**Table S1.** Sample name, variety or cultivar and location of mānuka trees included in the survey

| Tree | Variety/Cultivar | Longitude | Latitude | Location | Tree | Variety/Cultivar | Longitude | Latitude | Location |
|---|---|---|---|---|---|---|---|---|---|
| GCP004 | var. scoparium | 174.44 | -36.86 | BB | VB002 | var. scoparium | 178.20 | -37.60 | Potaka |
| GCP009 | var. scoparium | 174.83 | -36.60 | Army Bay | VB010 | var. scoparium | 178.20 | -37.60 | Potaka |
| GCP017 | var. scoparium | 173.62 | -35.33 | Horeke | VB014 | 'Martinii' | 175.30 | -37.80 | Hamilton |
| GCP020 | var. scoparium | 175.49 | -36.81 | Te Kouma | VB015 | 'Nanum Tui' | 175.32 | -37.79 | Hamilton |
| GCP021 | var. scoparium | 174.52 | -35.62 | Ngunguru | VB018 | 'Nanum Tui' | 175.32 | -37.79 | Hamilton |
| GCP022 | var. incanum | 174.70 | -36.71 | Albany | VB036 | ?'Sunraysia | 175.32 | -37.79 | Hamilton |
| GCP025 | var. incanum | 174.83 | -36.61 | Army Bay | VB042 | 'Nanum Tui' | 175.32 | -37.79 | Hamilton |
| GCP027 | var. incanum | 172.94 | -34.60 | Te Kao | VB050 | var. scoparium | 178.31 | -37.76 | Tikitiki |
| GCP028 | var. scoparium | 174.50 | -36.99 | Karekare | VB051 | var. scoparium | 178.29 | -37.75 | Tikitiki |
| GCP030 | var. scoparium | 174.83 | -36.60 | Army Bay | VB052 | var. scoparium | 178.31 | -37.76 | Tikitiki |
| GCP031 | var. incanum | 174.83 | -36.60 | Army Bay | VB053 | var. scoparium | 178.20 | -37.60 | Potaka |
| GCP033 | var. incanum | 173.28 | -34.90 | Kaimaumau | VB054 | var. scoparium | 178.20 | -37.60 | Potaka |
| GCP034 | var. incanum | 174.82 | -36.61 | Army Bay | VB055 | var. scoparium | 176.39 | -39.00 | Taharua |
| GCP035 | var. incanum | 174.65 | -36.91 | Glen Eden | VB065 | U.C. | 175.32 | -37.79 | Hamilton |
| GCP039 | var. incanum | 172.86 | -34.55 | Te Hapua | VB067 | var. scoparium | 178.29 | -37.72 | Te Araroa |
| GCP040 | var. scoparium | 175.46 | -36.58 | Waiaro | VB070 | var. scoparium | 178.29 | -37.72 | Te Araroa |
| GCP048 | var. scoparium | 175.65 | -36.76 | Otapaurau | VB071 | var. scoparium | 178.29 | -37.72 | Te Araroa |
| GCP049 | var. incanum | 172.87 | -34.43 | Te Hapua | VB074 | var. scoparium | 178.31 | -37.76 | Tikitiki |
| GCP055 | var. scoparium | 174.71 | -36.63 | Stillwater | VB077 | U.C. | 175.32 | -37.79 | Hamilton |
| GCP060 | var. scoparium | 174.51 | -35.58 | Matapouri | VB083 | var. scoparium | 178.29 | -37.75 | Tikitiki |
| GCP092 | var. scoparium | 174.71 | -36.63 | Stillwater | VB084 | 'Nanum Tui' | 175.32 | -37.79 | Hamilton |
| GCP111 | var. incanum | 172.86 | -34.55 | Te Hapua | VB088 | var. scoparium | 178.19 | -37.60 | Potaka |
| GCP113 | var. incanum | 172.69 | -34.60 | Cape Reinga | VB089 | var. scoparium | 178.20 | -37.60 | Potaka |
| GCP116 | var. incanum | 172.96 | -34.62 | Te Kao | VB090 | var. scoparium | 178.19 | -37.60 | Potaka |
| GCP119 | var. scoparium | 173.13 | -35.20 | Ahipara | VB091 | var. scoparium | 175.32 | -37.79 | Hamilton |
| GCP120 | var. scoparium | 174.75 | -36.39 | TP | VB093 | var. scoparium | 178.30 | -37.74 | Tikitiki |
| GCP124 | var. scoparium | 173.14 | -35.19 | Ahipara | VB099 | var. scoparium | 178.29 | -37.75 | Tikitiki |
| GCP125 | var. incanum | 174.82 | -36.61 | Army Bay | VB100 | var. scoparium | 178.29 | -37.75 | Tikitiki |
| GCP128 | var. scoparium | 174.20 | -36.46 | South Head | VB101 | var. scoparium | 175.32 | -37.79 | Hamilton |
| GCP129 | var. scoparium | 174.61 | -36.54 | Puhoi | VB102 | var. incanum | 178.29 | -37.72 | Te Araroa |
| GCP130 | var. incanum | 174.47 | -36.62 | Kaukapakapa | VB105 | var. scoparium | 178.29 | -37.75 | Tikitiki |

| Tree | Variety/Cultivar | Longitude | Latitude | Location | Tree | Variety/Cultivar | Longitude | Latitude | Location |
|---|---|---|---|---|---|---|---|---|---|
| GCP149 | var. scoparium | 173.30 | -34.88 | KP | VB114 | var. scoparium | 178.30 | -37.74 | Tikitiki |
| GCP154 | var. incanum | 172.94 | -34.60 | Te Kao | VB115 | var. scoparium | 178.30 | - | Tikitiki |
| GCP156 | var. scoparium | 174.27 | -35.55 | Whakapara | VB131 | var. scoparium | 178.19 | -37.60 | Potaka |
| GCP158 | var. incanum | 173.62 | -35.33 | Horeke | VB137 | var. scoparium | 178.29 | -37.72 | Te Araroa |
| GCP160 | var. incanum | 174.74 | -36.32 | Big Omaha | VB140 | var. scoparium | 178.19 | -37.60 | Potaka |
| GCP177 | var. scoparium | 173.21 | -34.99 | Paparore | VB142 | var. scoparium | 178.29 | -37.75 | Tikitiki |
| GCP178 | var. scoparium | 174.69 | -36.50 | MW | VB145 | var. scoparium | 178.30 | -37.74 | Tikitiki |
| GCP251 | var. scoparium | 173.38 | -34.97 | Lake Ohia | VB146 | var. scoparium | 178.19 | -37.60 | Potaka |
| L2253 | var. scoparium | 173.08 | -41.28 | Nelson | VB151 | U.C. | 174.91 | -37.01 | Manurewa |
| L3961 | var. scoparium | 175.48 | -36.86 | Manaia | VB162 | var. scoparium | 175.71 | -41.29 | Hinakura |
| L3962 | var. scoparium | 173.08 | -41.28 | Nelson | VB164 | var. incanum | 175.44 | -41.48 | Tora |
| L3969 | var. scoparium | 175.48 | -36.86 | Manaia | VB165 | var. scoparium | 175.71 | -41.29 | Hinakura |
| L3978 | var. scoparium | 175.48 | -36.86 | Manaia | VB167 | var. scoparium | 175.14 | -39.82 | Parikino |
| L3980 | var. scoparium | 175.48 | -36.86 | Manaia | VB168 | var. scoparium | 175.44 | -41.41 | Tuturumuri |
| L3986 | var. scoparium | 175.48 | -36.86 | Manaia | VB169 | var. scoparium | 175.44 | -41.48 | Tora |
| L3989 | var. scoparium | 175.48 | -36.86 | Manaia | VB170 | var. scoparium | 175.71 | -41.29 | Hinakura |
| L3990 | var. scoparium | 175.48 | -36.86 | Manaia | VB172 | var. scoparium | 175.44 | -41.48 | Tora |
| L3995 | var. scoparium | 173.08 | -41.28 | Nelson | VB173 | var. scoparium | 175.43 | -41.42 | Tuturumuri |
| L3998 | var. scoparium | 175.71 | -41.29 | Hinakura | VB175 | var. scoparium | 175.44 | -41.42 | Tuturumuri |
| L3999 | var. scoparium | 175.14 | -39.82 | Parikino | VB177 | U.C. | 174.91 | -37.01 | Manurewa |
| VB181 | var. scoparium | 178.29 | -37.75 | Tikitiki | VB201 | var. scoparium | 178.20 | -37.60 | Potaka |
| VB183 | var. scoparium | 178.30 | -37.74 | Tikitiki | VB202 | var. incanum | 178.29 | -37.72 | Te Araroa |
| VB184 | var. scoparium | 178.31 | -37.76 | Tikitiki | VB203 | var. scoparium | 178.20 | -37.60 | Potaka |
| VB185 | var. incanum | 175.14 | -39.82 | Parikino | VB204 | var. scoparium | 178.20 | -37.60 | Potaka |
| VB186 | var. scoparium | 178.31 | -37.76 | Tikitiki | VB205 | var. scoparium | 175.14 | -39.82 | Parikino |
| VB187 | U.C. | 174.91 | -37.01 | Manurewa | VB206 | var. scoparium | 178.20 | -37.60 | Potaka |
| VB188 | var. incanum | 178.31 | -37.77 | Tikitiki | VB207 | var. scoparium | 178.31 | -37.76 | Tikitiki |
| VB189 | var. scoparium | 175.71 | -41.29 | Hinakura | VB208 | var. scoparium | 178.20 | -37.60 | Potaka |
| VB190 | var. scoparium | 178.19 | -37.60 | Potaka | VB209 | var. scoparium | 178.29 | -37.72 | Te Araroa |
| VB191 | var. scoparium | 178.19 | -37.61 | Potaka | VB210 | var. scoparium | 178.30 | -37.72 | Te Araroa |
| VB192 | var. incanum | 178.31 | -37.76 | Tikitiki | VB212 | var. scoparium | 178.30 | -37.72 | Te Araroa |
| VB193 | var. incanum | 178.20 | -37.60 | Potaka | VB213/019 | var. scoparium | 178.30 | -37.75 | Tikitiki |
| VB194 | var. scoparium | 175.44 | -41.48 | Tora | VB214 | var. scoparium | 178.29 | -37.72 | Te Araroa |

| Tree | Variety/Cultivar | Longitude | Latitude | Location | Tree | Variety/Cultivar | Longitude | Latitude | Location |
|---|---|---|---|---|---|---|---|---|---|
| VB195 | var. scoparium | 178.20 | -37.60 | Potaka | VB216 | var. scoparium | 178.30 | -37.75 | Tikitiki |
| VB197 | var. scoparium | 178.19 | -37.60 | Potaka | VB217 | var. scoparium | 175.43 | -41.42 | Tuturumuri |
| VB198 | var. scoparium | 178.31 | -37.76 | Tikitiki | VB218 | var. scoparium | 176.96 | -39.15 | Putorino |
| VB199 | var. scoparium | 178.29 | -37.72 | Te Araroa | VB275 | var. scoparium | 178.15 | -37.60 | Potaka |
| VB200 | var. scoparium | 178.31 | -37.74 | Tikitiki | | | | | |

U.C. = unidentified cultivar TP = Tawharanui Peninsula MW = Mahurangi West
BB = Bethells Beach KP = Karikari Peninsula

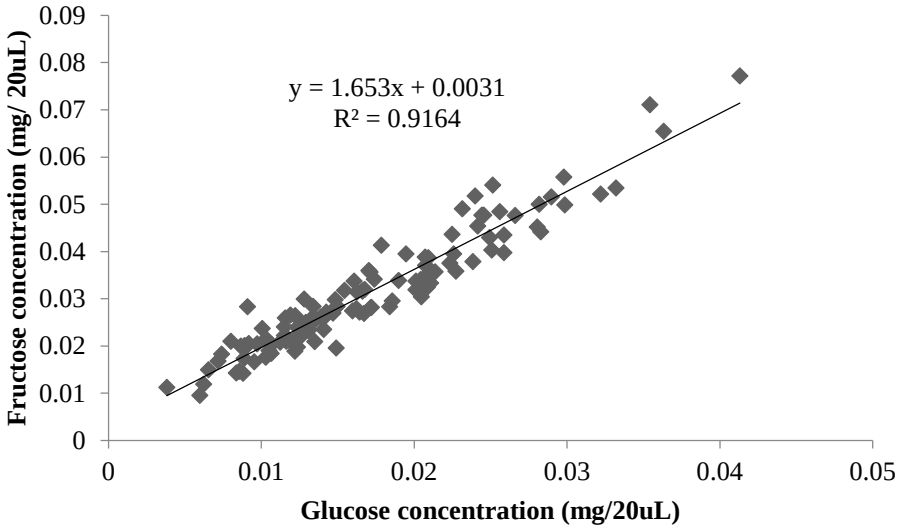


**Figure S1.** Concentration of fructose versus the concentration of glucose (per 20 µL of nectar/water extraction) of *L. scoparium* nectar samples collected in 2009-2010.

**Other factors that might affect DHA/Tsugar**

***Relationship between leaf oil and nectar DHA/Tsugar.*** The leaf oil properties of trees collected in the 2009 survey were investigated. The mean and range of the four triketone components in the leaf oil for the different regions studied are shown in Table S2.

**Table S2.** Mean and range of the four triketone components in the leaf oil for the different regions studied

| Area | Amount (mg/g) | Flavesone | Isoleptospermone | Leptospermone | Grandiflorone |
|---|---|---|---|---|---|
| East Cape | Range | 0-5.33 | 0-4.31 | 0-15.50 | 0-11.21 |
| | Mean | 2.42 | 2.16 | 8.53 | 5.00 |
| Coromandel | Range | 0-2.74 | 0-3.32 | 0-5.32 | 0-2.74 |
| | Mean | 1.70 | 2.04 | 3.80 | 1.24 |
| Nelson | Range | 1.95-2.50 | 1.26-1.74 | 5.66-7.55 | 0.71-4.79 |
| | Mean | 2.14 | 1.44 | 6.59 | 3.01 |
| Wairapa | Range | 0-1.02 | 0-1.61 | 0-3.80 | 0-0.93 |
| | Mean | 0.36 | 0.63 | 1.70 | 0.34 |
| Whanganui | Range | 0-0.32 | 0.13-0.65 | 0.54-1.07 | 0.01-0.06 |
| | Mean | 0.079 | 0.45 | 0.84 | 0.031 |
| Auckland | Range | 0-0.57 | 0-1.34 | 0-3.15 | 0-0.54 |
| | Mean | 0.076 | 0.19 | 0.47 | 0.068 |

Some degree of correlation occured between leaf oil and nectar DHA/Tsugar in some regions (East Cape and Wairarapa regions; adjusted $R^2$ of 80.7 and 88.9% respectively) with multivariate analysis of different leaf oils in different areas. This could reflect the chemotype variation reported by Douglas *et al.* (2004)[1] but it should be noted that this correlation could not be extended to all regions.

Seasonal variation in chemical composition and antimicrobial activity of leaf oils of *Leptospermum* cultivated in Brazil has been demonstrated[2] and so may occur also in New Zealand.

Some trees were found to have both high levels of antimicrobial triketones and a high nectar DHA/Tsugar (Fig. S2).

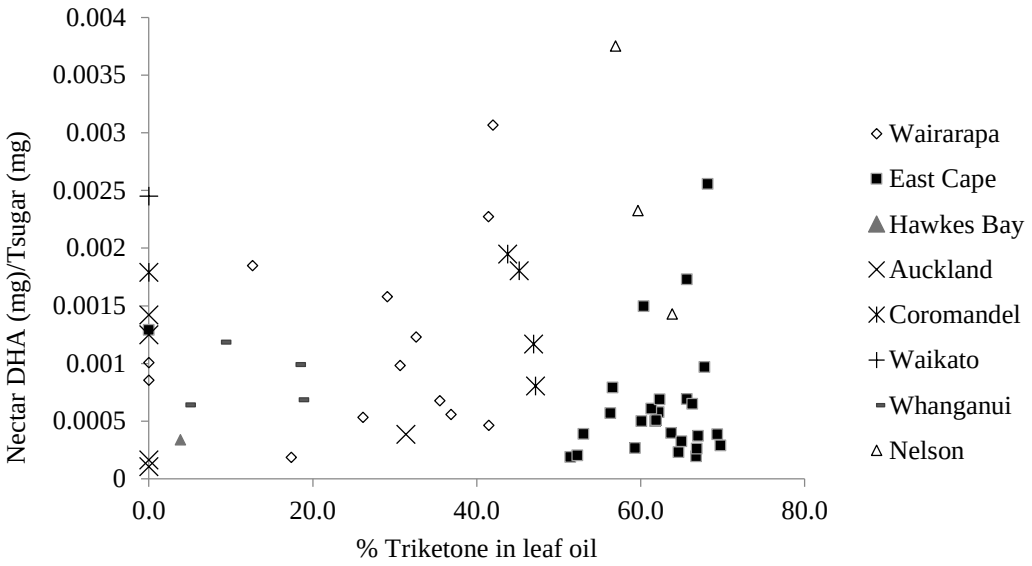


**Figure S2.** Percentage of triketone in leaf oil vs nectar DHA/Tsugar.

**Sex of the flower.** *L. scoparium* is andromonoecious, that is it bears both hermaphrodite and male-only flowers Fig. S3.

**Figure S3.** The andromonoecious nature of mānuka: hermaphrodite (upper) and male flowers (lower). The hermaphrodite flower is easily identified by a prominent stigma. Photo credit: John Tyrell 2013

To establish if the sex of the flower influences nectar DHA/Tsugar, male and hermaphrodite flowers from four different trees were tested (10F, n = 10 per gender). Total sugar did not vary

significantly between male and hermaphrodite flowers but nectar DHA/Tsugar did (paired sample t-test, $p < 0.05$) indicating that the difference is due to elevated DHA in the nectar of the male flowers relative to the hermaphrodite flowers.

**Age of the flower.** In flowers of *L. scoparium* the ovary roof comprises part of the hypanthium, which encloses the nectary tissue. The hypanthium is initially green in new flowers and as a general rule turns dark red with age (Fig. S4); the exposed hypanthium in this species means that this color change is prominent.

**Figure S4.** *L.* 'Red Ensign' flowers at different flowering stages exhibiting a green hypanthium (left) which deepens to red (right). Photo credit: Megan Grainger 2014

A hypanthium color change from green to red in another species of Myrtaceae has previously been hypothesized to act as a signal to insect pollinators for changes in the availability or quality of nectar.[3] To ascertain whether the nectar DHA/Tsugar changes with the age and color of the *L. scoparium* flower, three trees (one L. *'Martinii'*, two L. *'Nanum Tui'*) were tested (10F, $n = 10$) to compare the nectar of hermaphrodite flowers with green hypanthia to those with red. All three trees were in the high classification for nectar DHA/Tsugar. All the trees showed a significant increase (VB001 $p = 0.0001$, VB002 $p = 0.0000$ and VB015 $p = 0.0024$ paired t-test) in nectar DHA/Tsugar in flowers with red hypanthia from just in the high classification to very high (Fig. S5). Both DHA and Tsugar values were higher in flowers with red hypanthia which indicates that reddening of the hypanthium is not associated with reduced nectar flow as

is the case in other plants in the Myrtaceae family.[4] More detailed studies under controlled conditions of nectar flow and composition with aging under controlled environmental conditions will be reported in a separate publication.

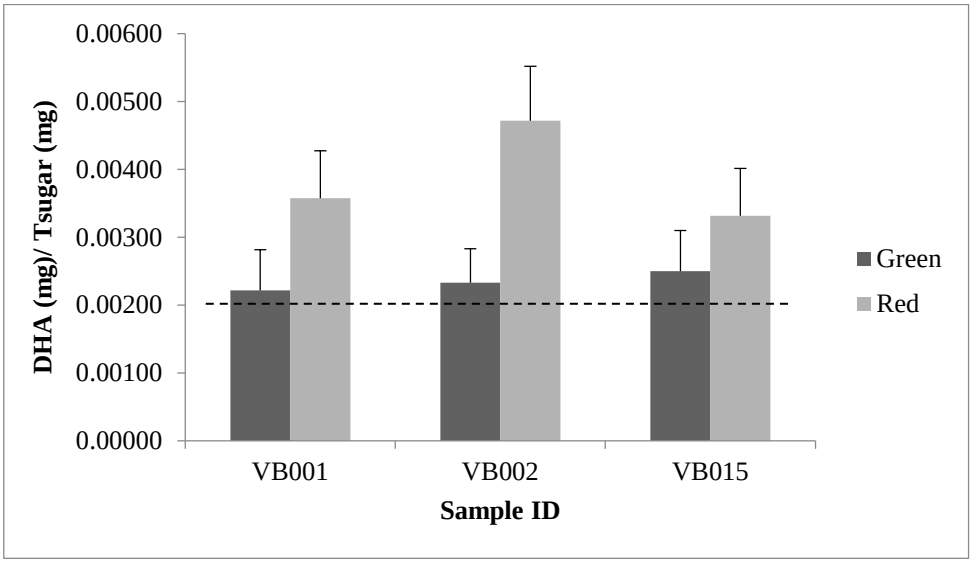


**Figure S5.** The DHA/Tsugar of mānuka flowers with either a green or red hypanthium. VB001 is *L.* 'Martinii' whereas VB001 and VB015 are *L.* 'Nanum Tui' (Error bars are standard error vales). The dotted line represents the high DHA/Tsugar (0.00200 mg/mg) classification.

*Soil Composition.* Quantifiable soil components were subjected to a linear regression test individually and then as groupings to see if there was any correlation to DHA/Tsugar. For the individual tests percentage $R^2$ values ranged from 0-8.2 and the data showed no trends. The best multi-variable regression test gave an adjusted $R^2$ of 26.5%; a trend was suggested but the lack of linearity and the scatter of data indicated that the soil, in which the tree grows, has little or no effect upon DHA/Tsugar.

This is similar to the observation that soil properties had no discernible effect upon leaf oil[1] or the activity of honey derived from a certain area.[5]

*Effect of sooty mold coverage.* The effect of stress on DHA production was investigated by comparing the nectar of trees infested with sooty mold (*Capnodium walteri* Sacc.),[6] growing

on the honeydew excreted by the scale insect *Eriococcus leptospermi* Maskell.[6] The removal of nectar by the scale insect is a stressor and the mold is an indicator of the extent of infestation. Sooty mold coverage was assigned on an arbitrary scale from 1-5 where 1 indicated no infestation and 5 indicated a thick layer of mold. The degree of sooty mold coverage on trees was not found to correlate with nectar DHA/Tsugar (Fig. S6).

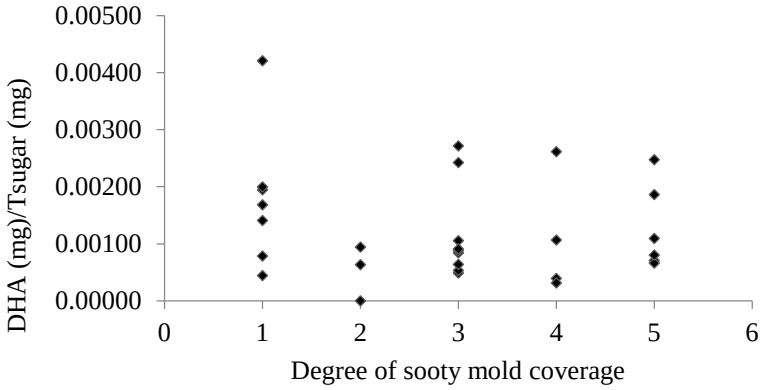


**Figure S6.** Nectar DHA/Tsugar related to sooty mold coverage of tree.

**Experimental**

*Materials.* Deionized water was obtained from a Barnstead Epure water system at 17.9 MΩ. Dichloromethane analytical grade was obtained from Ajax Finechem (Sydney, Australia) or purified using a Pure Solv solvent purification system Model, PS-SD-5 (Innovative Technology, Amesbury, MA). Diethyl ether (analytical grade), HCl (conc), NaOH (analytical grade) and *n*-heptane (HPLC grade) were obtained from Ajax Finechem (Sydney, Australia). Ethanol absolute (analytical grade) and methanol (analytical grade) were obtained from Scharlau (Barcelona, Spain). Aromadendrene (≥97%), 1, 8-cineole (pure) and *n* –dodecane (analytical standard) and isoamyl isovalerate (≥98% FCC Kosher) were obtained from Sigma Aldrich (Sydney, Australia)

Pure triketones, for standards, were extracted from bioactive mānuka oil sourced from the East Cape. The oil was dissolved in diethyl ether and the triketones were separated from the other oil components, by adding sodium hydroxide solution. The aqueous phase which contained the

triketones was removed and concentrated hydrochloric acid was added. Afterwards diethyl ether was added and the aqueous phase was discarded. The organic phase with the triketones was dried over sodium sulfate and the diethyl ether was removed under reduced pressure. The pure triketones were present as a yellow oily liquid.

*Leaf oil.* For the 2009 survey, foliage samples were collected and the leaf oil analyzed by GC-MS after ethanol extraction. Triplicate determinations were made for each tree. Individual compounds (85) were identified or categorized by their mass spectra (Supplementary Table 2) and were quantified using generic response factors for each of the four classes monoterpene, ester, sesquiterpene and triketone.

*Extraction of leaf oil.* Leaf samples were air dried at ambient temperature for one month. Entire leaves (100 mg ± 2 mg) were weighed into a vial, *n*-dodecane (internal standard, 7.000 mL, 0.005 g $L^{-1}$) in ethanol was added and the vial capped and shaken (70 $^{o}$C; 7 h) after which the solution (1 mL) was analyzed by GC-MS and GC-FID.

*Gas chromatography mass spectrometry (GC-MS) of leaf oils.* GC-MS was used for the identification of the compounds present in leaf oil extracts; a Hewlett Packard HP 6890 Series GC-system was coupled to a Hewlett Packard 5973 mass selective detector. The column used was a 30 m × 0.32 mm × 0.25 μm i.d. Phenomonex ZB5 gas capillary column. The temperature program was 45 °C held for 0.5 min, increased at 40 °C/min to 80 °C, increased at 8 °C/min to 295 °C and held for 10 min. Injections (1 μL) were made in splitless mode with a purge flow of 30 mL/min at 0.05 min.

*Gas chromatography with flame ionisation detection (GC-FID) of leaf oils.* GC-FID was used for quantification of the components of the leaf oil; an Agilent Technologies 7890A gas chromatograph was used with a 7683B Series split/splitless injector and 7683 Series autosampler. The column used was a 30 m × 0.320 mm × 0.25 μm i.d HP-5 gas capillary column (Agilent Technologies, Sydney, Australia). The inlet temperature was 250 °C and the

$H_2$ carrier gas flow was 4.168 mL/min. The temperature program was 45 °C held for 0.5 min, increased by 40 °C/min to 80 °C, increased by 8 °C/min to 295 °C and held for 10 min. Injections (5 μL) were made in splitless mode with a purge flow of 30 mL/min at 0.05 min.

*Quantification of leaf oil components.* Leaf oil components were quantified against an internal standard of *n*-dodecane and used a generic response factor for each class of compound. For the determination of generic response factors four substances were used: 1, 8-cineole as a monoterpene, aromadendrene as a sesquiterpene, isoamyl isovalerate as an ester and the extracted triketones (the areas of the four triketone components were added). The response of the four generic substances was measured over a concentration range of 0.001 to 0.05 mg/g and gave monoterpene: 0.86; sesquiterpene: 0.86, ester: 0.69 and triketone 0.28. Results are the mean of three replicate extractions.

*Analyses of soil samples.* Soil samples from the 2009 survey were defrosted and air dried. Color was determined using Munsell notation and texture was determined by hand. Presence or absence of allophane was determined using NaF and phenolphthalein. Soil was sieved successively (4 mm, 2 mm) and moisture content determined by oven-drying (105 °C, 12 h). pH was measured for an air-dried sample suspended in distilled water using a Jenway 3510 pH meter. Conductivity of the soil was determined using a Hanna Instruments EC215 multi-range bench top conductivity meter and total soluble salts were calculated from the conductivity. Phosphorus was determined using the Olsen P test. Total exchangeable bases (TEB) were determined by leaching with $NH_4Ac$ reaction of the eluate with HCl and back-titration with NaOH. Cation exchange capacity (CEC) was obtained by distillation of ammonia and titration of the distillate with HCl. Total carbon and total nitrogen were measured using a TruSpec CN elemental determinator from Leco Corporation. Trace element composition was determined using a Perkin-Elmer Elan DRC ll ICP-MS; samples were digested in *aqua regia* (36% HCI: 70% $HNO_3$, 1:3).

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