Research Article

Flavour and Fragrance Journal

Received: 13 March 2008, Revised: 8 December 2008, Accepted: 6 January 2009, Published online 12 February 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI 10.1002/ffj.1921

Beyond six scents: defining a seventh *Thymus vulgaris* chemotype new to southern France by ethanol extraction

Ken Keefover-Ring,[a]* John D. Thompson[b] and Yan B. Linhart[a]

ABSTRACT: The concept of plant chemotype has long been useful to describe secondary chemical phenotypes; however, the idea has practical limitations, especially when applied to ecological questions. This work reports the discovery of a new 1,8-cineole chemotype of *Thymus vulgaris* from a well-studied area in southern France. Multivariate statistical analysis of ethanol-extracted plant terpenes was used to describe this new chemotype and three others found at the site, and the results are used to discuss the chemotype concept. While the total amount of essential oils among these chemotypes showed no difference, the concentration of the main terpene differed significantly, with the 1,8-cineole and *cis*-sabinene hydrate chemotypes having the lowest amounts of their respective main components, and the linalool chemotype having the highest. The α-terpinyl acetate chemotype had intermediate levels of its main terpene. A factor analysis revealed four factors which explained almost 89% of the total variation in plant essential oils. Each factor represented a separate chemotype, including a *cis*-sabinene hydrate, linalool, α-terpinyl acetate and the new 1,8-cineole chemotype. Although the concept of plant chemotype is still valid, better definitions are important when evaluating the influences of a plant's secondary chemistry on other community members. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: *Thymus vulgaris*; 1,8-cineole; *cis*-sabinene hydrate; linalool; α-terpinyl acetate; multivariate analysis; chemotype concept; southern France

Introduction

Many plant species that produce secondary compounds contain individuals with distinct chemical phenotypes, commonly called chemotypes, often defined by the single dominant chemical an individual produces. These are usually qualitative designations, making the concept of chemotype somewhat arbitrary, due to large amounts of variation in the chemistry of any particular plant species. To address this ambiguity, researchers are increasingly using multivariate statistical analysis of intra-specific variation in plant secondary chemistry as a way to define chemotypes more precisely.[1–3]

A clear designation of chemotype is particularly useful within the family Lamiaceae, where many species have essential oil polymorphisms, i.e. the occurrence of individual plants whose various genotypes code for the production of different dominant terpenes.[4–7] Although the essential oil of a plant may contain multiple terpenes, many of which occur in trace amounts, its chemotype is usually defined by this single dominant terpene and other biosynthetically-related compounds.[8] *Thymus vulgaris* L. or common thyme in the South of France provides a classic example of a chemically polymorphic labiate.[9–11] Extensively studied populations found in and around the St-Martin-de-Londres basin contain individuals that produce one of six possible majority monoterpenes, geraniol (G), α-terpineol (A), sabinene hydrate (thuyanol, U), linalool (L), carvacrol (C) or thymol (T), as the main component of their total monoterpenes.[8,12] The production of the major monoterpene in individual plants involves an epistatic series of five loci with a set order of dominance, so that G > A > U > L > C > T.[12] In addition to their main terpenes, the various chemotypes have other predictable components; specifically, G, A and L plants can have considerable amounts of their primary monoterpene's acetate (geranyl, α-terpinyl and linalyl acetate, respectively); U plants often contain terpinen-4-ol, myrcen-8-ol and linalool in addition to the main monoterpene, *cis*-sabinene hydrate; and C and T plants have substantial levels of γ-terpinene and *p*-cymene.[8,13] The latter are precursors in the biosynthesis of carvacrol and thymol.[14,15]

This paper presents the discovery, using an ethanol solvent extraction, of a 1,8-cineole chemotype of *Thymus vulgaris* in southern France not previously reported by hydrodistillation of the essential oil. In addition to some of the chemotypes mentioned above, other studies of natural populations of *T. vulgaris* in Mediterranean France have also reported *p*-cymene and borneol chemotypes.[16] However, while a 1,8-cineole chemotype of this species has been documented to occur in Spain,[17–19] it was long thought to be absent in France.[20] Using multivariate analysis of plant terpenes, obtained by ethanol extraction, the

* Correspondence to: K. Keefover-Ring, Department of Entomology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA. E-mail: keefover@entomology.wisc.edu

[a] Department of Ecology and Evolutionary Biology, University of Colorado, N122 Ramaley CB 334, Boulder, CO 80309, USA

[b] Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175, CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France

Contract/grant sponsor: NSF; Contract/grant number: DEB 0091385; DBI 0500560 to YBL.

chemotypes found at a site in southern France, which clearly contains the 1,8-cineole (eucalyptol) chemotype, are described. These findings lead to a discussion of the chemotype concept and its limitations.

Experimental

Sample Collection and Preparation

Sampling was conducted on 14 October 2005 in the St-Martin-de-Londres basin in southern France, near the hamlet of Fraicinède, located about 20 km north of Montpellier. Samples consisted of approximately 1.5 cm of terminal foliage clipped from non-flowering plants and placed in 2 ml microcentrifuge tubes. The samples were refrigerated for 3 days, after which 1.0 ml neat ethanol, containing *m*-xylene as an internal standard (0.1 μl/ml), was added for terpene extraction. The foliage was submerged and soaked for 7 days. After extraction, the foliage was removed from the solution, air-dried and weighed, and the amount of each terpene per dry weight of foliage (mg terpene/g DW) was calculated, using terpene specific gravities.

Chemical Analysis

A portion of the solution from each sample was withdrawn for direct analysis, using a Hewlett-Packard 6890 gas chromatograph (GC), equipped with a flame ionization detector (FID), with helium as the carrier gas at a flow rate of 1.3 ml/min. Injector and detector temperatures were set at 260 °C and 250 °C, respectively. A DB-Wax capillary column (15 m × 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific) was installed in the GC and the oven profile consisted of an isothermal hold at 50 °C for 5 min, followed by a ramp of 6 °C/min to 125 °C, then a second ramp to 170 °C at 10 °C/min. Five μl of each sample was injected in the split mode with a split flow ratio of 50:1.

Compound amounts were determined by peak area comparisons of authentic standards where available (all standards were obtained from Sigma). Terpenes for which standards were not readily available, including myrcen-8-ol (2-methyl-6-methylene-2,7-octaien-1-ol) and myrcen-8-yl acetate, were quantified using the peak area of the nearest standard and assuming a linear response. In addition to *cis*-sabinene hydrate, U chemotype plants also contained small amounts of *trans*-sabinene hydrate (separated and identified by GC–MS, below). However, due to co-elution with linalool on the 15 m DB-Wax column, these compounds could not be quantified separately with GC–FID.

Additional compound identification analyses were carried out using an Agilent 6890N GC coupled with an Agilent 5975C inert mass selective detector with an ion source of 70.0 eV at 230 °C. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Injector temperature was set at 260 °C. An EC-Wax glass capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Alltech Associates, Inc.) was used for the analyses with oven conditions that included an isothermal hold at 60 °C for 5 min, followed by a ramp of 10 °C/min to 250 °C. Portions of the original ethanol extracts of selected samples of each chemotype were diluted 1:10 with *n*-hexane (GC^2, Burdick and Jackson) in small vials, vortex mixed and allowed to phase separate overnight. A small aliquot of the upper portion of the hexane/ethanol phase was removed and 1 μl was injected into the GC–MS in the splitless mode. Terpenes were identified using retention times of pure standards (all compounds, except myrcen-8-ol and myrcen-8-yl acetate), the NIST 2005 mass spectral library, Adams[21] and other published spectra.[22–24]

Linear retention indices were calculated on the same 15 m DB-Wax and 30 m EC-Wax columns used in the above analyses, and with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Aligent Technologies Inc.) installed on the GC–MS, for all terpenes using a continuous series of *n*-alkanes (C_8–C_{24}). All GC conditions were the same as above except for the oven profile, which for all three columns consisted of an initial temperature of 40 °C, followed by an immediate ramp of 3 °C/min to 200 °C. Calculated retention indices were compared to published values[21,24,25].

Statistical Analysis

All statistical analyses were carried out with SAS version 9.1 software[26]. We used the PROC GLM function to test whether the main terpene [terpene in the highest concentration (mg/g DW); 1,8-cineole, *cis*-sabinene hydrate, linalool and α-terpinyl acetate for the E, U, L and A chemotypes, respectively] and the total amount of oil (total mg terpenes/g DW) were different among plants of the four chemotypes, which were designated by their dominant monoterpene. We used a Ryan–Einot–Gabriel–Welsch multiple range test to examine all pair-wise comparisons for both main and total terpenes. Data for both variables were log-transformed to meet assumptions of normality and type III sums of squares were used, due to unequal sample sizes.

Next, the number of variables was reduced by performing a factor analysis on the concentration data (mg/g DW) of 11 terpenes that consistently occurred at >5% of plant oil composition, using PROC FACTOR with a PROMAX rotation. The first four factors, which had eigenvalues > 1, were accepted and the terpene scores of the first three factors were used for a graphical representation.

Results and Discussion

Using ethanol extraction, four chemotypes, defined by the major component in a plant's essential oil, were found at the study site: the new 1,8-cineole (E) chemotype (20 plants); a *cis*-sabinene hydrate (U) chemotype (26 plants); a linalool (L) chemotype (11 plants); and an α-terpinyl acetate (A) chemotype (6 plants) (Table 1). Although E plants tended to have lower mean total oil concentration, the difference for total terpenes among chemotypes was not significant ($F_{3,59} = 1.8$; $p = 0.15$). There was a highly significant difference ($F_{3,59} = 8.1$; $p = 0.0001$) in the amount of the main terpene (mean mg/g DW ± SE) among the chemotypes, with L (23.0 ± 4.0) and A (15.6 ± 2.0) chemotypes having the highest amounts of their respective major terpenes. The main component in L plants was significantly higher than both E (9.6 ± 0.8) and U (13.8 ± 1.3). Plants of the A chemotype had significantly more α-terpinyl acetate than E plants had 1,8-cineole, but were intermediate between U and L. Despite the fact that the statistical comparison was performed with only the single dominant monoterpenes, the results agreed with previous analyses of *T. vulgaris*, where L and A chemotypes had the greatest percentages of essential oil using a combination of their main terpene and its acetate.[8] The lower amounts of the main components in both E and U, relative to their total terpene concentrations, highlighted the fact that these chemotypes contained substantial amounts of other terpenes; however, the composition of these minor components was much less predictable in E plants (Table 1).

The 20 plants designated as the 1,8-cineole chemotype contained 23–46% of this monoterpene in their essential oil (Table 1) and, of the 63 plants analysed, 44 had 12% or more of their oil made up of 1,8-cineole, including some plants of all three other chemotypes. While no plants were found with such high levels of 1,8-cineole in an earlier collection at this site,[8] 38/40 individuals assayed at that time had detectable levels of 1,8-cineole and 10 had 10% or more of the terpene in their oil. In addition, 1,8-cineole was found in relatively high amounts in plants designated as A, U, and L chemotypes.[8]

The factor analysis produced four factors which together explained 88.8% of the total variation in thyme chemistry (factor

Table 1. Retention indices and percentages of the major terpenes found in the four designated chemotypes of *Thymus vulgaris* at the Fraicinède site in southern France

Compound	Retention indices (linear)			Mean % (SD) Range			
	15 m DB-Wax	30 m EC-Wax	30 m HP-5MS	E ($n = 20$)	U ($n = 26$)	L ($n = 11$)	A ($n = 6$)
β-Pinene	1090	1098	973	3.0 (0.5)	1.4 (0.8)	1.5 (0.9)	0.5 (0.6)
				2.3–4.0	0–2.5	0.1–2.7	0–1.4
Sabinene	1107	1111	970	2.9 (0.6)	1.9 (0.9)	1.1 (0.8)	1.9 (1.1)
				2.0–3.9	0–2.9	0–2.1	0–2.8
Myrcene	1145	1155	990	2.5 (1.6)	4.0 (2.2)	0.5 (0.4)	0.4 (0.4)
				0–6.0	0–6.5	0–1.2	0–0.9
Limonene	1177	1187	1026	1.2 (0.8)	1.6 (0.9)	0.4 (0.5)	1.0 (0.7)
				0–2.08	0–2.48	0–1.5	0–1.8
1,8-Cineole	1193	1195	1028	34.1 (6.5)	11.7 (10.1)	15.3 (11.9)	5.9 (8.4)
				23.5–45.7	0–27.0	0–27.4	0.3–18.8
γ-Terpinene	1225	1233	1056	1.0 (2.1)	0.6 (0.3)	0.3 (0.3)	0.2 (0.1)
				0.2–9.9	0.2–1.15	0–0.8	0–0.26
p-Cymene	1246	1256	1022	1.1 (1.0)	1.0 (0.5)	0.5 (0.4)	0.3 (0.1)
				0.2–4.8	0.2–2.0	0.1–1.7	0.1–0.5
cis-Sabinene hydrate	1445	1452	1064	18.0 (9.1)	36.8 (6.8)	4.6 (7.8)	8.9 (10.2)
				1.2–27.6	25.2–48.8	0.7–22.6	0.3–22.3
Linalyl acetate	1527	1548	1256	1.0 (1.2)	0.5 (0.5)	5.7 (2.4)	0.2 (0.2)
				0–4.5	0–2.1	2.5–9.3	0–0.7
Linalool/*trans*-sabinene hydrate	1529	1540/1532	1099/1096	15.6 (11.3)	15.4 (5.7)	61.0 (16.7)	6.2 (3.3)
				1.5–43.2	7.1–28.2	28.3–85.3	1.2–9.8
β-Caryophyllene	1558	1572	1416	1.5 (0.6)	2.0 (0.6)	1.7 (0.9)	0.9 (0.4)
				0.4–3.3	1.1–3.0	0.8–3.5	0.5–1.5
Terpinen-4-ol	1572	1583	1175	2.1 (1.4)	2.8 (1.5)	0.3 (0.6)	0.4 (0.6)
				0.1–4.7	0.7–5.9	0–1.6	0–1.2
α-Terpinyl acetate	1664	1676	1348	0.8 (0.5)	1.2 (0.4)	0.5 (0.3)	51.1 (12.5)
				0–2.0	0.6–1.8	0.00–1.2	37.5–70.6
α-Terpineol	1671	1678	1188	4.8 (0.8)	3.7 (0.6)	2.2 (1.0)	16.2 (4.8)
				3.2–6.1	2.4–4.9	0.4–3.5	11.0–22.5
Geranyl acetate	1730	1744	1384	0.1 (0.1)	0.2 (0.1)	0.2 (0.2)	0.1 (0.1)
				0–0.3	0.1–0.4	0–0.6	0–0.2
Myrcen-8-yl acetate	1737	1754	1348	3.1 (2.1)	5.8 (2.9)	1.1 (2.9)	2.4 (2.1)
				0–6.4	0.4–11.9	0–9.3	0–5.6
Geraniol	1829	1832	1255	0.3 (0.2)	0.3 (0.1)	0.1 (0.0)	0.1 (0.1)
				0–0.6	0–0.5	0–0.2	0–0.2
Myrcen-8-ol	1862	1867	1226	5.3 (3.1)	8.8 (1.7)	1.4 (3.2)	2.7 (2.0)
				0–10.3	5.6–12.9	0–9.4	0–5.2
Thymol	2167	2162	1292	1.5 (4.4)	0.4 (0.2)	1.5 (1.4)	0.2 (0.2)
				0–20.0	0.1–0.9	0.04–4.0	0–0.5
Carvacrol	2192	2187	1301	0.2 (0.5)	0 (0.1)	0.3 (0.3)	0.3 (0.3)
				0–1.7	0–0.4	0–0.6	0–0.7

Chemotypes represented by: E, 1,8-cineole; U, *cis*-sabinene hydrate; L, linalool; A, α-terpinyl acetate. SD, standard deviation.

1 = 41.3%; factor 2 = 21.5%; factor 3 = 16.2%; and factor 4 = 9.8%). Each factor described one of the four chemotypes present. Factor 1 was characterized by heavy loadings from the monoterpenes *cis*-sabinene hydrate, myrcen-8-ol, myrcen-8-yl acetate and myrcene, and can be described as a *cis*-sabinene hydrate chemotype factor. When initially described in the mid-1960s, the sabinene hydrate chemotype of *T. vulgaris* was thought to contain predominantly the *trans* isomer.[27] This designation was based upon the relationship of the methyl and isopropyl groups,[28] which occur at carbons one and four, respectively, of the five-membered ring. However, the carbon containing the methyl group also has a hydroxyl group present. Current IUPAC rules give priority to the hydroxyl group over the methyl, effectively reversing the former stereochemical designations, resulting in *cis* being the dominant isomer in *T. vulgaris* and probably most other *Thymus* species.[6,29] Factor 2 represented a linalool chemotype factor with high loadings from linalool and linalyl acetate. α-Terpineol and α-terpinyl acetate loaded the highest on factor 3, making it an α-terpinyl acetate chemotype factor, due to the high amounts of α-terpinyl acetate present (Table 1). Factor 4 was an 1,8-cineole factor with only that terpene showing a substantial loading (factor score of 0.81, with all other terpene scores below 0.13).

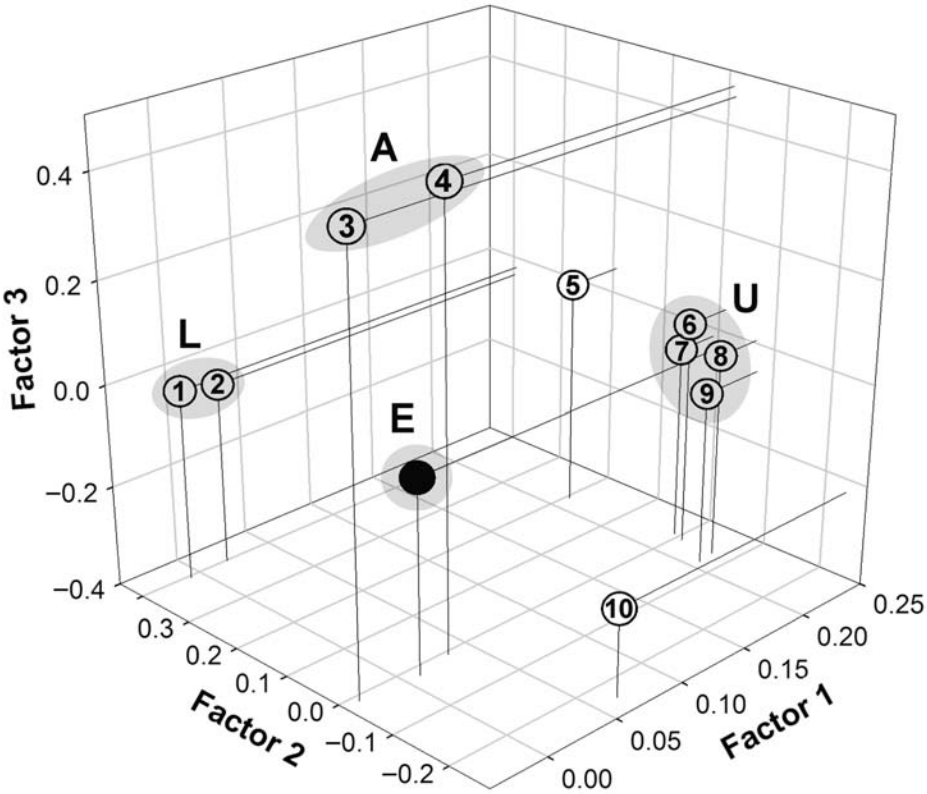

Figure 1. The first three factor scores of 11 main terpenes found in the four *Thymus vulgaris* chemotypes from the Fraicinède site in southern France. Black circle, 1,8-cineole; 1, linalyl acetate; 2, linalool; 3, α-terpineol; 4, α-terpinyl acetate; 5, β-caryophyllene; 6, myrcen-8-yl acetate; 7, myrcene; 8, myrcen-8-ol; 9, *cis*-sabinene hydrate; 10, terpinen-4-ol. The grey ellipses represent the four chemotypes found at the site: E, 1,8-cineole (eucalyptol); U, *cis*-sabinene hydrate; L, linalool; and A, α-terpinyl acetate

The relationships between the 11 terpenes subjected to factor analysis were clearly apparent when the first three factors scores of each terpene, which together explained 78.5% of the total variation, were plotted (Figure 1). The L chemotype was graphically represented by linalool and linalyl acetate, which clustered close to one another. The A chemotype was shown by the close association of α-terpineol and α-terpinyl acetate. Unlike the linalool plants, where linalyl acetate was present at much lower levels than its parent compound, these plants contained 2.1–4.0 times more α-terpinyl acetate than α-terpineol (Table 1). This pattern also occurs in other thyme species. Mockute and Bernotiene[30] found individuals of *T. pulegioides* that contained approximately 21 times more α-terpinyl acetate than α-terpineol. In *T. vulgaris*, the ratio of these compounds may be quite variable, as other putative α-terpineol chemotype individuals analysed by authors contained almost equal amounts of both monoterpenes, or greater levels of α-terpineol (K. Keefover-Ring and Y. B. Linhart, unpublished data). The terpenes *cis*-sabinene hydrate, myrcen-8-ol, myrcen-8-ol acetate and myrcene all clustered tightly together, illustrating their co-occurrence in plants of the U chemotype. Terpinen-4-ol, a compound usually associated with the U chemotype,[8,31,32] did not load particularly high on any of the factors and was isolated from the other U chemotype terpenes. The single sesquiterpene β-caryophyllene was also graphically separated from most of the other compounds, but showed some affinity to the U chemotype. Finally, the plot of the first three factor scores showed 1,8-cineole to be in an intermediate position among the other terpenes, emphasizing its occurrence in many of the plants tested, regardless of assigned chemotype.

The discovery of a new thyme chemotype in an area studied for so many years is surprising, especially given that almost 350 populations of thyme have been sampled.[33,34] The 1,8-cineole chemotype of *T. vulgaris* was already known to be present in Spain,[18,19] including a geographical subspecies occurring in north-eastern Spain.[35] In southern France, this chemotype may represent a recent mutation in *T. vulgaris*, hence its absence from studies carried out in the 1970s,[12,20] and the occurrence of some plants with a non-negligible proportion of 1,8-cineole (up to 21%) in the late 1990s.[8] In addition, this chemotype may have evaded detection in France, due to its rarity. Observations in the study region have not revealed the presence of this chemotype other than at the study site, except for a single thyme population < 2 km from the Fraicinède site, also known to contain U and L chemotypes, which has plants with very small amounts of 1,8-cineole in their oil.[8] A slow expansion of a new mutant has been proposed for other novel labiate chemotypes with a restricted range.[4]

Where the 1,8-cineole chemotype fits within the biosynthetic or genetic relationship of the other six *T. vulgaris* chemotypes found in southern France remains unknown. Wise *et al.*[36] demonstrated that while α-terpineol is involved in the production of 1,8-cineole in another labiate, *Salvia officinalis*, α-terpineol only occurs as an intermediate bound to the synthase active site. Furthermore, the factor analysis results showed a low correlation between α-terpineol and 1,8-cineole in thyme plants (Figure 1). Given that 1,8-cineole occurred with all of the other main chemotype monoterpenes, it is possible that this compound may be independent of the genetic control of the other six chemotypes.

Further studies using this new chemotype in controlled crosses would help answer this question.

The results of this work help refine the concept of plant chemotype. Twenty of the plants analysed were categorized as 1,8-cineole chemotypes, due to their high levels of this terpene. However, the results showed high variation in these plants with respect to the next most abundant terpene present (Table 1, Figure 1). This means that while two plants may share the same chemotype designation by name, they could have very different influences on herbivores, parasites or even pollinators, due to very different secondary and tertiary components. For instance, the 1,8-cineole *T. vulgaris* chemotype plants described in this study differ from those of the same species found at other locations. Blazquez and Zafrapolo[35] reported the analysis of an individual *T. vulgaris* ssp. *aestivus* whose essential oil consisted of 22% 1,8-cineole but also had high levels of geraniol (17%) and geranyl acetate (20%). These two terpenes were not found in any appreciable amount in the 1,8-cineole plants we tested (Table 1). In an ecological context, 1,8-cineole chemotype plants of these two profiles could have very different influences on other community members, since specific mixtures of secondary compounds can have different effects on organisms, due to the synergy of particular compounds together,[37,38] or it may be the minor component of a plant's secondary chemistry that acts as a deterrent or attractant.[39,40]

While most previous work describing 1,8-cineole chemotypes in *T. vulgaris* have used steam distillation for essential oil characterization, overall, the results from this study using ethanol extraction agree with earlier research. Percentages of the dominant monoterpenes in both the 1,8-cineole and linalool chemotypes of *T. vulgaris* reported by Torras *et al*.[18] and in the 1,8-cineole chemotype analysed by Blazquez and Zafrapolo[35] showed very similar amounts and ranges as found in this current study. This was also the case with the percentages of α-terpinyl acetate from individuals of another *Thymus* species.[30] In addition, when steam distillation and ethanol extraction methods were carried out on parallel samples of another aromatic plant, *Melaleuca alternifolia*, quite comparable levels of 1,8-cineole were recovered from the leaves.[41]

Dr Rolf Santesson, together with his son Johan, coined the word 'chemotype' in 1968[42] (R. Santesson, personal communication), defining the term as '. . . chemically characterized parts of a population of morphologically indistinguishable individuals'. Since that time the idea of assigning plants to specific chemotypes based on their secondary chemistry composition has been a useful convention for natural product chemists and chemical ecologists alike. However, we must be aware that this can be a very qualitative assessment of an individual's chemical profile, under which may be hiding significant chemical diversity. The use of multivariate analysis to assign chemotypes may help to mitigate this problem, since a more complete picture of the components of a particular chemotype is presented with this method.

Acknowledgements

Kasey Barton and Deane Bowers gave helpful input on early drafts of this paper. We also thank Dr Rolf Santesson and Dr Roland Moberg for their help in confirming Dr Santesson's origination of the term 'chemotype', and Chicita Culberson for helping to identify his original publication. Funding was provided by the NSF (Grant Nos DEB 0091385 and DBI 0500560, to Y.B.L.) and logistic support from CNRS-CEFE.

References

[1] J. D. Gbenou, M. Moudachirou, J. C. Chalchat, G. Figueredo, *J. Essent. Oil Res*. **2007**, *19*, 101–104.
[2] P. Grassi, M. J. Nunez, K. Varmuza, C. Franz, *Flavour Frag. J*. **2005**, *20*, 131–135.
[3] G. Agostini, F. Agostini, L. Atti-Serafini, S. Echeverrigaray, *Biochem. Syst. Ecol*. **2006**, *34*, 802–808.
[4] H. H. Marshall, R. W. Scora, *Can. J. Bot*. **1972**, *50*, 1845–1849.
[5] U. Ravid, E. Putievsky, *Planta Med*. **1985**, *51*, 337–338.
[6] E. Stahl-Biskup, F. Saez (eds). *Thyme: The Genus* Thymus. Taylor and Francis: London, **2002**.
[7] D. Vokou, S. Kokkini, J. M. Bessiere, *Biochem. Syst. Ecol*. **1993**, *21*, 287–295.
[8] J. D. Thompson, J. C. Chalchat, A. Michet, Y. B. Linhart, B. Ehlers, *J. Chem. Ecol*. **2003**, *29*, 859–880.
[9] J. D. Thompson, D. Manicacci, M. Tarayre, *Bioscience* **1998**, *48*, 805–815.
[10] J. D. Thompson. In *Thyme: The Genus Thymus. Population Structure and the Spatial Dynamics of Genetic Polymorphism in Thyme*, E. Stahl-Biskup, F. Saez (eds). Taylor and Francis: London, **2002**, 44–74.
[11] J. D. Thompson, *Plant Evolution in the Mediterranean*. University of Oxford Press: Oxford, **2005**.
[12] P. Vernet, P. H. Gouyon, G. Valdeyron, *Genetica* **1986**, *69*, 227–231.
[13] R. Granger, J. Passet, J. P. Girard, *Phytochemistry* **1972**, *11*, 2301–2305.
[14] A. J. Poulose, R. Croteau, *Arch. Biochem. Biophys*. **1978**, *187*, 307–314.
[15] A. J. Poulose, R. Croteau, *Arch. Biochem. Biophys*. **1978**, *191*, 400–411.
[16] J. Kaloustian, L. Abou, C. Mikail, M. J. Amiot, H. Portugal, *J. Food Sci. Agric*. **2005**, *85*, 2437–2444.
[17] E. Stahl-Biskup, In *Thyme: The Genus Thymus. Essential Oil Chemistry of the Genus Thymus—A Global View*, E. Stahl-Biskup, F. Saez, (eds). Taylor and Francis: London, **2002**, pp. 75–124.
[18] J. Torras, M. D. Grau, J. F. Lopez, F. X. de Las Heras, *J. Food Sci. Agric*. **2007**, *87*, 2327–2333.
[19] M. D. Guillen, M. J. Manzanos, *Flavour Frag. J*. **1998**, *13*, 259–262.
[20] T. Adzet, R. Granger, J. Passet, R. San Martin, *Biochem. Syst. Ecol*. **1977**, *5*, 269–272.
[21] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th edn. Allured: Carol Stream, IL, **2007**.
[22] J. A. Byers, F. Schlyter, G. Birgersson, W. Francke, *Experientia* **1990**, *46*, 1209–1211.
[23] B. Delpit, J. Lamy, F. Rolland, J. C. Chalchat, R. P. Garry, *J. Essent. Oil Res*. **2000**, *12*, 387–391.
[24] W. Jennings, T. Shibamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*. Academic Press: San Francisco, CA, **1980**.
[25] N. W. Davies, *J. Chromatogr*. **1990**, *503*, 1–24.
[26] SAS Institute. *SAS Version 9.1*. SAS Institute: Cary, NC, **2003**.
[27] R. Granger, J. Passet, M.-C. Pinede, *C. R. Acad. Sci. Paris Ser. D* **1968**, *267*, 1886–1889.
[28] W. F. Erman, *Chemistry of the Monoterpenes: An Encyclopedic Handbook*, vol. B. Marcel Dekker: New York, **1985**.
[29] E. Groendahl, B. K. Ehlers, K. Keefover-Ring, *J. Essent. Oil Res*. **2008**, *20*, 40–41.
[30] D. Mockute, G. Bernotiene, *Biochem. Syst. Ecol*. **2001**, *29*, 69–76.
[31] R. Granger, J. Passet, *C. R. Acad. Sci. Paris Ser. D* **1971**, *273*, 2350–2353.
[32] J. Passet, *Thymus vulgaris* L.: Chémotaxonimie et Biogénèse Monoterpénique. Doctoral Thesis, Faculte de Pharmacie: Montpellier, France, **1971**.
[33] P. H. Gouyon, P. Vernet, J. L. Guillerm, G. Valdeyron, *Heredity* **1986**, *57*, 59–66.
[34] P. Vernet, J. L. Guillerm, P. H. Gouyon, *Oecolog. Plantar*. **1977**, *12*, 159–179.
[35] M. A. Blazquez, M. C. Zafrapolo, *Pharmazie* **1990**, *45*, 802–803.
[36] M. L. Wise, M. Urbansky, G. L. Helms, R. M. Coates, R. Croteau, *J. Am. Chem. Soc*. **2002**, *124*, 8546–8547.
[37] R. G. Cates. In *Recent Advances in Phytochemistry, vol. 30, Phytochemical Diversity and Redundancy in Ecological Interactions*.

The Role of Mixtures and Variation in the Production of Terpenoids in Conifer–Insect–Pathogen Interactions, J. T. Romeo, J. A. Saunders, P. Barbosa (eds). Plenum: New York, **1996**, pp. 179–216.
[38] J. H. Langenheim, *J. Chem. Ecol*. **1994**, *20*, 1223–1280.
[39] D. C. Robacker, *J. Appl. Entomol*. **2007**, *131*, 202–208.
[40] W. N. Setzer, S. L. Stokes, A. Bansal, W. A. Haber, C. R. Caffrey, E. Hansell, J. H. McKerrow, *Nat. Prod. Commun*. **2007**, *2*, 685–689.
[41] G. R. Baker, R. F. Lowe, I. A. Southwell, *J. Agric. Food Chem*. **2000**, *48*, 4041–4043.
[42] R. Santesson, *Svensk Naturvetenskap* **1968**, *21*, 176–184.