Analysis of Juniper and Other Forest Tree Oil

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1 Introduction

Concurrent with the development of commercial gas chromatography in the late 1950's and early 1960's, botanists and chemists began to realize the value of a "new" suite of characters for the analysis and classification of plants — the terpenoids. Not only are the terpenoids under strong genetic control (Irving and Adams 1973), but the use of electronic digital integrators give quantitative characters amenable to multi-variate and geographic analyses (Adams 1970a; Adams 1972a). Given this "new" suite of quantitative chemical characters, the field of terpenoid chemosystematics rapidly expanded during the next two decades. The purpose of this chapter is to orient the novice to some of the basic procedures of analyses of terpenoids (particularly steam volatile components) and to show the utility of these compounds for the analyses of patterns of variation within and among forest tree taxa.

2 Sample Collection

Because the collection of wood samples has been examined in the chapter on cedarwood oil (Adams. this Vol.), I shall refer the reader interested in wood oils to that section. One should note that the wood and leaf oils are very different in *Juniperus* (and other Cupressaceae genera), whereas this is not the case in the pines, for example.

2.1 Sampling

Several general principles have been discovered over the past two decades in sampling for *Juniperus*, other conifers, and many (most?) forest trees. A preliminary study is generally needed if no work has been done on the genus or species of interest. One should take, for example, 3 (–5) leaf samples (100–200 g each) from each ordinal direction, from old and new foliage types (if present). Analyses of these 24 (–40) samples (3 reps, 4 sides, old/new leaves) will give an estimate of the within-tree variation. One can then usually decide if exposure, foliage type, etc. is a major factor (of course more samples may be needed depending on variability). In any case, it is desirable to get into the habit of always sampling in the same manner. For example, I collect eight to ten branchlets (15–20 cm long) for each juniper sample for steam distillation (plus material for herbarium

voucher), from about chest high on the south-facing side of the tree. I collect these eight to ten branchlets from at least three major limbs. Fruiting structures (cones in the case of conifers) should be separated from the foliage before analyses, as they often differ in their volatile oil from the foliage (Hernandez et al. 1987; Vernin et al. 1988).

The investigator is faced with two options at this point: keep the foliage fresh until distillation or dry the foliage and extract it in an air-dried condition. The vast majority of researchers have chosen to keep the foliage fresh because some portion of the more volatile monoterpenes would be lost upon drying. Whether you can obtain reproducible results from dried foliage must be determined by doing a test on the species of interest.

With the junipers, we have found no difficulties in keeping the foliage at room temperature for a few days. Normally, we freeze the foliage as soon as possible and thaw it just before (or during) distillation. This may not be possible for broadleafed deciduous trees, as the thin leaves tend to freeze together and stick together in a ball during distillation, resulting in poor extraction efficiency (see discussion on extraction below).

2.2 Sample Sizes

Sample sizes depend on the purpose of the study and on the variation among samples. For example, if the study is at the species level or higher, one generally finds qualitative differences, and numerous samples are not necessary. On the other hand, the examination of geographic variation or changes with seasons usually involves quantitative changes in composition, and larger sample sizes are needed. It is interesting to note that sampling 10 and then 20 *Juniperus virginiana* trees per population on successive years yielded the same geographic trends (Flake et al. 1969; 1973). Furthermore, the same geographic trend was found in *J. ashei* when using five samples as when using 15 samples per population (Adams and Turner 1970; Adams 1975a). It should be noted that both *J. ashei* and *J. virginiana* are dioecious (male and female trees), wind pollinated species. Different results might be expected with monecious and/or self-compatible species.

2.3 Diurnal, Seasonal, and Ontogenetic Variation

An important consideration in sampling is to collect materials that are comparable. Because one cannot make all the collections on one day at the same time, diurnal, seasonal, and ontogenetic variations must be considered.

2.3.1 Diurnal Variation

Hopfinger et al. (1979) found significant diurnal variation in leaf oil of Valencia orange trees. This variation is influenced by photosynthesis during the day (Fretz 1976); Lincoln and Langenheim 1977). In a study of diurnal variation in *J.*

scopulorum, analysis of 37 terpenoids revealed that 36 differed between trees, 11 varied between days, and 13 showed significant diurnal variation (Adams and Hagerman 1977). Four of these compounds are shown in Fig. 1. Notice that sabinene and methyl citronellate show patterns of gradual decline during the day into early evening (Fig. 1). In contrast, linalool, 4-terpineol, the eudesmols, elemol, etc., show increases starting at about 80% of their maximal values at 09.00 h and reaching their maximum values during the early evening, thence declining during the remainder of the night (Fig. 1). In order to judge the effects of these variations on genotype analyses, all of the 56 samples (four trees, seven sampling periods, 2 days) were subjected to principal coordinate analysis and the samples were found to cluster by genotypes (Fig 2). Thus, for *J. scopulorum*, it appears that the terpenoids are useful for the analysis of diurnal metabolic variation, but these variations do not mask the tree-to-tree variations. This study of diurnal variation was continued during the winter, using the same four trees of *J. scopulorum* (Adams 1979), and only one significant difference was found in the winter compared to 13 significant differences in diurnal variation in the summer. It was concluded that sampling during the dormant season (winter in that case) would greatly minimize or eliminate diurnal variation in *Juniperus* (and other conifers).

2.3.2 Seasonal Variation

Although wide seasonal variation of terpenoids is well known in herbaceous annuals such as the mints (Burbott and Loomis 1969) and sage (Fluck 1963), seasonal variation in the conifers is apparently much less. Von Rudloff (1972) examined the changes in volatile oils in leaves, buds, and twigs of white spruce

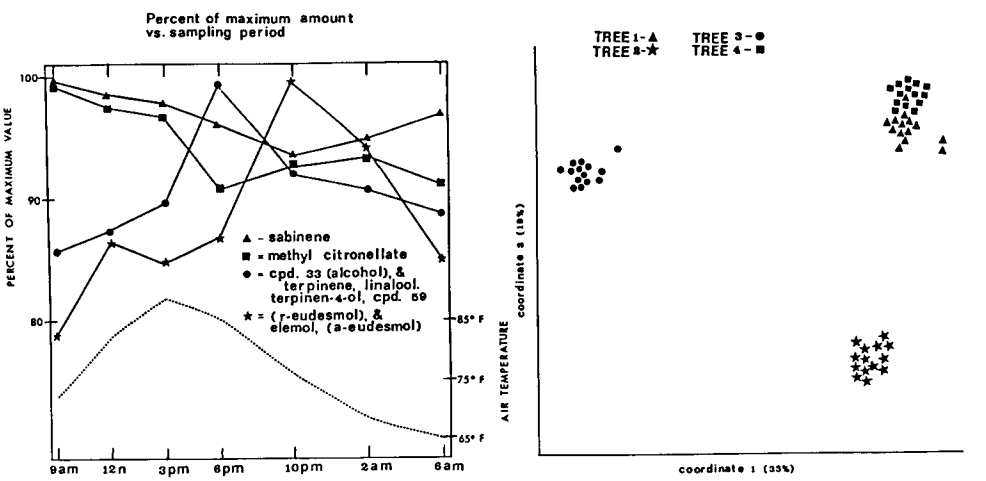
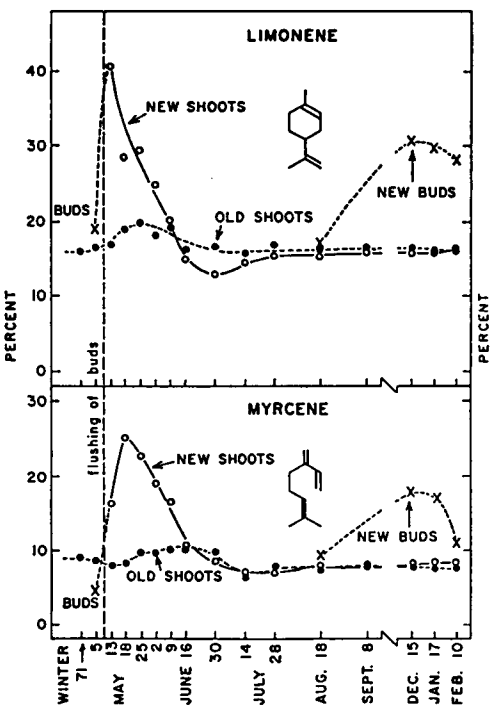


Fig. 1. *Left* diurnal variation in the percent maximum for four terpenoids representing the major trends in *J. scopulorum*. (Adams and Hagerman 1977)

Fig. 2. *Right* principal coordinate analysis for four *J. scopulorum* trees sampled 13 times over 2 days. Notice that the samples cluster by genotype, not by time sampled. (Adams and Hagerman 1977)

(*Picea glauca*). Figure 3 shows the seasonal variation in limonene and myrcene. The variation in camphor and bornyl acetate is depicted in Fig. 4. Notice that in all cases, the old shoots (2- to 4-year-old needles) are very constant throughout the year, with only a minor fluctuation during the onset of the growing season (May-June, Figs. 3,4). Sampling during the slow or no-growth time of the year (July-winter, in this case) effectively minimizes variation in white spruce. Seasonal variation in *Juniperus pinchotii* (Adams 1970b) indicated significant differences between summer and winter samples and that the variance among the summer samples was greater than the variance during the winter sampling. Adams (1970b) concluded that winter sampling of *J. pinchotii* was preferred. Analysis of seasonal variation in the terpenoids of *J. scopulorum* (Powell and Adams 1973) revealed significant seasonal variation and that seasonal variation was greater for components calculated on a weight basis (mg/g) than when calculated on a relative percent of the total oil basis. The use of relative percentage data was thus



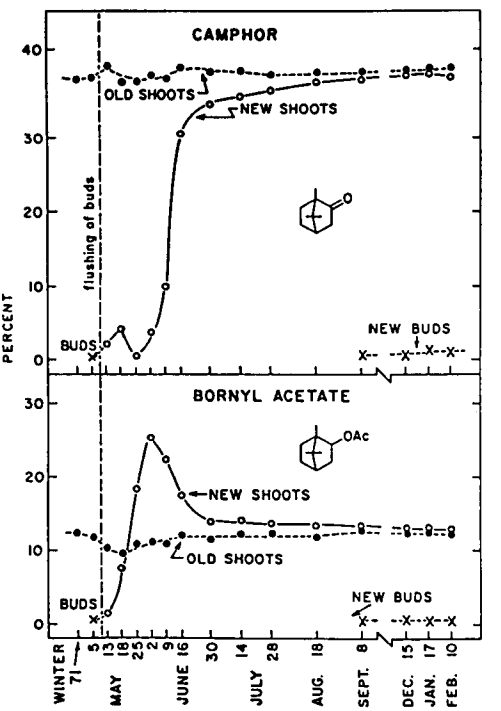


Fig. 3. *Left* changes in the relative percentage of limonene and myrcene in young and older white spruce (*Picea glauca*) shoot (needles) and buds. (von Rudloff 1972)

Fig. 4. *Right* comparisons of seasonal and ontogenetic variations in camphor and bornyl acetate from old and new shoots (needles) and buds in white spruce. (von Rudloff 1972)

encouraged for chemosystematic studies, although the weight basis would be preferred for biosynthesis studies.

Two recent papers deserve mention because they deal with seasonal variation in the terpenes of tropical rain forest trees. Whiffin and Hyland (1989) examined seasonal variation in *Litsea leefeana* and found that the three trees sampled showed no defined seasonal pattern (Fig. 5), with each tree showing its own pattern. Multi-variate clustering of the samples revealed that the terpenoid samples cluster by tree (Fig. 6). Whiffin and Hyland (1989) concluded that seasonal variation was not a problem in sampling Australian rain forest trees for volatile oil composition studies. A similar study on the Australian tree, *Angophora costata* (Leach and Whiffin 1989) concluded that quantitative changes in volatile oil composition over seasonal and diurnal periods were not significant in terms of chemosystematics studies, if immature leaves were excluded from the samples.

Nevertheless, it is prudent to minimize seasonal variation by sampling during the dormant (or least-growth) season, whether this be due to temperature or rainfall (i.e., samples taken during the middle or end of the driest season are least likely to be affected by metabolic changes).

2.3.3 Ontogenetic Variation

As previously mentioned, von Rudloff (1972) showed, rather dramatic changes in the volatile oils of new shoots (needles) in white spruce (Figs. 3, 4). In *Juniperus scopulorum* the young leaves (current new growth) were found to differ quantitatively from the mature leaves (Adams and Hagerman 1976) for 19 of 36 terpenoids

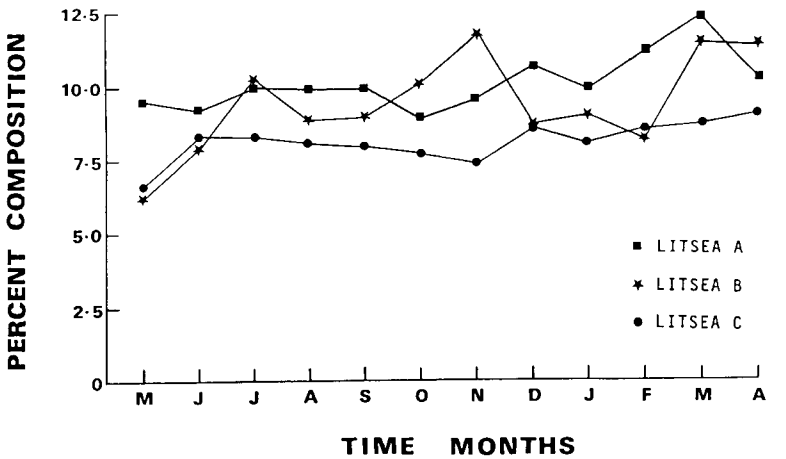


Fig. 5. Annual variation in a single terpenoid in three trees of *Litsea leefeana* in Australia. Note the rather constant, parallel levels from June to October, the Australian winter, and the larger variation during the rest of the year when growth is occurring. (Whiffin and Hyland 1989)

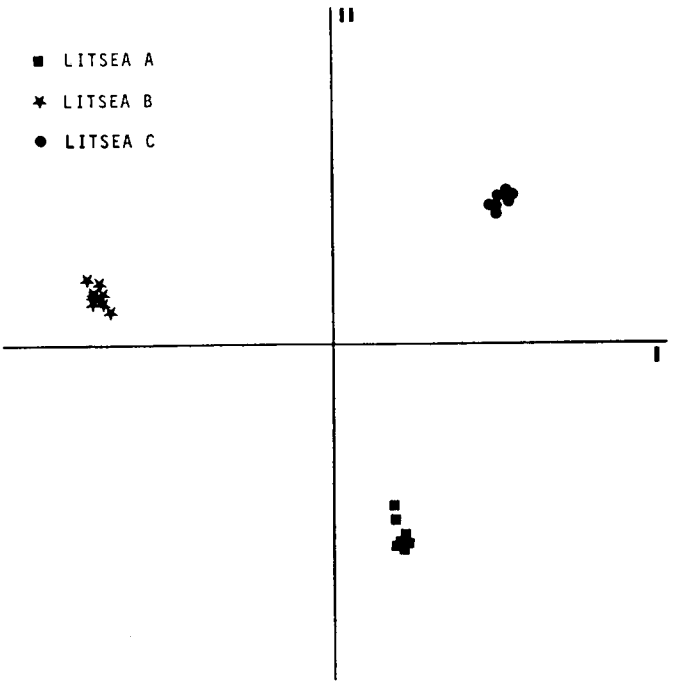


Fig. 6. Principal coordinate analysis of all the monthly samples of the three *Litsea leefeana* trees (graphed in Fig. 5). All of the monthly samples cluster by one of the three genotypes (*A*,*B*,*C*), not by month of collection. (Whiffin and Hyland 1989)

(Fig. 7). The elimination of juvenile or new growth from juniper leaf samples was recommended. However, analysis of the oil from juvenile and mature leaves of *J. horizontalis* (Adams et al. 1981c) revealed no significant differences in 39 terpenoids. Canonical variate analysis of the terpenoids of *J. scopulorum* and *J. virginiana* along with the mature foliage of *J. horizontalis* and coplotting an individual using the terpenoids from its juvenile leaves did not blur taxonomic distinctions (Fig. 8). Notice that individual 5A (adult leaves) and 5J (juvenile leaves) ordinate very much in the same position (Fig. 8).

Because almost all junipers are dioecious (male and female), an obvious question is "do males and females differ in their volatile leaf oils?". Examination of the oils from male and female *J. scopulorum* plants indicated (Adams and Powell 1976) that sexual differences are apparent during spring (rapid growth) but essentially no differences were found during the winter. Part of the differences in the spring may be due to the difficulty of finding and removing all of the small female cones, which may contribute a different oil profile. If one is working on a unisexual plant, this factor should be considered, but seems of no consequence in the junipers.

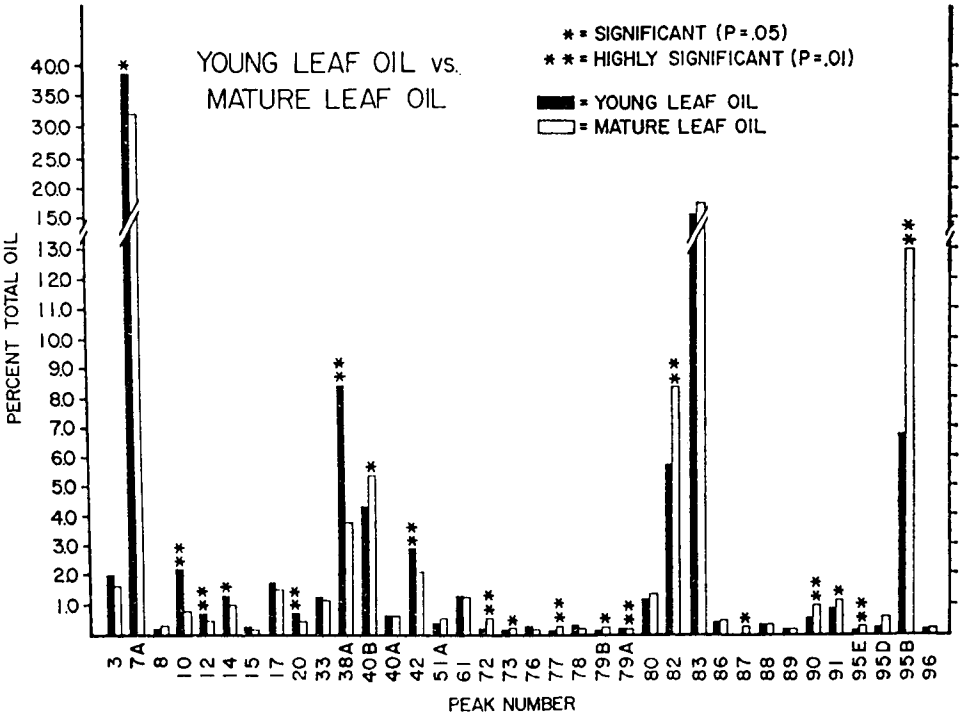


Fig. 7. Comparison of the major compounds in the leaf oils of young (juvenile) and mature (2–5 years old) leaves of *J. scopulorum* cv. *platinum* (Adams and Hagerman 1976)

For tropical evergreen trees, such as *Angophora costata*, Leach and Whiffin (1989) found that immature leaves must be eliminated from samples. That would appear to be good general advice.

One environmentally induced growth form of juniper has been analyzed. Near a natural burning coal vein in North Dakota, USA, the junipers are all very columnar in shape. These junipers were recognized (Fassett 1945) as a variety (*J. scopulorum* var. *columnaris* Fassett). Examination (Adams 1982a) of the volatile leaf oils from columnar and nearby, pyramidal (normal shaped) *J. scopulorum* trees revealed only one significantly different terpenoid. Canonical variate analysis showed that both the columnar and pyramidal *J. scopulorum* trees cluster together (Fig. 9). However, few transplant studies have been done where the terpenoids of conifer trees have been subsequently analyzed, so some caution must be advised in regards to the effects of edaphic factors on terpenes in trees.

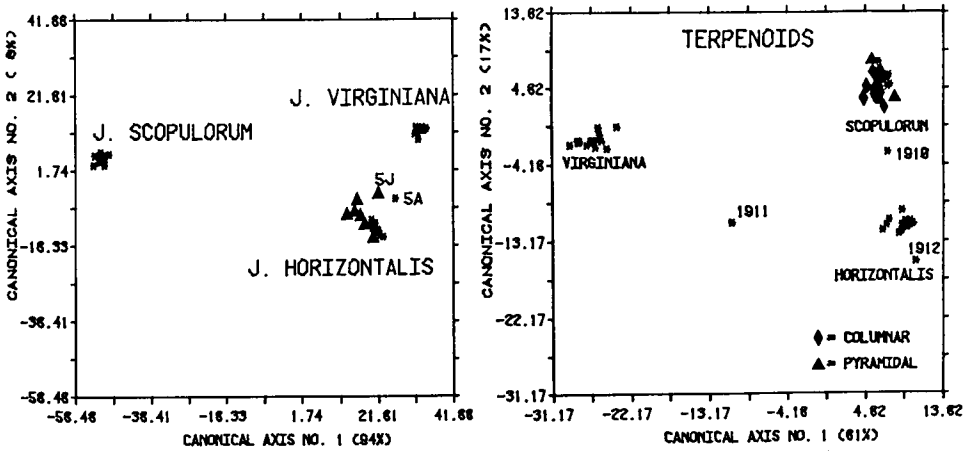


Fig. 8. *Left* canonical variate analysis of the volatile leaf oil from mature (*stars*) and juvenile foliage samples (*triangles*) of *J. horizontalis* co-analyzed with the oils from mature leaves of *J. scopulorum* and *J. virginiana*. Plant 5 (*5A* adult leaves; *5J* juvenile leaves) shows differentiation towards *J. virginiana*. (Adams et al.1981c)

Fig. 9. *Right* canonical variate analysis of the leaf terpenoids from columnar (caused by fumes from burning coal seams) and pyramidal-shaped (normal form) *J. scopulorum*. (Adams 1982a)

3 Oil Extraction

If wood is to be extracted, the reader is referred to the chapter on cedarwood oil (Adams, this vol.). Otherwise, I shall consider extraction of foliage. Providing some precautions are taken (see Adams, this Vol., notes on pH, etc.), steam distillation is a very useful method to obtain a good sample of the volatile oil. One might note, however, that if biosynthetic and/or physiological problems are to be addressed, one may want to treat the aqueous phase with β-glucodidase to release glycosidic bound terpenes (van den Dries and Svendsen 1989) to obtain a more quantitative estimate of the terpenes in the plant. The volatile oil can also be extracted by super critical fluids or solvent methods to minimize degradation (Cu et al. 1989). Recently, Craveiro et al. (1989) reported on the use of a microwave for steam distillation in 5 min. Although the method appears to be qualitative at present, further development could lead to quantitatively consistent extractions.

Assuming that steam distillation is used, one may want to separate the leaves from the woody stems, although this is not necessary in junipers because the small stems (3-8 mm diam.) do not contribute any quantity of oil (however, the weight of the stem wood may be very significant in computing yield data!). Normally, for junipers, we take a handful and orient all the stems downward to aid in the distribution of the upward steam column (see extraction apparatus in Adams, this Vol.). With junipers there is no need to cut up the leaves, but this is useful in conifers with resin ducts such as pine needles. Generally cutting the needles into 2-cm sections is sufficient.

Steam distillation for 2 h removes about 35% of the volatile oil in junipers and 24 h removes 95% of the oil. We normally do both. We remove the ether layer (see apparatus, Adams, this Vol.) after 2 h (and add new ether). The 2-h fraction is used for chemosystematic studies. After 24 h (22 h of additional distillation) we remove the ether layer. The excess ether from both fractions is evaporated in a hood with a jet of nitrogen. The samples are then weighed and the combined weights (i.e., 24 h total) are used to calculate the yield (see section on sample handing in Adams, this Vol.). After steam distillation, the foliage is placed in a tared, paper sack and dried for 48 h at 100 °C to determine dry wt. Oil yield is calculated as: oil wt./(dry distilled leaf wt. + oil wt.). Leaves that are dry and fragile or that have been frozen and thawed before distillation (and thus tend to stick together in a ball) are put into a nylon mesh bag, which is then put into the distillation chamber (see Adams, this Vol.). This prevents loose material from falling into the boiling water and/or clogging the steam intake. The reader is referred to Adams (this Vol.) for sample storage.

4 Chemical Analysis

The primary method for analysis is by gas chromatography either coupled to an electronic digital integrator or a computer.

4.1 Gas Chromatography

Gas chromatography has become an integral part of any essential oil analysis today. Bonded phase fused silica (or quartz) capillary columns, externally coated with polyamide resins to reduce breakage became commercially available about 1980. Third party manufacturers produce conversion kits so 1/4" injectors can be converted for use with capillary columns. One is almost without excuse for not converting to bonded phase, fused silica capillary columns today. For the nonpolar and moderately polar phases, the durability is tremendous. Columns last for years and can be washed out with solvents to regenerate them. Unfortunately, we cannot say that for the most popular phase for essential oil analyses, Carbowax 20M (PEG 20M). Our experience with the bonded Carbowax columns has been that the phases can be damaged easily by very small oxygen leaks and that the columns tend to change retention characteristics with age (Jennings 1978; Jennings and Shibamoto 1980; Adams 1980a) We have converted all of our primary analyses to a J & W DB-5, 30 m, 0.26 mm i.d., 0.25 micron coating thickness. The DB-5 phase is bonded methyl silicone with slight polarity and is equivalent to: BP-5; CP-Sil-8CB; DC-200; DC-560; Dexsil 300; GB-5; OV-3; OV-73; SE-52; SE-54; SPB-5; Ultra 2 and 007-2. Because extremely reproducible retention times (+/–2 s) are essential for identification of most terpenoids using GC/MS data systems (Adams 1989a), one's libraries become very valuable with time. If your column is changing characteristics, your library will not be useful. We have found less lot-to-lot

variation among new (i.e., replacement) commercial nonpolar columns than polar capillary columns. Another plus for the nonpolar bonded columns is that analysis can be performed up to 350 °C, so phyto-steroids, fatty acids, and waxes can be analyzed on the same column.

4.1.1 Carrier Gas

Much has been written about the theoretical efficiencies of carrier gases (see Jennings 1987). If all other factors are kept constant, then one sees increased resolution from nitrogen (poorest), to helium to hydrogen (greatest resolution). However, many capillary gas chromatography systems are not well enough tuned to high resolution chromatography to see any difference between using helium and hydrogen as carrier gases. Hydrogen presents a problem of being explosive. Helium (zero grade) is generally expensive. Nitrogen is usually very cheap, but may be contaminated with water and oxygen. We use helium as a compromise (but mostly because we are interfaced with an Ion Trap Mass Spectrometer which requires helium). The most important consideration is: can you obtain gas that is free of water and oxygen? In any case, the carrier gas should be routed through an indicating oxygen/water trap for final cleaning. It is a good idea to have a sample of the gas analyzed for water and oxygen before deciding on a gas vender. The flow rate is easily checked by injecting butane (from a lighter recharger) or methane from a natural gas source with the GC oven at 200 °C. For helium, a flow rate of 20 to 50 cm/s is within the maximum theoretical plates range (see Jennings 1978 for theoretical efficiency plots for helium, hydrogen, and nitrogen). If your column is connected directly to the source of an ion trap, then a flow of about 1 ml/min of helium is required. A 30 m × 0.26 mm i.d. column will deliver 1 ml/min of helium at about 30–32 cm/s (well within the maximum theoretical plates range, Adams 1989a).

4.1.2 Sample Injecting

Assuming you have a capillary injector, either splitting or splitless, one needs to pay careful attention to several things. Choose the best quality septa available. Heat the injector to about 250 °C with the GC oven at room temperature overnight and then make a blank (no injection) run the next morning. This will show you your septum bleed. Remove the septum and see if it is still pliable. Keep a written tally sheet of your injections for each septum. Change the septum after about 25 injections. The bleed of oxygen through an old septum can cause severe column damage.

Sample sizes depend on the diameter and phase coating thickness of your column. For a 0.26 mm i.d., 0.25 micron coating column, we dilute the oil to 10% with ether (i.e., 90% ether, 10% oil), and inject 0.1 µl, which is split 20:1. This results in about 500 ng on column. Components that are 25% or more of the oil will be overloaded (have a leading peak edge) under these conditions, but this allows one to detect trace components. We use 1-µl syringes with the plunger in the needle and clean the syringes by first working the plunger back and forth into

acetone and then heating in a syringe cleaner (cf. Hamilton) for 45–60 s. Regardless of how one cleans the syringe, you should check the procedure occasionally by injecting the "clean" syringe into the GC and making a blank run to look for carryover.

4.1.3 Temperature Programming

The separation of individual components can be greatly aided by temperature programming. A linear temperature program of 3°/min, from 60 to 240 °C is routinely used in our laboratory. If there are almost no monoterpenoids (as in cedarwood oils), a higher initial temperature can save analysis time; however, the retention time data will then not be comparable with a general library (i.e., that includes monoterpenes). Nonlinear temperature programming can be very effective in helping resolve difficult mixtures but, again, retention data may not be comparable to your library data. In the final analysis, one must experiment with the GC system to determine the best temperature program.

4.1.4 Detection

Detection has traditionally been by use of flame ionization detection (FID). FID sensitivity does vary, roughly according to carbon number. So, if exact mole concentrations are desired, response factors must be determined for each compound in the mixture. However, because data are often converted to a range-normalized basis, one seldom sees response factors and correction factors used in chemosystematic studies.

The other classical method for detection in GC analysis is thermal conductivity detection (TCD). Unfortunately, the development of micro-thermal conductivity detectors has not seemed to keep pace with the reduction in sample sizes in recent years, and one rarely reads of TCD being used with capillary columns.

The third and perhaps most rapidly growing detector is the ion trap detector (ITD or ion trap mass spectrometer, ITMS). Direct coupling of the capillary column to the source of the ion trap provides an efficient transfer and identifications can be made in concert with GC separation (Adams 1989a). Although little has been published on quantitation using the total ion counts, our experience has been favorable (Adams and Edmunds 1989).

5 Component Identification

The oils of juniper and other forest trees can be very complex, containing hundreds of terpenoids, aromatic compounds, and occasionally important amounts of aliphatic alcohols and aldehydes and more rarely, alkanes. Although liquid phase infrared (IR) analysis was the method of choice for identification in the 1960's, the introduction of capillary columns and the attendant small sample sizes has greatly reduced the use of liquid phase IR. Vapor phase IR identification promises

to be of great use as libraries improve. However, the principal method of identification of known compounds is generally combined GC/MS or GC/MS/computer searches.

5.1 GC/MC Computer Searches

A large library of mass spectra is readily available from sources such as the US NBS (National Bureau of Standards, formerly the EPA/NIH data base) with thousands of spectra. Unfortunately, searches from these large data bases, with the current technology (i.e., simple matching coefficients and no retention data) do not yield reliable identifications (see Adams et al. 1979 for discussion). Vernin et al. (1988) report on the use of SPECMA MS data bank for the identification of terpenes of *J. communis* berries and needles which apparently utilizes both MS data and Kovats indices.

Our library system [LIBR(TP), available form Finnigan Corp.] uses ion trap mass spectra (ITMS) and retention times on DB5. Although the ITMS spectra are generally quite similar to quadrapole mass spectra (Adams 1989a), there can be significant differences, so a reference library of ion trap spectra is essential. We use cedrol for ion trap tuning because it is very sensitive to space charging effects (overloading) and tuning (Adams 1989a).

Various juniper species leaf oil compositions are given in Table 1. Hopefully, the publication of these retention times will be useful for building libraries and for identification.

6 Applications of Terpenoid Data

There are scores of applications for terpenoid data but I would like to focus on three major areas: analyses of hybridization and introgression, geographic variation, and specific or evolutionary studies.

6.1 Analyses of Hybridization and Introgression

6.1.1 Juniperus

One of the earliest cases of the use of terpenoids was for the reexamination of the classical case of putative introgressive hybridization between *J. ashei* and *J. viginiana* (Hall 1952). Subsequent studies using terpenoid data showed clinal variation in *J. virginiana* but neither hybridization nor introgression with *J. ashei* (Adams 1977; Adams and Turner 1970; Flake et al. 1973; von Rudloff et al. 1967; von Rudloff 1975).

Terpenoids have been used to document hybridization between *J. scopulorum* and *J. horizontalis* (von Rudloff 1975; Adams 1983a; Adams 1982b), between *J.*

Table 1. Representative volatile leaf oil composition for junipers (*J. communis* var. *depressa* (USA); *J. foetidissmia* (Greece); *J. flaccida* var. *martinezii* (Mexico); *J. procera* (Kenya) and *J. virginiana* var. *virginiana* (USA). Compounds are listed in order of elution from a DB5 column. Compounds in parenthesis are tentatively identified. Data expressed as % total oil using total ion counts (TIC). T = less than 0.1% of the total oil.

RT Compound	*Communis* var. *depressa*	Flaccida var. *martinezii*	*Foetidissima*	*Procera*	*Virginiana*
1. 214 2-Hexenal	T	—	0.1	0.2	T
2. 301 Tricyclene	T	0.5	—	T	T
3. 307 α-Thujene	—	0.6	1.3	T	T
4. 319 α-Pinene	14.1	13.5	2.6	12.5	1.4
5. 337 α-Fenchene	—	—	T	0.1	T
6. 340 Camphene	0.2	0.6	T	0.1	T
7. 348 Thuja-2,4(–10)-diene	T	0.1	—	—	—
8. 363 [Bicyclo(3,2,1)oct-2-ene, 3-methyl-4-methylene]	—	1.8	—	—	—
9. 379 Sabinene	0.2	8.5	19.6	T	6.7
10. 383 1-Octen-3-ol	—	—	—	0.3	—
11. 386 β-Pinene	2.1	1.1	—	1.2	T
12. 408 Myrcene	4.4	4.0	2.7	1.2	0.9
13. 427 2-Carene	T	T	—	—	T
14. 435 α-Phellandrene	T	0.9	0.2	—	—
15. 444 3-Carene	0.2	T	T	6.1	T
16. 457 α-Terpinene	T	1.1	4.3	T	T
17. 465 o-Cymene	—	—	—	T	—
18. 471 p-Cymene	T	1.2	0.5	T	—
19. 474 Sylvestrene	—	—	—	0.1	—
20. 481 Limonene	1.1	1.6	0.9	0.2	18.9
21. 482 β-Phellandrene	1.1	5.0	0.6	0.8	T
22. 485 1,8-Cineole	—	—	0.2	T	—
23. 498 *cis*-Ocimene	—	T	—	—	—
24. 519 *trans*-Ocimene	—	0.4	—	—	T
25. 535 Pentyl isobutyrate <n->	T	—	0.1	—	—
26. 545 Γ-Terpinene	T	2.0	6.5	T	T
27. 560 *trans*-Sabinene hydrate	T	0.5	1.8	—	T
28. 574 *cis*-Linalool oxide	—	T	0.1	—	—
29. 604 (Eucarvone)	—	0.1	—	—	—
30. 605 Fenchone	T	—	—	—	—
31. 608 Terpinolene	1.4	0.9	1.9	1.1	0.5
32. 609 p-Cymenene	—	0.3	—	—	—
33. 626 α-Pinene oxide	—	1.9	—	—	—
34. 629 *cis*-Sabinene hydrate	—	0.5	1.9	—	T
35. 632 Linalool	2.0	3.0	1.0	0.5	4.4
36. 642 α-Thujone	—	—	18.6	—	—
37. 643 Nonanal <n->	T	—	—	—	—
38. 645 Isopentyl-isovalerate	0.4	—	—	—	—
39. 661 1,3,8-p-Menthatriene	—	—	—	T	—
40. 664 endo-Fenchol	0.2	—	—	—	—
41. 667 β-Thujone	—	—	3.5	—	—
42. 682 *cis*-p-Menth-2-en-1-ol	T	—	1.2	T	T
43. 692 α-Campholenal	2.3	0.4	—	T	—
44. 724 *trans*-Pinocarveol	1.2	0.7	—	0.1	—

Table 1. *(continued)*

RT Compound	*Communis* var. *depressa*	Flaccida var. *martinezii*	*Foetidissima*	*Procera*	*Virginiana*
45. 725 *trans*-p-Menth-2-en-1-ol	T	—	1.2	T	T
46. 727 *cis*-Verbenol	0.6	—	—	—	—
47. 734 Camphor	—	11.4	—	0.2	3.7
48. 735 *trans*-Verbenol	3.4	0.5	—	—	—
49. 746 Camphene hydrate	1.1	—	—	—	T
50. 758 Citronellal	0.5	—	—	—	—
51. 766 β-Pinene oxide	—	0.2	0.2	—	—
52. 775 *cis*-3-Pinanone	0.4	—	—	—	—
53. 781 Pinocarvone	0.4	T	—	—	—
54. 789 Borneol	1.8	0.9	—	0.2	0.8
55. 792 p-Mentha-1,5-dien-8-ol	0.4	—	—	—	—
56. 804 Nonanol	—	—	—	T	—
57. 820 4-Terpineol	2.5	8.2	17.6	0.1	1.5
58. 837 p-Cymen-8-ol	0.3	0.4	0.1	0.1	—
59. 852 α-Terpineol	3.9	0.7	0.7	0.5	T
60. 864 Myrtenal	2.2	0.2	—	—	T
61. 865 *cis*-Piperitol	—	—	0.3	—	—
62. 867 Myrtenol	—	0.2	—	—	—
63. 869 Estragole	—	—	—	—	T
64. 887 *trans*-3-Pinanone	0.6	0.3	—	—	—
65. 894 Verbenone	0.9	0.4	—	—	—
66. 896 *trans*-Piperitol	—	—	0.4	—	—
67. 923 *trans*-Carveol	0.6	0.1	—	—	—
68. 950 Citronellol	4.0	—	0.1	—	2.3
69. 967 Myrtenyl acetate	—	0.5	—	—	—
70. 968 Thymol methyl ether	0.1	—	—	—	—
71. 984 Carvone	0.3	0.1	—	—	T
72. 1009 *cis*-Myrtanol	0.1	0.4	—	—	—
73. 1011 Carvenone	0.3	—	—	—	—
74. 1011 Piperitone	—	0.4	—	—	T
75. 1018 Geraniol	0.4	—	—	—	—
76. 1023 Linalyl acetate	—	0.4	—	—	—
77. 1026 *trans*-Myrtanol	0.9	—	—	—	—
78. 1035 Methyl citronellate	1.3	—	0.1	—	—
79. 1099 Bornyl acetate	5.2	2.3	0.1	0.4	2.1
80. 1101 Safrole	—	—	—	—	10.9
81. 1113 Thymol	—	T	—	—	—
82. 1117 *cis*-Sabinyl acetate	—	—	0.9	—	—
83. 1119 *trans*-Verbenyl acetate	—	0.9	—	—	—
84. 1137 Carvacrol	—	0.1	—	—	—
85. 1229 *cis*-Isosafrole	—	—	—	—	6.7
86. 1240 4-Terpinenyl acetate	—	—	—	—	T
87. 1264 α-Terpinenyl acetate	—	0.5	—	—	—
88. 1275 Citronellyl acetate	0.5	—	—	—	—
89. 1303 Neryl acetate	0.2	—	—	—	—
90. 1334 α-Copaene	—	T	—	—	—
91. 1352 Geranyl acetate	1.9	—	—	—	—
92. 1371 β-Cubebene	—	T	—	—	—
93. 1375 β-Elemene	0.2	—	—	—	—
94. 1403 Methyl eugenol	—	—	—	—	2.9

Table 1. *(continued)*

RT Compound	*Communis* var. *depressa*	Flaccida var. *martinezii*	*Foetidissima*	*Procera*	*Virginiana*
95. 1442 Caryophyllene	0.2	0.2	0.1	0.5	T
96. 1467 Thujopsene	—	—	—	—	T
97. 1519 α-Cadinene	—	0.3	—	—	T
98. 1525 Geranyl acetone	0.2	—	—	—	—
99. 1527 α-Humulene	0.2	—	0.1	0.7	—
100. 1537 *cis*-β-Farnesene	0.3	—	—	—	—
101. 1577 β-Cadinene	—	0.3	—	—	T
102. 1594 Germacrene D	0.9	—	—	0.3	T
103. 1602 ar-Curcumene	0.2	—	—	—	—
104. 1634 α-Zingiberene	0.6	—	—	—	—
105. 1643 α-Muurolene	0.1	T	0.1	—	T
106. 1667 β-Bisabolene	0.8	—	—	—	—
107. 1676 Γ-Cadinene	0.2	1.0	0.1	—	T
108. 1695 Calamenene (1S,*cis*-)	—	T	—	—	—
109. 1700 δ-Cadinene	0.8	0.9	0.3	—	0.8
110. 1759 Elemol	—	0.7	0.1	4.3	8.2
111. 1772 Elemicin	—	T	—	—	T
112. 1777 Γ-Elemene	0.3	—	—	—	—
113. 1796 *trans*-Norelidol	3.8	—	—	—	—
114. 1821 Germacrene D-4-ol	1.2	—	0.1	—	T
115. 1825 Spathulenol	1.2	—	—	—	—
116. 1837 Caryophyllene oxide	0.2	0.2	T	0.5	—
117. 1876 Cedrol	—	—	3.2	—	—
118. 1898 β-Oplopenone	0.2	—	0.5	—	T
119. 1944 Cubenol	—	1.0	—	—	0.9
120. 1951 Γ-Eudesmol	—	0.2	—	1.4	2.8
121. 1973 τ-Cadinol	0.2	T	0.3	—	T
122. 1976 τ-Muurolol	0.2	0.2	0.3	—	2.4
123. 1984 Torreyol (=δ-cadinol)	0.1	T	0.1	—	—
124. 1993 β-Eudesmol	—	0.3	—	2.3	1.7
125. 2000 α-Eudesmol	—	0.3	—	3.8	3.1
126. 2003 α-Cadinol	0.8	—	1.0	—	—
127. 2034 (Elemol acetate)	—	—	—	1.3	—
128. 2079 epi-α-Bisabolol	3.2	—	—	—	—
129. 2141 *cis*,*cis*-Farnesol	0.4	—	—	—	—
130. 2159 *trans*,*trans*-Farnesol	1.6	—	—	—	—
131. 2201 *trans*,*cis*-Farnesol	0.4	—	—	—	—
132. 2306 Acetoxyelemol <8-α->	—	—	—	3.5	3.5
133. 2660 Manool <epi-13->	—	0.9	—	0.2	—
134. 2717 Manoyl oxide	—	0.9	0.2	0.5	—
135. 2841 Abietatriene	—	0.3	0.2	1.3	—
136. 2845 Manool	0.3	0.3	—	—	—
137. 2891 Abietadiene	—	—	—	15.4	—
138. 3253 (*cis*-) Totarol	—	—	—	0.6	—
139. 3275 (*cis*-) Abietal	—	—	—	1.7	—
140. 3300 (*trans*-) Totarol	—	—	0.4	21.4	—
141. 3330 Ferruginol	—	—	—	2.4	—

horizontalis and *J. virginiana* (Palma-Otal et al. 1983), between *J. scopulorum* and *J. virginiana* (Adams 1983a; Flake et al. 1978), and suggested between *J. virginiana* and *J. virginiana* var. *silicicola* (Adams 1986).

6.1.2 Other Forest Trees

The literature on the use of terpenoids is so vast that space does not permit any significant review. However, a few examples in various genera may be illustrative. In the spruce (*Picea*) one must mentioned the very early study on introgression of white and Engelmann spruce along the Bow River in Alberta (Ogilvie and von Rudloff 1968). Hybridization in *Pinus* has been subject of numerous studies including Zavarin et al. (1980); Snajberk et al. (1982); Bailey et al. (1982); and Neet-Sarqueda et al. (1988).

The analysis of hybridization between *Eucalyptus crenulata* and *E. ovata* provides a good example of the use of terpenoids for an angiosperm forest tree (Simmons and Parsons 1976). The intermediate chemical profiles of the hydrids are also shown in their analyses (Simmons and Parsons 1976).

6.2 Studies of Geographic Variation

6.2.1 Juniperus

Over the past 20 years several important studies have been made on geographic variation in *Juniperus*. Geographic variation studies of *J. phinchotii* (Adams 1972b, 1975b) revealed fairly uniform populations except where the taxon is sympatric with *J. erythrocarpa* in the trans-Pecos Texas area. Previous suggestions of hybridization with *J. ashei*, *J. depeana*, and *J. monosperma* were refuted.

Populational studies in *J. ashei* (Adams and Turner 1970; Adams 1975a; 1977) revealed ancestral populations of *J. ashei*. Fig. 10 shows that the composite of the terpenoid pattern, factored by principal coordinate analysis, accounted for 50% of the variation among populations. The divergent populations were shown to bear affinities to the sibling, ancestral species, *J. saltillensis* in Mexico. Furthermore, the observed terpenoid pattern (Adams 1977) supports the post-glacial migration into the Edwards plateau and northward (Fig. 11). Without the use of continuous, quantitative terpenoid data, analysis of the ancestral history of *J. ashei* would have been almost impossible.

Another major study on Pleistocene refugia and recolonization was performed on *J. scopulorum* (Adams 1983a). Canonical variate analysis and contour mapping of the population coordinate scores were used to describe infraspecific variation and correlate these patterns with the Wisconsin maximum ice advance and subsequent recolonization by *J. scopulorum* (Adams 1983a).

Comer et al. (1982) showed that even the oil from juvenile leaves could be utilized if very controlled conditions were used. They analyzed the leaf oils from seedlings (juvenile foliage) of *J. virginiana* and *J. scopulorum* grown in a common garden and determined patterns of geographic variation between *J. scopulorum*

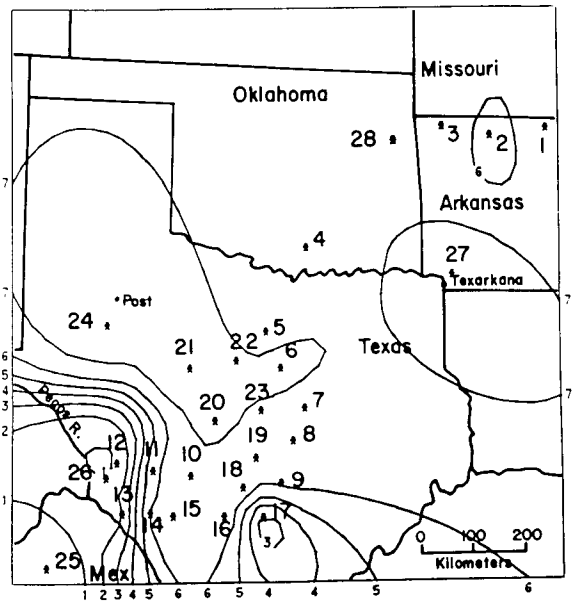


Fig. 10. Principal coordinate analysis of the leaf terpenoids of *J. ashei* showing that the major geographic trend (50% of the variation) is due to the ancestral (relictual) genotypes in populations 12, 13, 25, and 26 arising from the Mexican highlands. (Adams 1977)

and *J. viginiana* throughout the Great Plains of the United States (Comer et al. 1982).

Finally, I would like to mention a study on *J. silicicola* and *J. virginiana* in the southeastern United States that resulted in the substantiation of the recognition of *J. virginiana* var. *silicicola* (Adams 1986). The two taxa are scarcely distinct morphologically or chemically. As can be seen in Fig. 12, canonical variate analyses of the terpenoids clearly shows that the two taxa form a continuum. In fact one population (WT from Texas) that had previously been called *J. silicicola* (due to the flattened tree crowns), is clearly most similar to *J. virginiana* from nearby Bastrop, Texas (note WT and BT, Fig. 12). Due to the clinal gradation into *J. virginiana*, specific status for *J. silicicola* could not be supported but because *J. silicicola* occupies unique sites for junipers (sand dunes) and thus probably has some unique physiological genes for that adaptation, the varietal status (*J. virginiana* var. *silicicola*) was chosen for that taxon.

6.2.2 Other Forest Trees

Terpenoids have been used in numerous studies on geographic variation of other forest trees. Many of these studies have been on conifers such as *Abies grandis* (Zavarin et al. 1977), *Picea glauca* (Wilkinson et al. 1971), *Pseudotsuga menziesii*

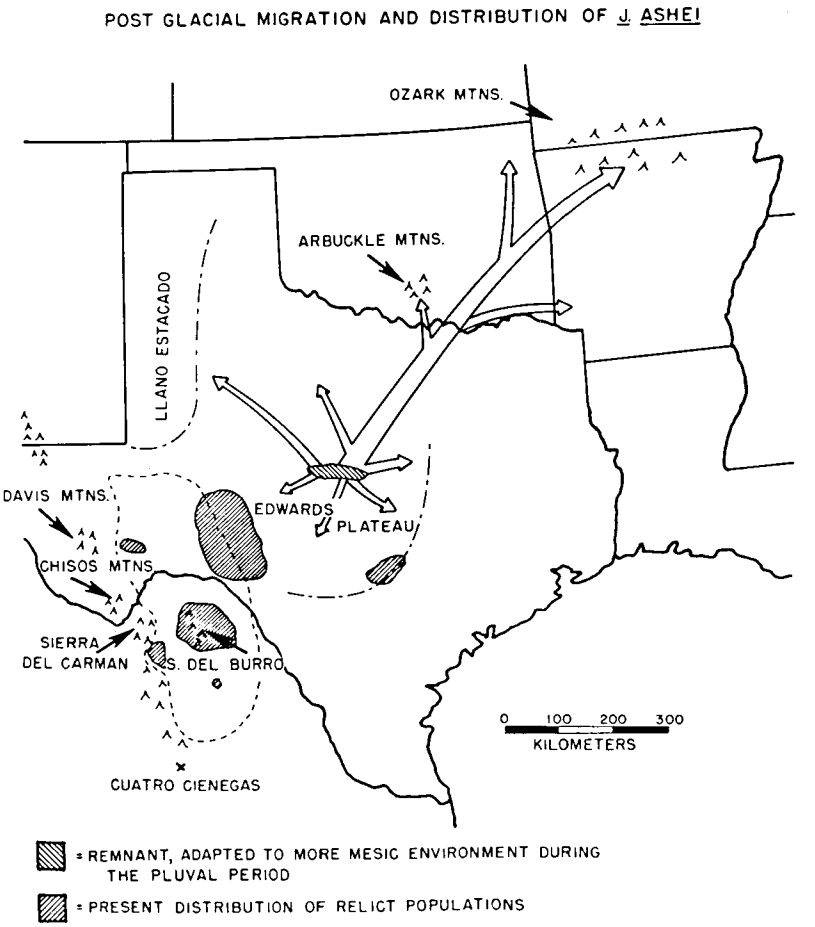


Fig. 11. The post-glacial migration of *J. ashei* into its present distribution, based on the terpenoids and morphology. The congruence of the terpenoids and morphology greatly strengthens the postulate. (Adams 1977)

(von Rudloff 1973, 1975; Zavarin and Snajberk 1975) and *Pinus* (Adams and Edmunds 1989; Hunt and von Rudloff 1977; Smith and Preisler (1988); Zavarin et al. (1989).

For example, von Rudloff (1975) found that a clinal pattern from the interior range of *Pseudotsuga menziesii* (Douglas fir) to the coastal locations (Fig. 13). Notice cpd. 7 (β-pinene) ranges from about 5% in the interior population to over 40% in the coastal population. Likewise, bornyl acetate (cpd. 33) is over 30% in the interior population and decreases to almost zero in the coastal populations (Fig. 13).

Analyses (Zavarin et al. 1989) of the wood monoterpenes of pinyon pine (*Pinus edulis*) from 18 locations revealed that the populations from Arizona and

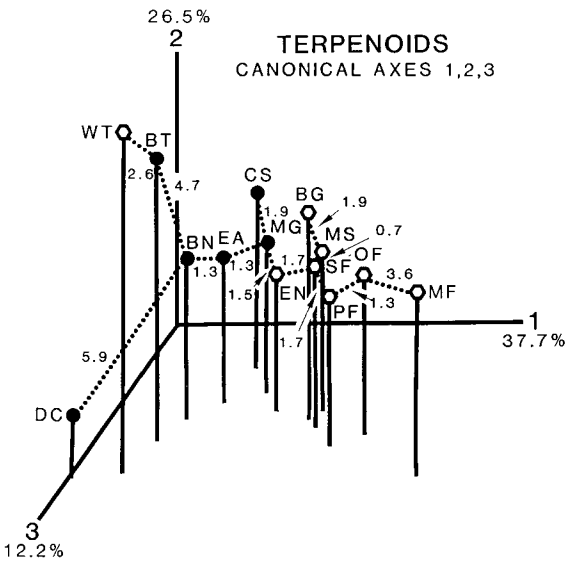


Fig. 12. Ordination of populations of *J. virginiana* (*solid circles*) and putative *J. virginiana* var. *silicicola* (*open hexagons*). Note the putative var. *silicicola* from West Columbia, Texas (WT), is clearly most similar to *J. virginiana* from Bastrop, Texas (BT) and is not *J. virginiana* var. *silicicola*. (Adams 1986)

New Mexico differed considerably from populations in other areas (Fig. 14). These populations were named the Apache chemical race. Interestingly, a presumed, recently established (ca. a few hundred years ago) population in northern Colorado (popn. 26, Fig. 14), clustered strongly with the Arizona and New Mexico, Apache race group. Because the native Americans used (and continue to use) pinyon nuts for food, it is thought that this outlying population was established during north-south migrations by the native Americans. This paper serves as a good example of the potential use of volatile oil characters to address anthropological questions as well as the broad applicability of terpenoid data.

6.3 Taxon Level Differences and Evolutionary Studies

6.3.1 Juniperus

One of the common applications of terpenoid data is for the determination of species limits and for identification of unknown plants. A recent example is that of the cultivated tree referred to as *J. excelsa* at the Royal Botanic Garden, Kew, London. Foliage was collected and compared with native trees in Greece and the tree of unknown origin at Kew was definitely established as being *J. excelsa* (Adams 1990).

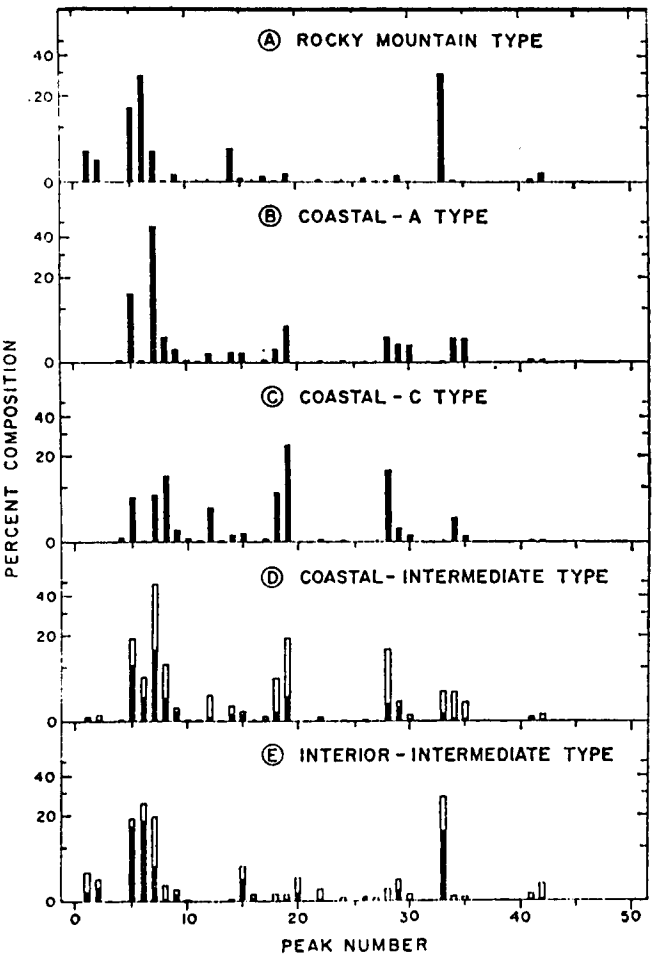


Fig. 13. Bar histograms showing mean leaf oil compositions of the rocky mountain and coastal varieties of Douglas fir and intermediate types. (von Rudloff 1975)

The leaf terpenoids have been used in North America to analyze forms of *J. monticola* (now *J. sabinoides*) (Adams et al. 1980a), varieties of *J. flaccida* (Adams et al. 1984a), varieties of *J. deppeana* (Adams et al. 1984b), and ancestral and derived species (*J. saltillensis* and *J. ashei*) (Adams et al. 1980b).

A number of comparisons have been made between species such as: *J. durangensis* and *J. jaliscana* (Adams et al. 1985a); *J. comitana*, *J. gamboana* and *J. standlevi* (Adams et al. 1985b); *J. lucayana* and *J. saxicola* (Adams et al 1987); *J. blancoi*, *J. horizontalis*, *J. virginiana*, and *J. scopulorum* (Adams et al., 1981a); *J. californica*, *J. monosperma*, *J. occidentalis* and *J. osteosperma* (Adams et al. 1983); *J. oxycedrus*, *J, thurifera* and *J. sabina* (Hernandez et al. 1987); *J. dahurica*, *J.*

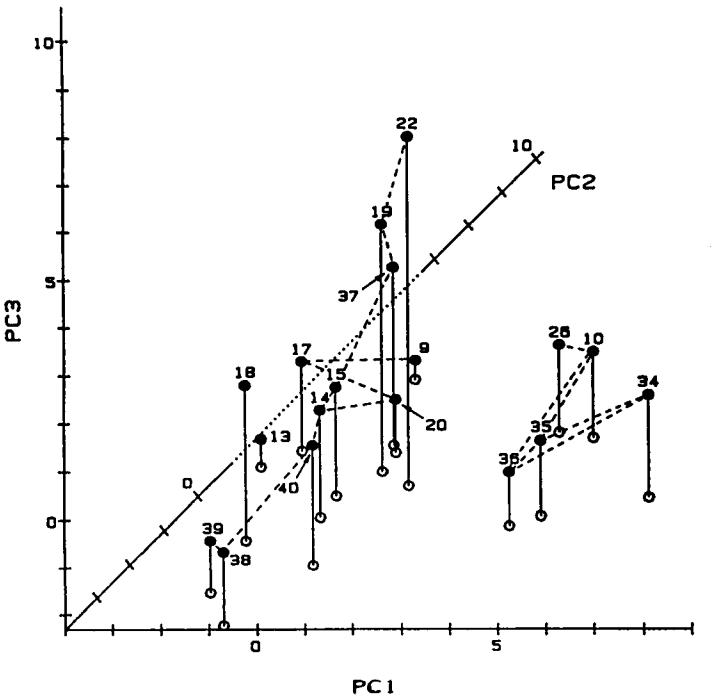


Fig. 14. Principal component analysis of biosynthetically-transformed monoterpenes for populations of pinyon pine (*Pinus edulis*) showing clustering by region. The cluster of 10, 26, 34, 35, and 36 was named the Apache chemical race. (Zavarin et al. 1989)

pseudosabina, *J. sabina* and *J. sibirica* (Satar 1984), and *J. erythrocarpa*, *J. monosperma* and *J. pinchotii* (Adams et al. 1981b).

Several studies have compared numerous species spanning a region: Caribbean junipers (Adams and Hogge 1983; Adams 1983b; Adams 1989b) and the junipers of Mexico and Guatemala (Zanoni and Adams 1976).

The Caribbean junipers are interesting in that they are a monophyletic group of relative recent origin, and they differ by only a few morphological characters. However, there has been considerable divergence in their leaf volatile oils. The leaf oils have proved very useful in analysis of the origin of the group (Adams, 1989b). Figure 15 shows the proposed routes of speciation for the group, based on principal coordinate analysis and a minimum spanning network using terpenoid data. The pathways and relationships in Hispaniola are so complex that additional characters and/or more sampling will be needed to resolve the situation.

The fact that terpenoids can be readily quantitated is a key factor in their use, as they are amenable to multivariate analysis. Underlying trends can be discovered and random variation reduced by multivariate analyses.

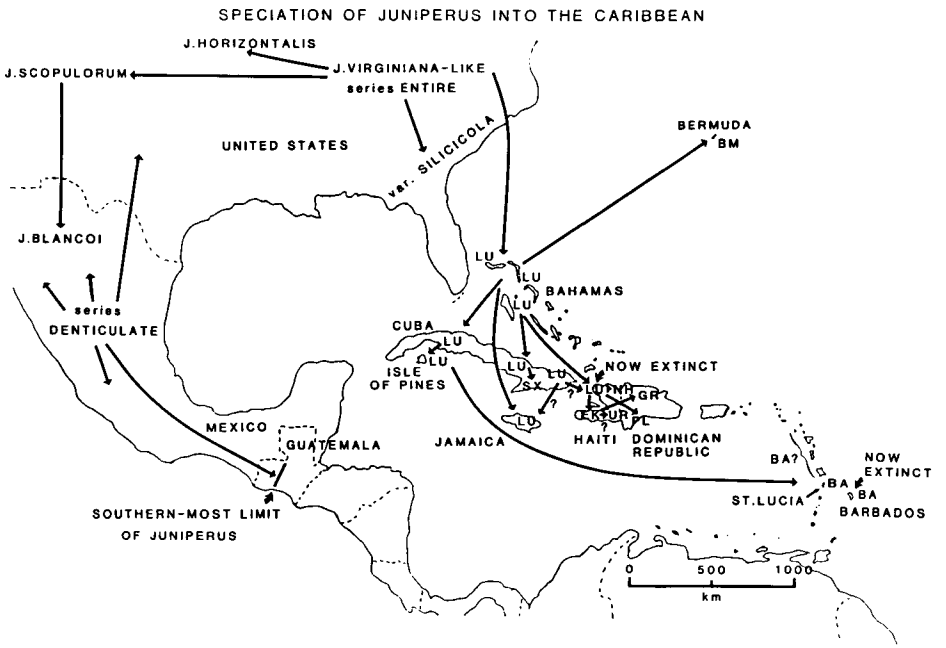


Fig. 15. Proposed speciation of *Juniperus* into the Caribbean from *J. virginiana* (or ancestral stock). The analysis was primarily based on terpenoid data because of the lack of morphological features separating the taxa. (Adams 1989b)

6.3.2 Other Forest Trees

Langenheim and her students (see Langenheim et al. 1982) have carefully researched both specific differences and co-evolution with herbivorous insects in *Hymenaea* (a tropical legume genus). They have found the leaf resin terpenoids to be of considerable taxonomic use, but also of use for the analyses of co-evolution with insects. Work on co-evolution has resulted in the formation of a subdiscipline, chemical ecology. The number of papers in chemical ecology is now so great that is beyond the scope of this review.

Carman and Sutherland (1979) analyzed the leaf diterpenes of several accessions of *Cupressus macrocarpa* and *C. arizonica* and individual samples from *C. tortulosa* and *C. sempervirens*. All of the 27 trees were non-native and had been placed into cultivation in Australia. The identities of two of the *C. macrocarpa* were uncertain. The diterpenes generally divided the individuals into two groups: high in isophyllocladene, phyllocladene, and isohibaene, or high in abietadiene and abietatriene. Although the study was limited in samples and particularly in the knowledge concerning the origin of the trees sampled, it does provide some indication that diterpenes could be useful for systematic studies.

Pauly et al. (1983) examined the volatile leaf oils from two closely related *Cupressus* species (*C. dupreziana*, *C. sempervirens*) and found their leaf oils to be almost identical. They concluded that *C. dupreziana* was a subspecies, derived from *C. sempervirens*. The leaves were ground (which I would not recommend) and steam distilled for 3 h (cf. 24 h by Carman and Sutherland 1979). Only a trace of the diterpenoid manool was found, so perhaps the extractions were too short to give a good yield of diterpenes.

Lastly, it should be noted that von Rudloff has published numerous papers on specific differences in the leaf oils of conifers (see von Rudloff 1975 for a review). Figure 16 shows specific differences in the volatile leaf oils of North

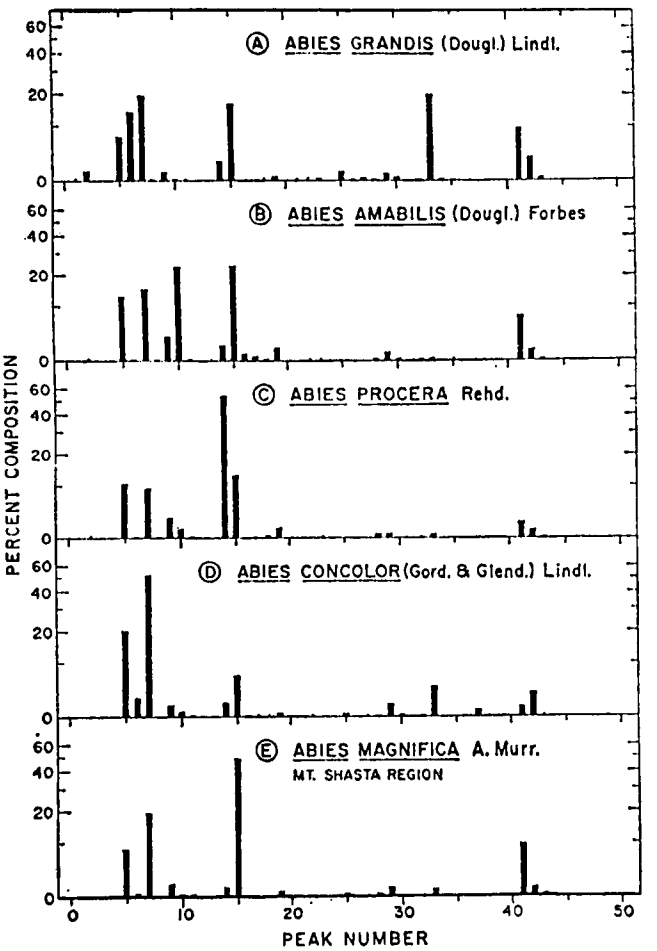


Fig. 16. Bar histograms showing average leaf oil terpene compositions for *Abies* species (firs) Each species has a unique profile of components which can be used for chemotaxonomic studies. (von Rudloff 1975)

American firs (*Abies*). This is but one example of many that he presents to show the utility of volatile leaf oils in analyzing evolutionary patterns among species (von Rudloff 1975). One should note, however, that his work was preceded by detailed baseline studies on ontogenetic, seasonal, and sampling studies. Although we can now be more assured of the impact of ontogenetic, seasonal, and sampling studies, one must still do some preliminary research before embarking on a terpenoid-based study.

References

Adams RP (1970a) Differential systematics and contour mapping of geographical variation. Syst Zool 19:385–390

Adams RP (1970b) Seasonal variation of terpenoid constituents in natural populations of *Juniperus pinchotii* Sudw. Phytochemistry 9:397–402

Adams RP (1972a) Numerical analyses of some common errors in chemosystematics. Brittonia 24:9–21

Adams RP (1972b) Chemosystematic and numerical studies of natural populations of *Juniperus pinchotii* Sudw. Taxon 21:407–427

Adams RP (1975a) Gene flow versus selection pressure and ancestral differentiation in the composition of species: analysis of populational variation in *Juniperus ashei* Buch. using terpenoid data. J Mol Evol 5:177–185

Adams RP (1975b) Numerical-chemosystematic studies of infraspecific variation in *Juniperus pinchotii* Sudw. Biochem Syst Ecol 3:71–74

Adams RP (1977) Chemosystematics — analysis of populational differentiation and variability of ancestral and modern *Juniperus ashei.* Ann Mo Bot Gard 64:184–209

Adams RP (1979) Diurnal variation in the terpenoids of *Juniperus scopulorum* (Cupressaceae)—summer versus winter. Am J Bot 66:986–988

Adams RP (1982a) The effects of gases from a burning coal seam on morphological and terpenoid characters in *Juniperus scopulorum* (Cupressaceae). Southwest Nat 27:279–286

Adams RP (1982b) A comparison of multivariate methods for the detection of hybridization. Taxon 31:646–661

Adams RP (1983a) Infraspecific terpenoid variation in *Juniperus scopulorum* evidence for Pleistocene refugia and recolonization in western North America. Taxon 32:30–46

Adams RP (1983b) The junipers (*Juniperus*: Cupressaceae) of Hispaniola: comparisons with other Caribbean species and among collections from Hispaniola. Moscosa 2:77–89

Adams RP (1986) Geographic variation in *Juniperus silicicola* and *Juniperus virginiana* of the southeastern United States: multivariate analyses of morphology and terpenoids. Taxon 35:61–75

Adams RP (1989a) Identification of essential oils by ion trap mass spectroscopy. Academic Press, New York

Adams RP (1989b) Biogeography and evolution of the junipers of the west Indies. In: Woods CA (ed) Biogeography of the West Indies. Sandhill Crane Press, Gainesville, FL, pp 167–190

Adams RP (1990) The chemical composition of the leaf oils of *Juniperus excelsa* M.-Bieb. J Ess Oil Res 2:45–48

Adams RP, Edmunds GF Jr (1989) A reexamination of the volatile leaf oils of *Pinus ponderosa* Dougl. ex. P. Lawson using ion trap mass spectroscopy. Flav Frag J 4:19–23

Adams RP, Hagerman A (1976) The volatile oils of young versus mature leaf oils of *Juniperus scopulorum:* chemosystematic significance. Biochem Syst Ecol 4:75–79

Adams RP, Hagerman A (1977) Diurnal variation in the volatile terpenoids of *Juniperus scopulorum* (Cupressaceae). Am J Bot 64:278–285

Adams RP, Hogge L (1983) Chemosystematic studies of the Caribbean junipers based on their volatile oils. Biochem Syst Ecol 11:85–89

Adams RP, Powell RA (1976) Seasonal variation of sexual differences in the volatile oil of *Juniperus scopulorum.* Phytochemistry 15:509–510

Adams RP, Turner BL (1970) Chemosystematic and numerical studies in natural populations of *Juniperus ashei* Buch. Taxon 19:728–751

Adams RP, Granat M, Hogge L, von Rudloff E (1979) Identification of lower terpenoids from gas chromatograph-mass spectral data by on-line computer method. J. Chromatogr Sci 17:75–81

Adams RP,von Rudloff E, Hogge L, Zanoni TA (1980a) The volatile terpenoids of *Juniperus monticola* f. *monticola*, f. *compacta*, and *f. orizabensis*. J Nat Prod 43:417–419

Adams RP, von Rudloff E, Zanoni TA, Hogge L (1980b) The terpenoids of an ancestral/advanced species pair of *Juniperus*. Biochem Syst Ecol 8:35–37

Adams RP, von Rudloff E, Hogge L, Zanoni TA (1981a) The volatile terpenoids of *Juniperus blancoi* and its affinities with other entire leaf margined junipers of North America. J Nat Prod 44:21–26

Adams RP, Zanoni TA, von Rudloff E, Hogge L (1981b) The southwestern USA and northern Mexico one-seeded junipers: their volatile oils and evolution. Biochem Syst Ecol 9:93–96

Adams RP, Palma MM, Moore WS (1981c) Examination of the volatile oils of mature and juvenile leaves of *Juniperus horizontalis*: Chemosystematic significance. Phytochemistry 20:2501–2502

Adams RP, von Rudloff E, Hogge L (1983) Chemosystematic studies of the western North American junipers based on their volatile oils. Biochem Syst Ecol 11:189–193

Adams RP, Zanoni TA, Hogge L (1984a) The volatile oils of *Juniperus flaccida* var. *flaccida* and var. *poblana*. J. Nat Prod 47:1064–1065

Adams RP, Zanoni TA, Hogge L (1984b) Analyses of the volatile leaf oils of *Juniperus deppeana* and its infraspecific taxa: chemosytematic implications. Biochem Syst Ecol 12:23–27

Adams RP, Zanoni TA, Hogge L (1985a) The volatile leaf oils of two rare junipers from western Mexico: *Juniperus durangensis* and *Juniperus jaliscana*. J Nat Prod 48:673–675

Adams RP, Zanoni TA, Hogge L (1985b) The volatile leaf oils of the junipers of Guatemala and Chiapas, Mexico *Juniperus comitana*, *Juniperus gamboana* and *Juniperus standlevi*. J Nat Prod 48:678–681

Adams RP, Almirall AL, Hogge L (1987) The volatile leaf oils of the junipers of Cuba: *Juniperus lucayana* Britton and *Juniperus saxicola* Britton and Wilson. Flav Frag J 2:33–36

Bailey, DK, Snajberk K, Zavarin E (1982) On the question of natural hybridization between *Pinus discolor* and *Pinus cembroides*. Biochem Syst Ecol 10:111–119

Burbott AJ, Loomis WD (1969) Evidence of metabolic turnover of monoterpenes in peppermint. Plant Physiol 44:173–179

Carman RM, Sutherland MD (1979) Cupressene and other diterpenes of *Cupressus* species. Aust J Chem 32:1131–1142

Comer CW, Adams RP, Van Haverbeke DR (1982) Intra- and interspecific variation of *Juniperus virginiana* L. and *J. scopulorum* Sarg. seedlings based on volatile oil composition. Biochem Syst Ecol 10:297–306

Craveiro AA, Matos FJA, Alencar JW, Plumel MM (1989) Microwave extraction of an essential oil. Flav Frag J 4:43–44

Cu J-Q, Perineau F, Delmas M, Gaset A (1989) Comparison of the chemical composition of carrot seed essential oil extracted by different solvent. Flav Frag J 4:255–231

Fassett NC (1945) *Juniperus virginiana*, *J. horizontalis* and *J. scopulorum* V. Taxonomic treatment. Bull Torrey Bot Club 72:480–482

Flake RH, von Rudloff E, Turner BL (1969) Quantitative study of clinal variation in *Juniperus virginiana* using terpenoid data. Proc Natl Acad Sci USA 62:487–494

Flake RH, von Rudloff E, Turner BL (1973) Confirmation of a clinal pattern of chemical differentiation in *Juniperus virginiana* from terpenoid data obtained in successive years. In: Runeckles VC, and Marbry TJ (eds) Terpenoids: Structure, biogenesis, and distribution. Academic Press, New York, pp 215–228 Recent advances in phytochemistry series, Vol 6

Flake RH, Urbatsch L, Turner BL (1978) Chemical documentation of allopatric introgression in *Juniperus*. Syst Bot 3:129–144

Fluck H (1963) Intrinsic and extrinsic factors affecting the production of natural products. In: Swain T (ed) Chemical plant taxonomy. Academic Press, London, pp 167–186

Fretz TA (1976) Effect of photoperiod and nitrogen on the composition of foliar monoterpenes of *Juniperus horizontalis* Moench. cv. Plumosa. J. Am Soc Hortic Sci 101:611–613

Hall MT (1952) Variation and hybridization in *Juniperus*. Ann Mo Bot Gard 39:1–64

Hernandez EG, Martinez MDCL, Villanova RG (1987) Determination by gas chromatography of terpenes in the berries of the species *Juniperus oxycedrus* L., *J. thurifera* L. and *J. sabina* L. J Chromatogr 396:416–420

Hopfinger JA, Kumamoto J, Scora RW (1979) Diurnal variation in the essential oils of Valencia orange leaves. J Bot 66:111–115

Hunt RS, von Rudloff E (1977) Leaf-oil-terpene variation in western white pine populations of the Pacific northwest. For Sci 23:507–516

Irving RS, Adams RP (1973) Genetic and biosynthetic relationships of monoterpenes. In: Runeckles VC, Mabry TJ (eds) In: Terpenoids: structure, biogenesis, and distribution. Academic Press, New York, Recent advances in phytochemistry series, vol 6.

Jennings W (1978) Gas chromatography with glass capillary columns. Academic Press, New York

Jennings W (1987) Analytical gas chromatography. Academic Press, New York

Jennings W, Shibamoto T (1980) Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Academic Press, New York

Langenheim JH, Lincoln DE, Stubblebine WH, Gabrielli AC (1982) Evolutionary implications of leaf resin pocket patterns in the tropical tree *Hymenaea* (Caesalpinioideae: Leguminosae). Am J Bot 69:595–607

Leach GJ, Whiffin T (1989) Ontogenetic, seasonal and diurnal variation in leaf oils and leaf phenolics of *Angophora costata*. Aust Syst Bot 2:99–111

Lincoln DE, Langenheim JH (1977) Effect of light and temperature on monoterpene yield and composition in *Satureja douglasii*. Biochem Syst Ecol 6:21–32

Neet-Sarqueda C, Plummettaz Clot A.-C, Becholey I (1988) Mise en évidence de l'hybridation introgressive entre *Pinus sylvestris* L. et *Pinus uncinata* DC. en Valais (Suisse) par deux methodes multi-variées. Bot Helv 98:161–169

Ogilvie RT, von Rudloff E (1968) Chemosystematic studies in the genus *Picea* (Pinaceae). IV. The introgression of white and Englemann spruce as found along the Bow River. Can J Bot 46:901–908

Palma-Otal M, Moore WS, Adams RP, Joswiak GR (1983) Morphological chemical and biogeographical analyses of a hybrid zone involving *Juniperus virginiana* and *J. horizontalis* in Wisconsin. Can J Bot 61:2733–2746

Pauly G, Yani A, Piovetti L, Bernard-Dagan C (1983) Volatile constituents of the leaves of *Cupressus dupreziana* and *Cupressus sempervirens*. Phytochemistry 22:957–959

Powell RA, Adams RP (1973) Seasonal variation in volatile terpenoids of *Juniperus scopulorum* (Cupressaceae). Am J Bot 60:1041–1051

Satar S (1984) Mono- and sesquiterpenes in the essential oil of mongolian *Juniperus* species. Pharmazie 39:66–67

Simmons D, Parsons RF (1976) Analysis of a hybrid swarm involving *Eucalyptus crenulata* and *E. ovata* using leaf oils and morphology. Biochem Syst Ecol 4:97–101

Smith RH, Preisler HK (1988) Xylem monoterpenes of *Pinus monophylla* in California and Nevada. Southwest Nat 33:205–214

Snajberk K, Zavarin E, Derby R (1982) Terpenoid and morphological variability of *Pinus quadrifolia* and the natural hybridization with *Pinus monophylla* in the San Jacinto Mountains of California. Biochem Syst Ecol 10:121–132

van den Dries, Svendsen AB (1989) A simple method of detection of glycosidic bound monoterpenes and other volatile compounds occurring in fresh plant material. Flav Frag J 4:59–61

Vernin G, Boniface C, Metzger J, Ghiglione C, Hammoud A, Suon K, Fraisse D, Parkanyi C (1988) GC-MS-SPECMA bank analysis of *Juniperus communis* needles and berries Phytochemistry 27:1061–1064

von Rudloff E (1972) Seasonal variation in the composition of the volatile oil of the leaves, buds, and twigs of white spruce (*Picea glauca*). Can J. Bot 50:1595–1603

von Rudloff E (1973) Geographical variation in the terpene leaf composition of the volatile oil of Douglas fir. Pure Appl Chem 34:401–410

von Rudloff E (1975) Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem syst Ecol 2:131–167

von Rudloff E, Irving R, Turner BL (1967) Reevaluation of allopatric introgression between *Juniperus ashei* and *Juniperus virginiana* using gas chromatography. Am J Bot 54:600

Whiffin T, Hyland BPM (1989) The extent and systematic significance of seasonal variation of volatile oil composition in Australian rainforest trees. Taxon 38:167–177

Wilkinson RC, Hanover JW, Wright JW, Flake RH (1971) Genetic variation in the monoterpene composition of white spruce. For Sci 17:83–90

Zanoni TA, Adams RP (1976) The genus *Juniperus* in Mexico and Guatemala: Numerical and chemosystematic analysis. Biochem Syst Ecol 4:147–158

Zavarin E, Snajberk K, (1975) *Pseudotsuga menziesii* chemical races of California and Oregon. Biochem Syst Ecol 2:121–129

Zavarin E, Snajberk K, Critchfield WB (1977) Terpenoid chemosystematic studies of *Abies grandis*. Biochem Syst Ecol 5:81–93

Zavarin E, Snajberk K, Derby R (1980) Terpenoid and morphological variability of *Pinus quadrifolia* and its natural hybridization with *Pinus monophylla* in Northern Baja California and adjoining United States. Biochem Syst Ecol 8:225–235

Zavarin E, Snajberk K,Cool L (1989) Monoterpenoid differentiation in relation to the morphology of *Pinus edulis*. Biochem Syst Ecol 17:271–282