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A Comparison of the Volatile Oils of Mature Versus Young Leaves of *Juniperus scopulorum*: Chemosystematic Significance

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Key Word Index—*Juniperus scopulorum;* Cupressaceae; terpenes; variation; chemosystematics; ontogeny.

Abstract—The volatile oils of leaves of clonal, greenhouse grown trees of *J. scopulorum* cv. *platinum* were examined from young (juvenile or whip leaves) and mature (adult or scale leaves) foliage. Of 36 compounds subjected to analysis of variance, 19 showed significant differences between young and old leaves. In general, the hydrocarbon terpenoids were higher in concentration in new leaves while the oxygenated compounds were higher in the older leaves. This same pattern has been reported in several other gymnosperms. Numerical taxonomy using data on the volatile oils from young and mature leaves yielded the same relative similarities among the five trees examined. Surprisingly, the plants were more similar in the volatile oil from the young leaves than from the mature leaves. The mixing of different ages of leaves in samples for chemosystematic studies is, however, to be discouraged.

Introduction

The widespread use of terpenoid data for chemosystematic purposes demands constant attention to the analysis of sources of errors and ways to minimize them [1–4]. In the genus *Juniperus*, we have reported on sampling differences [1], seasonal differences [5–6], sexual differences [7], diurnal variation [8] and, of course, population variation [9–12].

Von Rudloff has been tireless in cautioning chemosystematists about using mixed samples of young and mature foliage (see [13]), and in early work on *Tanacetum vulgare* L. [14] mentioned that young leaves produced a quite different array of terpenes than mature ones. Scora and Torrisi [15] analyzed 2-month-old vs 9-month-old leaves of *Citrus sinensis* var. *valencia* for 23 terpenoids: they found the concentrations of 12 compounds were highly significantly different (1% level) and two other compounds were significantly different (5% level). Interestingly, quantities of all the monoterpenes were smaller in the young leaves while all the oxygenated terpenes were larger in the adult leaves.

In the gymnosperms, Zavarin *et al.* [16], using seven monoterpenes, found the young leaves (<1 yr old) of *Pinus ponderosa* had larger amounts of 3-carene and terpinolene and less β-pinene than mature leaves (1–3 yr old). No differences were observed between the latter. They recommended omitting young leaves from samples used for chemosystematic studies in order to reduce variability. Levinson *et al.* [17] investigated the young and mature leaves of *Sequoiadendron giganteum* and found several differences in the volatile oils. Most noticeable was the absence of allyl phenyl ethers (safrole, eugenol, *O*-methyl eugenol and elemicin) in the young foliage. These compounds were thought to be associated with lignification and increased as the leaves matured. In a careful study on young and older foliage of white spruce (*Picea glauca*), von Rudloff [18] reported substantial differences in terpenoid composition between young and older foliage. This coincided with the flushing of new buds and major growth (May and June). By the end of June, the new needles appeared to be chemically similar to the older shoots.

Powell and Adams [6] found that significant seasonal variation occurred in many terpenoid compounds of *Juniperus scopulorum.* These changes were mostly correlated with growth. No effort was made in this study to distinquish between new leaves and older foliage since the purpose was to measure changes in typical samples which might be collected by chemosystematists. Many of the observed differences, however, were thought to be due to differential terpene concentration in the young (juvenile) leaves and the purpose of this paper is to report on these differences between the young and mature leaf oils in *J. scopulorum*

Results

Of the 68 compounds detected during analysis,

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32 were discarded because of their small concentration (never larger than 0·1% of the total in either sample set). Of the 36 compounds used in analysis of variance (AVOVA), 19 showed significant differences (Table 1).

TABLE 1. CONCENTRATIONS OF 36 COMPOUNDS FROM THE LEAF OILS OF *J. SCOPULORUM* WITH AN AVERAGE GREATER THAN 0·1% TOTAL OIL IN EITHER OLDER OR YOUNG LEAVES

Compound	Older leaves	Young leaves	*F* ratio	Significance*
3 α-Pinene/α-thujene	1·58	2·01	4·90	n.s.
7A Sabinene	32·56	39·18	8·70	*
8	0·27	0·12	1·58	n.s.
10 Myrcene	0·70	2·18	71·49	**
12 α-Terpinene	0·38	0·67	34·53	**
14 limonene	0·91	1·21	10·70	*
15 β-Phellandrene	0·11	0·14	0·09	n.s.
17 γ-Terpenene	1·47	1·68	4·71	n.s.
20 Terpinolene	0·39	0·66	74·49	**
33 (Alcohol)	1·25	1·31	0·29	n.s.
38 (Linalool)	3·75	8·40	20·94	**
40B Methyl citronellate	5·33	4·29	6·54	*
40A	0·65	0·51	2·04	n.s.
42 Terpine-4-ol	2·13	2·93	16·12	**
51A	0·46	0·31	3·65	n.s.
61	1·20	1·29	0·40	n.s.
72	0·50	0·12	48·71	**
73	0·13	0·03	10·75	*
76	0·08	0·14	1·34	n.s.
77	0·14	0·08	13·31	**
78	0·09	0·18	2·10	n.s.
79B	0·12	0·01	6·86	*
79A	0·27	0·12	16·27	**
80	1·43	1·33	0·76	n.s.
82 Elemol	8·39	5·74	24·29	**
83	17·72	15·61	4·65	n.s.
86	0·37	0·37	0·001	n.s.
87	0·19	0·00	5·35	*
88	0·24	0·23	0·003	n.s.
89	0·12	0·08	0·15	n.s.
90 } α/β-Eudesmol	0·93	0·45	14·45	**
91 }	1·19	0·74	10·75	*
95E	0·13	0·01	40·92	**
95D	0·42	0·71	3·05	n.s.
95B Acetate II	12·86	6·69	116·80	*
96	0·11	0·01	4·13	n.s.

F 0·01 = 11·26, *F* 0·05 = 5·32 (d.f. = 1/8).

Some of the compounds differed by large factors (compounds 10, 38, 72, 95E, 95B).

Figure 1 shows a bargraph of all compounds occurring in concentrations larger than 0·1% for young vs adult foliage. (The peaks are listed in the order of elution on a PEG column.) It is not difficult to identify *J. scopulorum* from the juvenile foliage alone, but one will notice a different pattern develops as the leaves age. The C_{10} hydrocarbons are found (Fig. 1) from peak 3 to about peak 20 with oxygenated terpenes and a couple of sesquiterpenes hydrocarbons between peaks 33 and 81 and oxygenated sesquiterpenes from about peak 82 onward. Almost all of the terpene hydrocarbons are found in larger quantities in the younger foliage. In contrast, almost all of the oxygenated and sesquiterpenoid compounds are in greater amounts in the older, adult foliage. Does this imply that the terpene hydrocarbons are produced first, then oxygenated? To examine this question further, we have examined quantitative terpenoid data on new vs old leaves from studies on spruce [18], redwood [17], ponderosa pine [16], and Valencia oranges [15] and compared these with the data reported here (Table 2). The trend in all four gymnosperms is for the hydrocarbon terpenes to be larger in the new foliage and for the oxygenated compounds to be larger in the older foliage. The trend in the single angiosperm (orange) is just the opposite. The data on *Pinus ponderosa* is not completely comparable since oxygenated terpenoid compounds were not analyzed, but it is interesting to note that all but one of the terpene hydrocarbons were larger in the new foliage while methyl chavicol (from the shimikic acid pathway) was larger in the older foliage. The data on *Sequoidadendrum* giganteum also includes compounds from the shimikic acid pathway, namely safrole, elemicin, eugenol, and *O*-methyl eugenol.

Relationships among taxa have been examined many times using terpenoid data. Figure 2(a) shows phenograms of 10 different samples of *J. scopulorum* examined here (five young and five old leaf samples) using equal weights for each of 36 compounds. Note that all five of the juvenile foliage samples cluster together. The level of similarity is not absolute but highly relative. Nevertheless, the order of entry seems significant. Examination of the similarity matrix (for Fig. 2a) revealed that the OTU M1177 (mature) came into the cluster via OTU M1178. Thus, the order of clustering appeared to be the same whether young or mature foliage was utilized. The young leaf samples cluster more closely, than do the adult leaf samples. This is surprising but it may be due to the fact that these plants were all rapidly growing and we had difficulty finding "pure" samples of mature foliage on most plants.

Figure 2(b) shows the phenogram of the same 10 OTUs but using 29 compounds with *F* ratios greater than or equal to 1·0. These characters were weighted by $F-1$ in the calculation of similarity ratios. This phenogram shows the young and mature leaf oils to be more distinct, but the *a priori* analysis of variance of young vs mature leaf oils and the subsequent use of *F* ratio weighting tends to

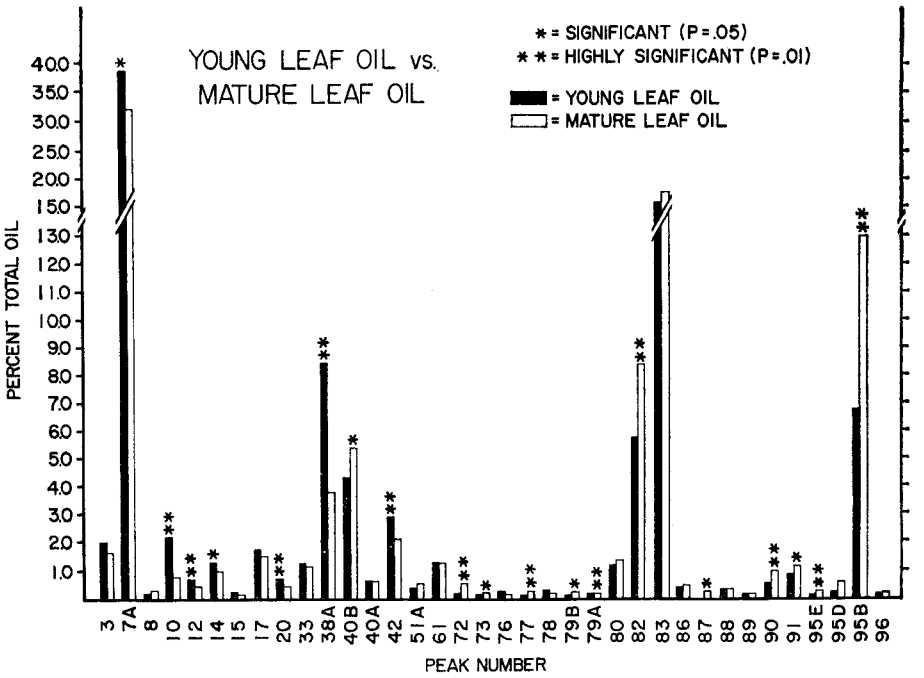

FIG. 1. A COMPARISON OF THE CONCENTRATIONS OF VARIOUS MAJOR COMPOUNDS IN THE LEAF OILS OF YOUNG AND OLD LEAVES OF *J. SCOPULORUM* CV. *PLATINUM*. Note that the hydrocarbons (peak 3–20) are larger in the young leaves whereas the oxygenated compounds are larger in the mature leaves. The level of significance is denoted by one or two asterisks for $P = 0{\cdot}05$ and $P = 0{\cdot}01$, respectively.

TABLE 2. COMPARISONS OF THE CONCENTRATIONS OF VOLATILE COMPOUNDS IN THE OILS FROM NEW AND OLD FOLIAGE FOR *SEQUOIADENDRON GIGANTEUM* [17], *PICEA GLAUCA* [18], *PINUS PONDEROSA* [16], *JUNIPERUS SCOPULORUM* (PRESENT STUDY) AND *CITRUS SINENSIS* VAR. *VALENCIA* [15]

Sequoiadendron	Highest in old	Highest in new	*Picea* (15 Dec. sample)	Highest in old	Highest in new	*Juniperus*	Highest in old	Highest in new	*Pinus*	Highest in old	Highest in new	*Citrus*	Highest in old	Highest in new
α-pinene		×	non-terpene		×	sabinene		×	β-pinene	×		α-pinene	×	
unknown	×		tricyclene		×	3-carene		×	3-carene		×	Phellandrene	×	
unknown		×	α-pinene		×	myrcene		×	myrcene		×	limonene	×	
myrcene		×	β-pinene		×	cpd. 13		×	limonene		×	γ-terpinene	×	
limonene	×		myrcene	×		terpinolene		×	β-phellandrene		×	ρ-cymene	×	
terpine-4-ol		×	limonene		×	(linalool)		×	terpinolene		×	octanal		×
caryophyllene	×		β-phellandrene		×	cpd. 40	×		methyl chavicol	×		methyl heptenone		×
α-terpineol	×		terpinolene		×	terpine-4-ol		×	(monoterpenes			octyl acetate		×
humulene		×	camphor	×		cpd. 72	×		+methyl			decanal		×
citronellol	×		terpine-4-ol	×		cpd. 73	×		chavicol only)			linalool		×
unknown	×		borneol	×		cpd. 77	×					neryl formate		×
safrole	×		α-terpineol	×		cpd. 79B	×					decyl acetate		×
O-methyl eugenol	×		piperitone	×		cpd. 79A	×					citronellol		×
elemicin	×		bornyl acetate	×		elemol	×					geranial		×
			cadinenes		×	cpd. 87	×							
			cadinols		×	(α/β eudesmols)	×							
						cpd. 95A	×							
						acetate II	×							

push the groups farther apart. The order of entry into the clusters differs from young to mature oil samples in contrast to Fig. 2(a) where the entry was the same. It is doubtful if one could consistently get the same relationships using young leaves as using mature leaves. The young leaf oils cluster a little closer than the mature leaf oils as in Fig. 2(a), but probably not significantly different.

Conclusion

Differences in the quantities of certain terpenoids in the volatile oil in the young and mature leaves in *J. scopulorum* were quite

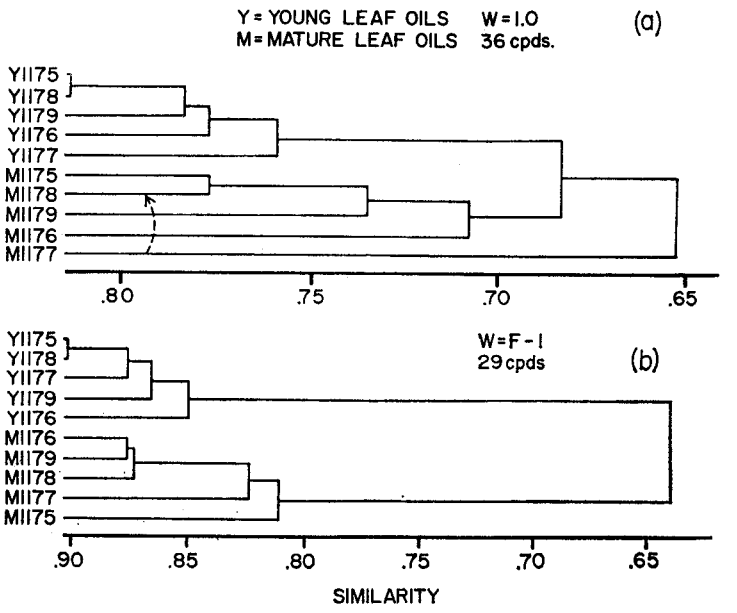

FIG. 2. (a). A SINGLE LINKAGE UNWEIGHTED PHENOGRAM OF 10 SAMPLES FROM *J. SCOPULORUM* CV. *PLATINUM*. The tree numbers preceded by a Y are the young leaf samples and those preceded by an M are the mature leaf samples. The dashed lines from OTU M1177 to OTU M1176 indicates that the former OTU entered the cluster via its similarity to OTU M1176. (b). A SINGLE LINKAGE, $F-1$ WEIGHED PHENOGRAM OF THE SAME 10 SAMPLES AS IN (a). Note that the reduced character set (29 compound swith F ratios larger than 1·0) and character weighting ($F-1$), leads to a larger split between young and mature leaf oils. The order of entry into clusters is changed from (a).

significant. These differences must be taken into consideration when sampling for chemosystematic studies. The results indicate, however, that different similarities could be obtained whether young or mature foliage was used, according to weighting. It is obvious that comparing young foliage from one plant to mature foliage to another plant would distort such similarities and should be avoided. The young leaf samples seem to be more uniform than the mature leaf samples and this may have been due to sampling. Further work is needed to clarify the situation. In *J. scopulorum,* the hydrocarbon terpenes are higher in concentration in the new leaves than in old leaves and the oxygenated compounds show the opposite behaviour. This agrees with observations on other gymnosperms, but the reverse trend has been reported in Valencia oranges.

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Experimental

Clonal plants of *Juniperus scopulorum* cv. *platinum* were used to minimize extraneous variation. Plants were obtained from Willis Nursery in 1973 and grown in the greenhouse to eliminate climatic differences. In July, 1975, five trees were sampled. Since juvenile foliage in *Juniperus scopulorum* is characterized by large whip leaves whereas the mature foliage has leaves which are very small and scale-like, the young and mature leaves can be separated. The foliage was collected, separated into young and mature leaves, frozen within minutes, and steam distilled (as outlined in [6]) within 5 hr of collection. The volatile oil was separated by capillary GLC (see [6] for column conditions) and quantitated by an electronic digital integrator. The peaks were then analyzed by analysis of variance to determine if significant differences occurred between young and mature foliage samples and to compute F ratios for use in character weighting. Identification of the compounds follows Powell and Adams [6] and von Rudloff and Couchman [19].

Similarity measures were computed using an unweighted and weighted ($F-1$) mean character difference [1] and single linkage clustering to examine the effects of the young and mature leaf oils on the phenetic similarity of these plants.

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