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CHEMOSYSTEMATIC STUDIES OF THE VOLATILE OILS OF *JUNIPERUS HORIZONTALIS*, *J. SCOPULORUM* AND *J. VIRGINIANA*

ERNST VON RUDLOFF

Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Sask. S7N OW9, Canada

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Key Word Index—*Juniperus*; Cupressaceae; foliage mono- and sesquiterpenes; quantitative variation; population differences; species differentiation; hybrid swarms; chemotaxonomy.

Abstract—The volatile oil from the foliage of individual plants from different populations of *Juniperus horizontalis*, *J. scopulorum* and *J. virginiana* has been analyzed qualitatively and quantitatively. Some analytical problems are discussed and several new constituents have been identified. *J. horizontalis* can be differentiated from the other two species by the presence of relatively large percentages of cadinane-type sesquiterpenes and less of the elemol–eudesmol type. The ratio of methyl citronellate to citronellol may also have diagnostic value, as may be the virtual absence of aromatic ethers of the safrole-type. The oil of *J. scopulorum* is virtually devoid of the cadinol type sesquiterpenes but differentiation from that of *J. virginiana* is difficult. A useful measure of clinal variation within different populations of each species is the ratio of sabinene and limonene percentages. The occurrence of aromatic ethers in *J. scopulorum* and *J. virginiana* is erratic; in *J. horizontalis* they are present in trace amounts only. Provided an adequate foliage sample size is taken during the dormant season, population studies based on the means of the percentages of the foliage oil components are feasible. Two hybrid swarms of *J. horizontalis* and *J. scopulorum* were sampled and all plants had intermediate foliage oil compositions.

INTRODUCTION

About 30 juniper species of the section Sabina are found on the N. American continent. This group presents a complex taxonomic and phylogenetic problem, subdivision becoming at times arbitrary because of the many morphological similarities [1]. However, subdivision on the basis of serrated or non-serrated (entire-margin) scale-like leaves is easily possible [1, 2]. The group with non-serrated leaves consists of three species, *Juniperus horizontalis* Moench. (creeping juniper), *J. scopulorum* Sarg. (Rocky Mountain juniper) and *J. virginiana* L. (red juniper or eastern red cedar), and these are sometimes referred to as the Virginiana complex. Their natural ranges extend from the Atlantic to the Pacific coasts and from southern Canada to Texas and New Mexico (see Fig. 1) [1–3]. In his early treatment of this group, Engelmann [4] considered *J. scopulorum* as a western subspecies of *J. virginiana*, but Fassett [2] states that all 3 species can be clearly distinguished by a number of morphological characteristics except in areas where they grow together. *J. horizontalis*, being a prostrate shrub, is distinguishable by its low, spreading form, but somewhat similar low forms of *J. scopulorum* have been described [5, 6]. Fassett [2] commented on the high degree of variability of the morphological characters within each species and found that statistical treatment was essential. He also described hybrid swarms of *J. horizontalis* and *J. scopulorum* in the Banff region of Alberta (Fig. 1, Site A), in the Big Horn Mountains of Wyoming (Site B) and the Little Missouri Badlands of North Dakota (Site C) as well as those of *J. scopulorum* and *J. virginiana* in the Missouri River basin

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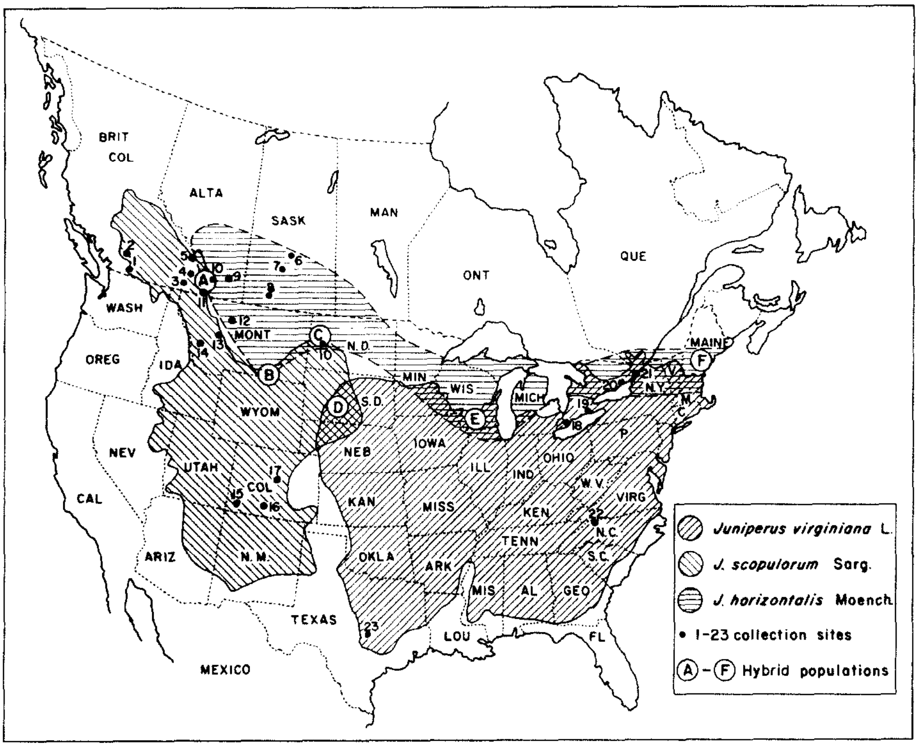

Fig. 1. Approximate natural ranges of the 3 junipers of the Virginiana complex, areas of hybrid swarms, and collection sites of foliage samples.

(Nebraska, and South Dakota, Site D) and those of *J. horizontalis* and *J. virginiana* in the Driftless area of Wisconsin (Site E) and near the coast of Maine (Site F). More recently, Van Haverbeke [6] made a thorough morphological analysis of the juniper populations of the Missouri River Basin and, on the basis of statistical analysis, concluded hybrid origin for these junipers. The strong tendency towards bimodality indicated the presence of 2 species, but neither of the pure parental forms of *J. scopulorum* and *J. virginiana* was found in this area. The morphological data indicate a more rapid trend towards *J. scopulorum* in the west than towards *J. virginiana* in the east and this could be interpreted as evolutionary divergence from *J. scopulorum* rather than hybridization with *J. virginiana*. Evidence for introgressive hybridization of *J. scopulorum* and *J. horizontalis* in Montana and northern Wyoming was also obtained.

In addition to morphological characters, Van Haverbeke also used quantitative infrared spectroscopy on the crude lipid extracts of the cone pulp, but no useful chemosystematic data were obtained. No explanation for the choice of this method and crude lipid extracts was given. In our experience [7] the terpenes found in conifer leaves and foliage offer a useful chemosystematic approach that can also be applied to the junipers. Zavarin and Snajberk [8, 9] and others [7] have found that the terpenes of the cortical oleoresin of some conifers may be used with equal success. Mirov's well-known studies [10] on the turpentines of pines indicate a minimum of seasonal variation, but aside from other disadvantages [7] this method cannot be applied to junipers. Erdtman [11] and his coworkers have investigated the wood extractives of many junipers and several chemosystematic conclusions at the order or family levels were drawn. However, distinction of closely related junipers appears to be difficult and analysis of the wood extractives is not as convenient as that of the terpenes of the leaves or oleoresins when a large number of samples has to be considered. A chemosystematic analysis based on terpenes offers as a particular advantage that the means from many trees or shrubs of the same population can be easily obtained. However, it is essential to minimize any quantitative variations caused by environmental or other extraneous factors [7]. Physiological changes must be at a minimum at the time of sample collection [12].

Some contradictory data on seasonal variation of the quantitative amounts of the terpenes found in junipers have been reported [7, 13–16]. Tatro *et al.* [16] found diurnal variations in the junipers of California when collections were made in November, but the temperature fluctuation from a low of 16° to a high of 30° was such that a state of dormancy is unlikely. In all conifer foliage that we collected from more northern latitudes, little if any quantitative changes were found when this was collected in late fall or during winter (September to March or April) [7]. Powell and Adams [13] found with the foliage of *J. scopulorum* from Colorado that the terpenes varied also during winter when a weight basis was used, but this was minimal on the relative percentage basis commonly employed in terpene analysis by GLC.

Previously, we have analyzed the foliage oils from different populations of *J. virginiana* and statistical analysis (computerized numerical classification) showed a clinal variation [17–19]. The smallest divergence between different samples was found in the central Appalachian region and a larger one in the more distant populations of the Southwest (Texas) and North (Ontario). No evidence for the putative hybridization of *J. virginiana* and *J. ashei* Buchholz [1, 20] could be found in the samples from Texas and our chemical results are in good accord with the views of Barber and Jackson [21]. The foliage oil of *J. ashei* [22] is characterized by the predominance of camphene–camphor type monoterpenes and the virtual absence of the typical sesquiterpenes of *J. virginiana* [23]. It may be significant that in other junipers of the sabina group with serrated leaves the camphene–camphor group is also found [7, 16, 24]. The latter were not found in the foliage oils of *J. horizontalis* [25] or *J. scopulorum* [26] and only traces of bornyl acetate and borneol in that of *J. virginiana* [23].

In view of the usefulness of the foliage oil terpenes in chemo-systematic studies of closely related conifer species and their hybrids [7], it was of interest to determine their qualitative and quantitative variation in *J. horizontalis* and *J. scopulorum* within individual plants, as well as populations. In the present study, the terpene composition was reexamined to identify more of the minor components, to determine the individual and overall quantitative variability, to determine whether the species differences were large and consistent enough to describe populations in areas of overlap of the natural ranges, and to determine whether intermediate forms in hybrid swarms can be detected by terpene analysis. The various sites of collection (No. 1–23) are shown in Fig. 1. The 2 hybrid swarms from Banff, Alberta (Site A), and Little Missouri Badlands, North Dakota (Site C), were chosen because of accessibility during winter and easy distinction of low, intermediate and tree-like forms.

RESULTS

The chemical composition of the foiliage oils of the creeping, Rocky Mountain and red junipers was found to be more complex than previously indicated. In addition to the 16–18 major (1–50%), 4–6 minor (0·1–1%) terpenes and 3–4 aromatic ethers of the safrole–eugenol type identified previously [23, 25, 26], α-terpinene (peak 6)*, *trans*-sabinene hydrate (peak 17), *cis*-sabinene hydrate (peak 21, labile), T- and α-cadinol as well as T-muurolol (peaks 48–52), and 2 cadinyl acetate-isomers (peak 42 and 45b) were isolated. Also, elemyl acetate (peak 45), which was found in *J. virginiana* and *J. scopulorum* (Acetate 1) may be an artefact. When Acetate 2 (peak 55) was rechromatographed, considerable amounts of elemyl acetate were formed. GLC of elemyl acetate produced no secondary products, but considerable losses owing to decompositon or polymerization took place. Acetate 2 has PMR and IR spectra that are similar to those of elemyl acetate. Jones and Sutherland [27] have shown that at 100° elemol may be formed from hedycaryol. Thus, it cannot be ruled out that elemol and its acetate as isolated after steam-distillation of these juniper oils are artefacts. This creates large problems for the determination of quantitative variations in these sesquiterpenoid constituents. However, since hedycaryol, elemol and the eudesmol isomers may be derived from *trans*-farnesol via the same hypothetical intermediate [28],

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* The GLC peak numbers are those reported for *J. virginiana* [23].

this analytical difficulty can be overcome by combining the recorded percentages of these elemol–eudesmol type terpenes. Similarly, the cadinol–muurolol type sesquiterpenes which may be derived via a common intermediate from *cis*-farnesol [28] can be combined as a second group. However, this complex mixture of sesquiterpene alcohols and their acetates is not fully resolved on any of the GLC columns used previously. We have now obtained a satisfactory degree of resolution by analysis on 3 to 4 m long columns with liquid phases of different polarities (PEG/QF-1, PEG/OV-17 and SE-30; see experimental) using relatively low temperature programming rates (from 50 to 210° at 2 to 3°/min). However, not only is the analysis time per run increased to over 80 min, but decomposition of the more labile sesquiterpenes (e.g. sabinene hydrate, elemol, elemyl acetate) is more pronounced. For this reason, the quantitative variation within individual plants was determined as before [17–19, 23] and only the combined samples from each population were analyzed on the above mentioned columns.

Previously, we found that in the leaf oil of the coastal Douglas-fir α- and γ-terpinene, terpinolene and terpinen-4-ol correlate positively [7, 29]. These chemically closely related monoterpenes may also be derived from a common precursor [7]. To simplify the tables of results of this study, the relative percentages of α- and γ-terpinene and terpinolene were combined, but that of terpinen-4-ol is reported separately. Similarly, since the various cadinene and muurolene isomers may be derived from germacrene-D [30], and also are very difficult to separate, their percentage is reported as one; those of *trans*- and *cis*-sabinene hydrate were also combined.

A somewhat different analytical problem is the erratic occurrence of safrole, methyleugenol and elemicin in these juniper foliage oils (in *J. virginiana* also estragole and methylvinylanisole [23]). These aromatic ethers are of the phenylpropanoid type and they are derived by a different biosynthetic pathway than the terpenes [31]. Therefore, these foliage oil components should be considered separately from the terpenes. Also, since the relative percentages are obtained by summation of peak areas, the terpene percentages in those samples which contain these aromatic ethers will be correspondingly lower. Hence, the aromatic ether peak percentages were deducted and all terpene percentages recalculated in those samples that contained more than 0·5% of the ethers.

During this study it was found that whereas 6 hr steam-distillation of the macerated foliage of *J. scopulorum* and *J. virginiana* sufficed to give a near-quantitative recovery of the volatile oil, in several samples of *J. horizontalis* less than 80% yield was obtained. When the distillation was continued for a further 18–24 hr near-quantitative yields were obtained, but the additional oil consisted mainly of the cadinene–muurolene isomers and their alcohols and acetates. Extended steam-distillation of the foliage of *J. virginiana* gave a small percentage of volatile oil which contained these cadinane-type sesquiterpenes as well, but their relative amounts were invariably less than 2% of the total oil. Similar treatment of the foliage of *J. scopulorum* gave an oil with only traces of the cadinol isomers, but the cadinene isomers were isolated. It is not known whether this difference upon extended steam-distillation is the result of a difference in leaf anatomy [32], or because different biosynthetic sites within the resin ducts or glands are involved. Another possible explanation is that the same labile precursor rearranges in a different manner in each juniper species (e.g. by virtue of different catalysis). Since cadinene–muurolene type sesquiterpenes are known to be formed from germacrene-D [30], and elemene-type sesquiterpenes are known to be in thermal equilibrium with the corresponding ten-membered ring isomers [33], a germacrene-type precursor is likely, but determination in routine GLC analysis for chemosystematic studies would present considerable problems. This aspect remains to be solved.

Because the scale-like leaves of these junipers are tightly pressed against the green stems it is not feasible to separate the leaves from the stems in sufficient amounts for chemosystematic studies [7]. All woody branches were removed before steam-distillation and none of the typical juniper wood terpenes [11, 34] (thujopsene, widdrene, cedrene, etc.) were isolated. However, some cadinane-type sesquiterpenes have been found in the wood of some juniper species [11]. The yields of volatile oil (6 hr distillation) were 0·6–1·1% in *J. horizontalis* (0·6–1·5% after 24 hr), 0·4–2·2% in *J. virginiana* and 1·6–4·3% in *J. scopulorum*, as based on fr. wt of the foliage. The highest yields of oil (2·3–4·3%) were obtained from the second collection of *J. scopulorum* near Keremeos, British Columbia (Site 1, Fig. 1) which was after a period of severe drought. Part, but not all of the higher yield can be explained by a lower moisture content.

Within-tree variability

Seasonal variability in the relative terpene percentages was found to be at a maximum in the new growth during June and July and this decreased during August to reach a minimum from September through to March or April. This agrees with our previous findings on *Picea glauca* (Moench) Voss [35] and other conifers [7], as well as those on *J. scopulorum* from Colorado by Powell and Adams [13]. In more southerly latitudes one has to expect a longer growing season and a correspondingly shorter rest period during which reproducible data can be obtained. However, if the sample size of foliage is below a certain minimum (see below) within-tree variability is detectable also during winter. All further sampling in this investigation was carried out between September and April to eliminate any effects of seasonal variation as far as possible.

The within-tree or plant variation of the relative terpene percentages from one branch to another are shown in Table 1. The variation recorded in 3 different branchlets (a, b and c) is in excess of the experimental error (0·3–1%, higher with the decomposing peaks) and this demonstrates that single branchlets with 10–20 g foliage provide insufficient plant material reproducible data. It is necessary to collect several branchlets from different sides and heights of each tree or plant and combine these to give a total sample of at least 50 g. Several collections of branches from different heights of *J. scopulorum* and *J. virginiana* showed the vertical variation in terpene percentages to be small. Differences on the north and south sides of individual juniper plants were found to be insignificant by Tatro *et al.* [16] and previously we obtained similar results with single trees of *Picea glauca* [35] and Douglas-fir [7, 29]. However, in highly variable species (e.g. *Picea engelmannii* [7]) a gradient from the lower to upper branches was found.

Table 1. Relative percentages* of the major components of the volatile oil of the foliage from different branches (a, b and c) of juniper plants

	J. horizontalis†			*J. scopulorum*‡			*J. virginiana*§		
	a	b	c	a	b	c	a	b	c
α-Thujene α-Pinene	5	5·5	5	3	3·5	4·5	2	2·5	2·5
Sabinene	47	46	51	48·5	51	51	30·5	30·5	31·5
Myrcene	3·5	4·5	4·5	1	1	1·5	2	2·5	2·5
Limonene	4	4	7	2	2	1	1	1	1
Terpinenes‖	3	3·5	2	4	4·5	4·5	2·5	3·5	3
Terpinene-4-ol	12·5	15	8	4·5	5·5	6·5	5	4	6
Citronellol	2·5	1·5	2·5	0·5	0·5	1	0·5	1	1
Methyl Citronellate	0·2	0·1	0·3	4	4	3	6	5	7
Cadinenes	3	2·5	3·5	1	3·5	2	2	2·5	2
Cadinols + acetates	18	16·5	14·1	less than 0·5			less than 1		
Elemol + acetates	less than 2			13	11	9	36	36·5	33·5
Aromatic ethers	less than 0·5			10	12	10	less than 0·5		

* Percentage of terpenes calculated after deduction of aromatic ether peak areas. † Saskatoon population. ‡ Alberta provenance growing at Saskatoon. § Ontario provenance growing at Saskatoon. ‖ α- and γ-terpinene and terpinolene.

An aspect that has to be considered with junipers is the occasional occurrence of leaves in the juvenile form on adult trees or plants. Foliage with juvenile form of the leaves from two *J. horizontalis* and two *J. scopulorum* plants had the same terpene composition as that in the normal, mature form, but one sample from a *J. virginiana* tree contained less than 0·1% volatile oil and its terpene composition differed markedly from that of the normal foliage. Hence, all foliage in the juvenile form was avoided in the sample collections.

Tree-to-tree variability

From our previous studies on *J. virginiana* [17–19] one may conclude that the tree-to-tree variation in any given population is relatively high. The variation between 5 trees each from North Carolina and southern Ontario is shown in Table 2. The largest degree of variation is found in the relative amounts of aromatic ethers. Separate calculation and evaluation of these appears therefore to be necessary, especially in view of their different biosynthetic origin than the terpenes [31]. The variation in the relative amounts of the mono- and sesquiterpenes is fairly high but within limits that indicate that population studies are entirely feasible, especially when computerized statistical methods are used [17, 19]. A noteworthy difference between the trees from North Carolina and Southern Ontario is the relatively low percentages of sabinene and high ones of limonene in the former as compared with much more variable amounts in

Table 2. Tree-to-tree variation of the relative percentages* of the major components of the foliage oils of 2 different *Juniperus virginiana* populations

	North Carolina†					Southern Ontario‡				
Tree no.	1	2	3	4	5	1	2	3	4	5
α-Thujene α-Pinene	1	1	0·5	0·5	0·5	1	1	1	1	2
Sabinene	1	4	6	0·5	0·5	11	3·5	6	7	31·5
Myrcene	1	0·5	0·5	0·5	0·5	0·5	0·5	1	2·5	2
Limonene	30	28	24	36	28	17	33·5	35·5	39	3
Terpinenes§	0·5	0·5	1	0·5	0·5	2	0·5	1·5	2·5	2·5
Terpinen-4-ol	0·5	1	1·5	0·5	0·5	3	0·5	2·5	2·5	4·5
Sabinene hydrates	6·5	1	2	5	4	2	1	2·5	2·5	2·5
Citronellol	1	0·5	1	1	1	0·5	1	0·5	1	1·5
Methyl Citronellate	2·5	1	2	5	4	7	1·5	6·5	6	3
Cadinenes	3·5	2	1·5	1·5	1	2	5	2	2·5	5
Cadinols + acetates	← all less than 2% →									
Elemol + acetates	43·5	57·5	45	47	51·5	45	48	39	32·5	40·5
Aromatic ethers	6	22	29·5	19	10·5	10	0·5	4	3	0·5

* Percentage of terpenes calculated after deduction of aromatic ether peak areas. † Alamance County, January 1968 (about 30 g foliage each sample). ‡ St. Williams, December 1968 (50–80 g foliage each sample). § α- and γ-terpinene and terpinolene.

the latter. Reviewing some of our previous results on *J. virginiana* populations, it appears that three distinct chemical types, or chemovars, are present. The first, type A, has less than 10% sabinene and well above 20% limonene. Most trees of the central Appalachian region have this ratio and this may be the typical chemovar of *J. virginiana*. The second chemovar, type B, is one with more than 20% sabinene and less than 10% limonene (e.g. tree 5 from southern Ontario, Table 2). The third chemovar, type C, is that with intermediate percentages of both monoterpenes (e.g. tree 1, southern Ontario, Table 2). In all three chemovars the ratios of the other monoterpenes remain approximately the same. Also, the amounts of the two sabinene hydrate isomers do not correlate well with that of sabinene itself. In contrast, the terpinenes and terpinolene are usually present in about the same amount as terpinen-4-ol. About one third to half of the terpenes are sesquiterpenes and in all *J. virginiana* populations these are predominantly of the elemol–eudesmol type.

As pointed out previously [17], at least 10 trees per collection site should be sampled and a better gradation of the cline in going from the central Appalachian region to Texas or Ontario is obtained when 20 trees per site were sampled [19]. No consistent differences between the foliage oil composition of female and male trees were recorded.

In Table 3 the results obtained from 10 trees of *J. scopulorum* from Keremeos, southern British Columbia, are listed. This population is sufficiently far removed from the natural ranges of *J. horizontalis* and *J. virginiana* to exclude any effects of hybridization, but whether this northwestern location represents the typical *J. scopulorum* genotype is not known. Again, the percentages of the aromatic ethers are highly variable. Sabinene was invariably the major component and limonene was below 3% (i.e. only chemovar type B appears to be present). The relative amounts of the other monoterpenes are somewhat higher than in *J. virginiana* and those of the oxygenated sesquiterpenes correspondingly lower. The amounts of the latter appear to be more variable, and the cadinol type are almost absent. In contrast, the cadinene–muurolol isomers were present in similar amounts as in *J. virginiana*. Again, no consistent difference between male and female plants was found.

The plant-to-plant variation of the foliage oil terpenes of *J. horizontalis* (see Table 4) is of the same order as in *J. virginiana* and *J. scopulorum* but the aromatic ethers are virtually absent. As in *J. scopulorum* sabinene is the major component, but the amounts of limonene are much more variable, and chemovars B and C are present in about

Table 3. Tree-to-tree variation of the relative percentages* of the major components of the foliage oils of 10 *Juniperus scopulorum* trees from south-western British Columbia (Keremeos district)

Tree no.	1	2	3	4	5	6	7	8	9	10
α-Thujene α-Pinene	3	4·5	6	5	4·5	4·5	6	4	3·5	4·5
Sabinene	34	45	48	32	40·5	37·5	35	40	36·5	50
Myrcene	1	1	1·5	1	1	1	1	1	1	1
Limonene	1·5	1·5	2·5	1·5	2·5	1	1	1	1·5	1·5
Terpinenes†	6	6·5	6·5	4·5	5·5	3·5	4	4	4·5	5
Terpinen-4-ol	8	9	8·5	11	15	12	12	8·5	13·5	10
Sabinene hydrates	2	2	0·5	1·5	1	1·5	1·5	2	1	1·5
Citronellol	← less than 0·5% in all →									
Methyl citronellate	4	3·5	8	5·5	6·5	3·5	4·5	7	5·5	9
Cadinenes	2·5	3	1	2·5	3·5	5	1·5	2	1·5	1·5
Cadinols + acetates	← less than 0·5% in all →									
Elemol + acetates	37	21·5	18	31	20	25	30	27·5	27·5	10
Aromatic ethers	1	1	6·5	1·5	31·5	29	26	29	10	27·5

* Percentage of terpenes calculated after deduction of aromatic ether peak areas.

† α- and γ-terpinene and terpinolene.

Table 4. Plant-to-plant variation of the relative percentages of the major components of the foliage oils of 10 *Juniperus horizontalis* plants from the Saskatoon area*

Plant no.	1	2	3	4	5	6	7	8	9	10
α-Thujene α-Pinene	4·5	4·5	5	5	4	5·5	4	5	5	4
Sabinene	35	24·5	38·5	42·5	41	32·5	41	39	39	32·5
Myrcene										
Limonene	11·5	15·5	0·5	0·5	0·5	13·5	0·5	2	2	9·5
Terpinenes†	4·5	3·5	2·5	1·5	2·5	2	2	2·5	1	2
Terpinen-4-ol	6	4·5	5	2	2	1·5	2	1·5	1·5	2
Citronellol	5·5	8	1	2	2·5	3	2·5	7·5	7	3
Methyl citronellate	← all less than 0·5% →									
Sabinene hydrate	1	0·5	0·5	0·5	1	0·5	1	0·5	0·5	0·5
Cadinenes	2·5	4	8	11	11	9·5	11	8	7·5	12
Cadinols + acetates	21	23	26	25	24	24	22	19	21	24
Elemol + acetates	3	5	1	0·5	1	1	2·5	1	2	1
Aromatic ethers	← all less than 0·5% →									

* Saskatchewan River bank, Saskatoon to Beaver Creek, October 1970.

† α- and γ-terpinene and terpinolene.

equal numbers. The content of methyl citronellate was below 0·5% in each sample and citronellol appears to be present in larger relative amounts than in the other 2 juniper species. The most distinguishing feature is the presence of about 20% cadinol-type sesquiterpenes, 3–12% cadinene isomers and only 5% or less of elemol and eudesmol isomers. In most populations no consistent differences were found between female and male plants, but one male plant from a northern location in Saskatchewan had a non-typical foliage oil composition.

It follows that in all 3 juniper species the tree-to-tree variation is relatively high and that at least 10 trees per population or site should be sampled to obtain representative means (see below).

Variation between populations

The locations of the different collection sites of *J. scopulorum* (No. 1–5, 13–17), *J. horizontalis* (No. 6–12) and *J. virginiana* (No. 18–23) as well as 2 hybrid populations (A and C) analyzed in this study are shown in Fig. 1. The Keremeos (No. 1) and Saskatoon (No. 7) populations (different trees or plants) were resampled after several years to determine possible variations over longer periods. Also, 2 adjacent collection sites in a given population (Keremeos, No. 1; Saskatoon, No. 7; and Rondeau, No. 18) of each species were sampled to determine the variability of the means from 10 trees within a given population.

In Table 5 the means of 10 plants each from eight different collections of *J. horizontalis* are listed. Collection sites 6 (Sandy Lake) and 7 (Saskatoon district) are similar ecologically (Saskatchewan River banks) and hence may be considered to be one population. However the means of the terpene percentages are not the same. Even the means from the collections from the Saskatoon area in the fall of 1970 and 1973 (No. 7a and 7b) show variations of several per cent. This is owing to different plants having been sampled in the repeat collections; when the same plants were resampled the degree of variability was much lower. Thus, the means from 10 plants still reflect the relatively high variation from one plant to another. The differences between the means from Saskatchewan River crossing (No. 8) and the Saskatoon-Sandy Lake areas are of the same order, which is as expected from the overall similarity in ecology of these collection sites. The overall terpene patterns from these three collection sites are similar and one may conclude that when 10 samples per site are analyzed a "typical" mean for *J. horizontalis* of these sites of Saskatchewan is approached. If one compares this with the means

Table 5. Mean percentages of the major components of the foliage oils of 10 plants each of *Juniperus horizontalis* from 8 different collection sites

Site no.	6	7a†	7b†	8	9	10	11	12
α-Thujene	1·5	1·5	1·5	1·5	1·5	1	1	1
α-Pinene	3	3	3	4·5	5·5	4·5	3·5	5
Sabinene	40	36	39	46	42	35	25	32
Myrcene	3·5	3	3	4	4·5	3·5	3·5	4
Limonene	2·5	6	1	5·5	15	18	34	16
Terpinenes	5	2·5	3·5	4·5	3	2	2·5	2·5
Terpinen-4-ol	10	3	3	6	3	1·5	2	2
Sabinene hydrates	0·5	1	0·5	← less than 0·5 →				
Citronellol	3	4	3	2	3	1	1	1
Methyl citronellate	← all less than 0·5% →							
Cadinenes	7	8·5	11	6	6	8	6	8
Cadinols + acetates	18	22·5	24	14	15	17	15	18
Elemol + acetates	1	2	2	2	1·5	3	1	3·5
Aromatic ethers	← all less than 0·5% →							

* No. 6 = Sandy Lake, No. 7 = Saskatoon district, No. 8 = Saskatchewan River Crossing, Saskatchewan; No. 9 = Drumheller area, No. 10 = Gap-Kananaskis, No. 11 = Cowley, Alberta, No. 12 = Missouri River crossing and Grass range Montana (see also Fig. 1).

† 7a, October 1970; 7b October 1973.

Table 6. Mean percentages* of the major components of the foliage oils of 10 trees of *Juniperus scopulorum* of each of 10 different collection sites†

Site no.	1a	1b	2	3	4	5	13	14	15	16	17
α-Thujene	1·5	2	1·5	2	1·5	1·5	2	2	2	1·5	1
α-Pinene	2·5	3	2	5	2·5	3	3	2·5	3·5	3·5	3
Sabinene	40·5	44·5	34	34	29	42	38	36	40·5	48	46·5
Myrcene	1	1	1	1	1	1·5	1	1	2	2	2
Limonene	1·5	2	4	11	10·5	9	2	6·5	10·5	2·5	2
Terpinenes	8	4·5	2·5	2·5	3	5·5	3·5	5·5	7·5	7	6
Terpinen-4-ol	11	6	5·5	2·5	3·5	7	4·5	5	8	9	6·5
Sabinene hydrates	2	2	0·5	4	2	2	2·5	1	1·5	2	2
Citronellol	0·5	1	1	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Methyl citronellate	6	5	2	3·5	1·5	6·5	0·5	0·5	0·5	0·5	0·5
Cadinenes	2·5	2	1	0·5	1·5	2	1·5	1·5	1	0·5	1·5
Cadinols + acetates	← less than 0·5 →					1·5	← less than 0·5 →				
Elemol + acetates	18	23	39	32	41	16	37	35	18	17	23
Aromatic ethers	19	18	6	2	1·5	3·5	0·5	0·5	0·5	0·5	0·5

* Percentages of terpenes calculated after deduction of aromatic ether peak areas.

† No. 1 = Keremeos (a) March 1970, (b) October 1973, No. 2 = Trapp Lake, No. 3 = Dutch Creek, No. 4 = Windermere and No. 5 = Golden, British Columbia; No. 13 = Billings and No. 14 = Garrison, Montana; No. 15 = Cortez, No. 16 = Alamosa and No. 17 = Manitou Springs regions, Colorado.

from the three sites in Alberta (No. 9–11) and one from Montana (No. 12), the overall similarity in means is maintained except with respect to sabinene and limonene. The content of the latter is markedly higher in the samples from Alberta and Montana and it appears that this is a good measure of possible differences in creeping juniper populations. In several plants from the Cowley area of Alberta (No. 11) the limonene content approached 40%; that of sabinene varied between 20 and 30%. In all samples the cadinene type sesquiterpenes predominated markedly over the elemol–eudesmol type and the content of methyl citronellate was less than 0·5%. This, as well as the practical absence of the aromatic ethers, appears to be a species characteristic of *J. horizontalis*. The content of citronellol and sabinene hydrate isomers was lower in the samples from Alberta than in those from Saskatchewan, but because of the relatively large plant-to-plant variability this requires many more samples and statistical treatment for further evaluation.

The means from 2 collections in different years from the Keremeos area of southwestern British Columbia (No. 1) are shown in the first 2 columns of Table 6. These agree fairly well, but those from the Trapp Lake area (No. 2), which is ecologically similar, differ more markedly. The means from Dutch Creek (No. 3) and Windermere (No. 4) of southeastern British Columbia are fairly similar but those from Golden (No. 5), which is again an ecologically similar region, differ more extensively. However, the Golden area may already be influenced by gene exchange with the hybrid population at Banff (Site A). The finding of a mean of 1·5% cadinol isomers would lend support to this (of the individual 10 trees sampled near Golden, 7 had the *J. scopulorum* terpene characteristics, but 3 showed distinct trends towards *J. horizontalis*). If one compares the means from the populations from British Columbia with those from Montana (No. 13 and 14) and Colorado (No. 15–17) then a noteworthy difference in the aromatic ether content is obvious. A similar difference exists also in the content of methyl citronellate. Since the cadinol isomer content was less than 0·5% in all samples, this cannot be ascribed to gene exchange with *J. horizontalis* via hybrid populations at the overlap of their natural range.

Table 7 lists the means of 6 different *J. virginiana* populations. Duplicate collections were made in the vicinity of Rondeau (southern Ontario, No. 18a and 18b), Alamance County (North Carolina; No. 22a and 22b) and near Austin (Texas, No. 23a and 23b). Again, the variation between two sites

Table 7. Mean percentages* of the major components of the foliage oils of 10 trees of *Juniperus virginiana* from 6 different collection sites

Site no.	18a	18b	19	20	21	22a	22b	23a	23b
α-Thujene	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
α-Pinene	3	6	2·5	4·5	2·5	0·5	1	1·5	1
Sabinene	9	6	11	28	14·5	1·5	4·5	22	11
Myrcene	1	1	1·5	1·5	1	1	1	1	1
Limonene	17	22	23	12	9·5	29·5	28	4	20
Terpinenes	2	1	1·5	4	1·5	1	1	2·5	1·5
Terpinen-4-ol	2·5	1·5	2·5	7·5	4·5	2·5	2	3·5	2
Citronellol	1·5	1	0·5	1	1	0·5	0·5	0·5	0·5
Methyl citronellate	5·5	2·5	4·5	1·5	3·5	4·5	3·5	3·5	3
Cadinenes	2·5	3·5	4·5	2·5	3·5	3	2·5	2	4
Cadinols + acetates	← all less than 2% →								
Elemol + acetates	42·5	39·5	36·5	35	42	42·5	41·5	41·5	44·5
Aromatic ethers	11	11	4·5	4·5	5	18·5	18·5	16·5	10·5

* Percentage of terpenes calculated after deduction of aromatic ether peak areas.

† 18 = Rondeau, 19 = St. Williams, 20 = Napanee, and 21 = Thousand Islands, Ontario; 22 = Alamance County, North Carolina; 23 = Austin, Texas.

that can be considered as being in the same population is as large as that between more distant ones, except in the case of those from Alamance County, North Carolina. If one compares the terpene patterns of *J. virginiana* from this area with those of *J. scopulorum* from British Columbia, the respective mean percentages of sabinene and limonene, and possibly also those of the terpinenes and terpinen-4-ol, may serve as distinguishing chemical characteristics. If, however, one takes the means from the *J. virginiana* populations of southern Ontario or Texas, differentiation becomes more difficult. This result would lend some support to the possibility that the populations found in Texas may be the result of a more ancient introgression between *J. virginiana* and *J. scopulorum* [19]. Clearly, for all future studies involving these juniper species at least 20 samples per collection site and computerized treatment [17, 19] of the terpene percentages should be considered.

Hybrid populations

Juniperus horizontalis and *J. scopulorum* are known to hybridize in several areas where their natural ranges overlap [5, 6]. Samples from the hybrid swarms at Banff, Alberta (Site A) and the Little Missouri Badlands, North Dakota (Site C) were obtained and their terpene compositions are shown in Tables 8 and 9. At both locations prostrate (creeping) plants, trees of 2–5 m height, and intermediate forms may be found. Three trees of each form were sampled near Banff and the foliage oil compositions of all clearly indicate intermediacy if one accepts that the sesquiterpenes may be used as a measure of this. However, no correlation between low, intermediate and tall forms and the percentage of the cadinol isomers is evident; in fact one tall tree contained 7% of these whereas all 3 low, spreading plants contained 3–5%. In contrast, the content of aromatic ethers appears to be more indicative in this respect. However, because of their sporadic presence in *J. scopulorum* this may only be a useful measure when the means from many hybrid plants are compared. The percentages of the monoterpenes are highly variable and no distinctive correlations are apparent. The same overall conclusions may be drawn for those from the second hybrid swarm (Table 9) although in all three low, prostrate plants the content of the cadinol isomers was about 15%, which is typical for *J. horizontalis*.

Table 8. Percentages of the major components of low, intermediate, and tall juniper plants from the hybrid population at Banff, Alberta*

	Low, spreading			Intermediate			Tall, tree-like		
	a	b	c	a	b	c	a	b	c
α-Thujene	0·5	1	1	0·5	1	1·5	1	1·5	1·5
α-Pinene	2	2·5	3·5	3	3	3·5	2·5	2·5	3·5
Sabinene	26	49·5	33·5	49·5	32·5	41·5	50	46·5	36
Myrcene	1	3	2	2	2	1·5	2·5	1·5	1
Limonene	27	10	1	1	20	1·5	2	1·5	1
Terpinenes	3	4·5	1·5	5	3·5	3	6	5·5	3
Terpinene-4-ol	5	5	1·5	6·5	4·5	3·5	7	9	5·5
Sabinene hydrates	2·5	2·5	0·5	6	1·5	0·5	4·5	2	1
Citronellol	0·2	0·3	0·2	0·2	0·1	0·2	0·5	0·2	0·3
Methyl citronellate	3·5	0·5	0·5	0·5	0·5	2	1	2	2
Cadinenes	2	1·5	3	0·5	4	1	1·5	0·5	1·5
Cadinols + acetates	4	3	5	1·5	7	3	3	1·5	3·5
Elemol + acetates	19	15	36	22	13	35	15	18	34
Aromatic ethers	0·2	0·3	0·1	0·2	4·5	0·2	0·2	12	4

* Site A, Fig. 1; three plants of each type (a, b, c).

Table 9. Percentages of the major components from 10 juniper plants of the hybrid population in the Little Missouri Badlands, North Dakota*

Sample no.	1	2	3	4	5	6	7	8	9	10
α-Thujene	1	1·5	1·5	2·5	2·5	2	2·5	3	3	3
α-Pinene	4·5	6·5	3·5	3	2·5	4	7·5	3·5	2·5	4
Sabinene	26	29	39	35	29	34·5	38·5	35	27.	43
Myrcene	4	4	5·5	2·5	1·5	2	3	2	1·5	2
Limonene	27·5	15	1·5	1·5	3	2	2	3·5	3·5	2
Terpinenes	5	8	12·5	10	11·5	9	8	10	11·5	5
Terpinen-4-ol	5	8	9·5	11	12	9·5	9	12	15·5	11
Sabinene hydrates	1	1	0·5	1	1	1	1	1·5	1	0·5
Citronellol	← all less than 0·5 →									
Methyl citronellate	0·5	0·5	0·5	2	2	3	2·5	trace	3	3·5
Cadinenes	3·5	5	6	1	0·5	4	2·5	1	0·5	0·5
Cadinols + acetates	14	16	16	3	0·5	3	4	3	1	1
Elemol + acetates	3	2	2	18	31	23	16	21	20	16
Aromatic ethers	0·1	0·1	0·2	1	0·5	0·5	0·5	0·5	1	0·5

* Site C, Fig. 1.

† No. 1-3: low prostrate shrubs; No. 4–6: intermediates; No. 7–10: tall, tree-like plants.

Unfortunately, no samples from possible hybrid swarms of *J. horizontalis* and *J. virginiana* could be obtained. Although their natural ranges are reported to overlap in southern Ontario (see Fig. 1) only typical *J. virginiana* trees were found at collection sites 20 and 21.

Detection of hybrids between *J. scopulorum* and *J. virginiana* may be more difficult on a chemical basis. Just as with the morphological characters [5, 6] the terpene patterns of "typical" parent populations of *J. scopulorum* from British Columbia or Montana on the one hand (see Table 6) and *J. virginiana* from the central Appalachians on the other (see Table 7) are fairly well differentiated, but several *J. virginiana* trees from either southern Ontario or Texas have terpene patterns that approach those of *J. scopulorum*. In view of the relatively high tree-to-tree variability in both species, extensive sampling and statistical treatment, preferentially by algorithms [13, 36] are prerequisites for further population studies. However, a simple chemical technique is the determination of the number of trees in each population which have the sabinene–limonene ratios of chemovars A, B and C respectively, since chemovar A predominates in the *J. virginiana* populations of the central Appalachians but is absent in the *J. scopulorum* trees thus far analyzed.

DISCUSSION

The within-plant variation of the relative terpene percentages is at acceptable limits for population studies provided each plant is adequately sampled during the dormant season. The complex composition of the foliage oils and the labile nature of some components create some resolution problems and the reproducibility is not as high as that in other conifer species (e.g. *Picea glauca*, Douglas-fir, *Abies* species [7]). Resolution can be improved by lower temperature programming rates, but the longer residence time in the GLC column causes more decomposition of the labile components thus reducing the reproducibility. However, if the method of combining the relative amounts of the closely related components is acceptable, shorter GLC columns can be used, which reduces decomposition and errors and decreases the analysis time.

The within-population variation is relatively high in all three juniper species and 10 samples per collection site may not suffice in all instances to obtain representative means. However, if only the means are required, combining the foliage samples from one site, irrespective of their numbers, requires only one steam-distillation and one set of analytical operations. This is a major advantage over the use of morphological characters.

The 3 juniper species of the Virginiana complex can be distinguished chemically by comparing the means of the foliage oil composition of homogeneous populations of each species. *J. horizontalis* is characterized by the relatively large percentages of the cadinane-type sesquiterpenes and less than 3% of the elemol–eudesmol types. In *J. scopulorum* and *J. virginiana* their ratios are reversed, less than 0·5 and 2% of the cadinol isomers being characteristic respectively. It appears that methyl citronellate and citronellol may have some diagnostic value as well. Of particular interest is the ratio of the relative amounts of sabinene and limonene. As pointed out above, three different chemical types or chemovars (A, B, C) may exist in *J. virginiana* but only one (type B) predominates in *J. scopulorum*. Types B and C are found in *J. horizontalis*, but their ratio appears to be different in the populations from Saskatchewan (more of type C) than in those from Alberta (more of type B). Just as with different populations of *J. virginiana*, measurement of this ratio may provide a simple indicator of clines within *J. horizontalis* and possibly also in *J. scopulorum*. In the *J. virginiana* populations of the central Appalachian region the sabinene percentage is below 10% in all samples and limonene above 25%. In the divergent populations of the Southwest (Texas) or North (Ontario) the ratio becomes much more variable. Detailed statistical analysis of the percentage data of all the individual components of the foliage provides a more refined, albeit much more complex, method of population analysis. A recent improvement in the use of algorithms by Flake and Turner [36] confirmed the clinal variation in *J. virginiana* populations. In addition it showed a more distinct clustering of the populations from the southwest (Texas, Oklahoma), i.e. the chemical data appears to reflect division into northeastern and southeastern races as found by Hall [1] on the basis of habital features. Flake and Turner are currently applying this refined analysis to a study of different populations of *J. scopulorum*.

EXPERIMENTAL

Foliage samples were transported in ice-cooled insulated boxes and stored at −20° until steam-distillation. Woody branches were removed, frozen foliage macerated and steam distilled for 6 hr and the volatile oil was recovered as described before [23, 37]. In some expts the distn was continued for a further 18–24 hr. In addition to the standard 3·5 m polyethylene glycol (PEG) and SE-30 GLC columns, those with 2% PEG plus 1% OV-17 (mixed in soln before application to solid support, 70–80 mesh, DCMS-treated high performance Chromosorb G) and 2% PEG plus 1% QF-1 as liquid phase were used. Relative percentages of the individual components were obtained by summation of peak areas with digital electronic integrators. Preparative separations were carried out as in refs. [23, 37]. Identity of the known components was confirmed by comparison of IR and PMR spectra. Peaks No. 6, 17 and 21 were found to be α-terpinene, *trans*- and *cis*-sabinene hydrate. Several cadinyl type sesquiterpenes were found in the elemol–eudesmol series of peaks (Nos. 42–52). Larger amounts were obtained from *J. horizontalis* foliage oil (extended distillation): Peak 42:—a cadinyl acetate: IR 885, 1648 and 3080 cm^{-1} ($>C{=}CH_2$) 1240 and 1710 cm^{-1} ($-COMe$); PMR τ 5·47 (2H, *d* 9 cps, $>C{=}C\underline{H}_2$), τ 7·91 (3H, *s*, $-COC\underline{H}_3$), τ 8·22 (3H, *s*, $>C{=}C{-}C\underline{H}_3$), τ 9·35 and 9·07 (6H, 2x*d*, 6·5 cps, $-CH(C\underline{H}_3)_2$). Peak 44–45:—a labile cadinyl acetate: PMR τ 5·00 (1H, *s*, $>C{=}C\underline{H}$) τ 7·72 (3H, *s*, $-COC\underline{H}_3$), τ 8·80 (3H, *s*, $-C(OAc)C\underline{H}_3$) τ 9·15 and 9·07 (6H, 2x*d*, 6·5 cps, $-CH(C\underline{H}_3)_2$). Peaks 48–49 contained a mixture of *T*-cadinol and *T*-muurolol and peaks 51–52 α-cadinol as determined by comparison of PMR spectra [38].

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