneration after foliage harvest, and time of flowering, are important in foliage production for commercial essential oil plantings but can only be properly assessed in adequately spaced, 2–3-m-high trees. Although it may be possible to begin identification of elite plants with assessment of some plant and foliage characters in the second growth season, reliable confirmation of those identifications and an adequate analysis of the dynamics of oil composition in mature plants may take some 4–5 growth seasons. An approach used to identify elite plants of *Boronia megastigma* by means of a ranking index combining chemical and plant criteria (Smale et al. 1995) is being modified for use with manuka. When identification of elite lines has been reliably confirmed by evaluation over several seasons, improved clonal lines from *in vitro* propagation from individual plants can be established in intensive cultivation to replace the wild harvest system currently being used for commercial production.

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