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SEASONAL VARIATION IN THE VOLATILE TERPENOIDS OF JUNIPERUS SCOPULORUM (CUPRESSACEAE)[1]

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ABSTRACT

Fifteen trees in a natural population of *Juniperus scopulorum* Sarg. were sampled at 4-week intervals throughout a year and the terpenoid constituents were extracted from the foliage. Several environmental variables were measured. Correlations with changes in volatile oil composition (computed as both relative percentages and as weights) were examined. Significant seasonal variations were discovered in both the relative percentage and weight data. These seasonal changes in the volatile oil composition were correlated with linear growth, temperature, yield of volatile oil, and dry weight of foliage. Seasonal variation in the terpenoids computed on a weight basis was found to be greater than the seasonal variation of the terpenoids computed as relative percents of the total oil. Evidence is presented which indicates that there is no stable period to sample on a weight basis, but sampling in the late fall and winter periods minimizes variation in the relative percentage data. The use of relative percentage data is thus encouraged for chemosystematic studies.

WITHIN THE LAST TWO DECADES a number of secondary plant constituents have been successfully utilized in resolving biosystematic problems. The widespread distribution, structural heterogeneity, chemical complexity, and apparent physiological stability of volatile terpenoids render them particularly useful in establishing phenetic and phylogenetic relationships. Yet there is a widespread contention among many chemosystematists that the volatile oils are "waste products" which are somehow immune to seasonal and ontogenetic changes.

The quantity and composition of volatile oils reflect the influence of genetic (Hanover, 1965, 1966, 1971; Hefendehl and Murray, 1971; von Rudloff, 1972b) as well as certain nongenetic variables. Diurnal fluctuations in the volatile oil of sage (*Salvia officinalis*) have been reported (Fluck, 1963). Scora and Torrisi (1966) encountered significant differences in the foliage essential oil of Valencia oranges when coastal and inland populations were compared. Fluck (1963) cited evidence which suggested that the terpenoids of several plant taxa were strongly influenced by climatic and edaphic conditions. Burbott and Loomis (1967) reported environmentally induced variations in the monoterpenes of peppermint (*Mentha piperita*) and, more recently (1969), discussed evidence for the metabolic turnover of monoterpenes, as have Croteau, Burbott, and Loomis (1972). Burbott and Loomis suggested that in peppermint the monoterpenes are utilized as substrates for energy metabolism when more suitable stored substrates become depleted within the secretory cells.

Several investigations on seasonal variations in the volatile oil composition have been made. The results are contradictory and reflect strongly the taxa and tissues examined. Von Rudloff and Underhill (1965) were unable to detect significant compositional variations in the volatile oil of mature tansy plants (*Tanacetum vulgare*) before, during, or after flowering. The volatile oil from young plants was more variable; evidence indicated an early synthesis of the sesquiterpenes and a labile C_5 alcohol. The volatile oil composition of mature wild mint plants (*Mentha arvensis* var. *glabrata*) was found to be remarkably consistant prior to, during, and after flowering (von Rudloff and Hefendehl, 1966). These results differed considerably from those obtained by Battaile and Loomis (1961) in their work on the biosynthesis of monoterpenes in peppermint (*Mentha piperita* 'Mitcham'). They provided evidence for the de novo synthesis of terpenes in young tissues with terpene interconversions restricted to older tissues.

Seasonal variations in the volatile oil composition of *Pinus* are poorly documented and contradictory. In a classicial study of pine oleoresins, Mirov (1961) found little seasonal variation in turpentine composition. Similar results were reported by Smith (1964) from his work on volatile oil variability in ponderosa pine (*Pinus ponderosa*) oleoresins. Zavarin et al. (1971) concluded that although major seasonal fluctuations in the volatile oil of ponderosa pine were restricted to the current year's foliage, increased metabolic activity

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during needle growth influenced mature as well as juvenile needles.

Von Rudloff (1962) noted significant variations in the volatile oils from white spruce (*Picea glauca*) and blue spruce (*Picea pungens*) foliage harvested at different times throughout the year. He concluded that α-pinene and 3-carene were produced in the early stages of growth. A somewhat later accumulation of camphor, free borneol, and possibly tricyclene, camphene, and *p*-cymene was observed. Recently, in a detailed study of seasonal variations in the volatile oil composition of white spruce leaves, buds, and twigs, von Rudloff (1972b) reported that major changes occurred only in the juvenile foliage. Minor variations were detected in the older foliage during the early summer months.

Adams (1969) reported seasonal variations in the volatile oil extracted from the foliage of a *Juniperus ashei* tree sampled at monthly intervals throughout a one-year period. Similar results were observed in a study of seasonal variation in the volatile oil composition of *Juniperus pinchotii* (Adams, 1970). Analysis revealed that the relative percentages of 25 of the 70 terpenoids composing the foliage volatile oil were quite significantly different when summer and winter collections were compared.

Most recently, Tatro et al. (1973) in a study of the volatile oil of *Juniperus occidentalis*, *J. osteosperma*, and *J. californica* concluded that "significant" seasonal variation was not occurring in these taxa, yet they present evidence that diurnal variations are occurring! In examination of the graphs (p. 238) it appears that there is a significant difference ($P = .05$) between the January and the June sample for sabinene in *J. californica*. It was unfortunate that variance intervals were plotted rather than 95% confidence limits, so that other compounds could be checked. In any case, their data (for percent calculations) might be interpreted to encourage or discourage random seasonal sampling and further clarification is needed.

The purposes of this study were: (1) to determine if the volatile oil composition of *J. scopulorum* varies throughout the year; (2) if changes do occur, how these changes in the volatile oil quantity and composition correlate with environmental and growth factors; (3) should individual compounds be quantified on the basis of weight per dry foliage or as percentages of the total oil extracted, in order to minimize the effects of seasonal changes when sampling for systematic studies?

MATERIALS AND METHODS—Fifteen trees of *Juniperus scopulorum* Sarg., appearing in good vigor, were selected from a natural population occurring approximately 13 miles southwest of Ft. Collins, Colorado. These were permanently labeled and fresh foliage samples were collected (as outlined by Adams, 1970) at 4-week intervals beginning May 1, 1971 and continuing through April 28, 1972 (i.e., 14 sampling periods). The fresh foliage samples were sealed in plastic bags and, within 2 hr of collection, stored at 0 C, pending steam distillation.

Prior to the initial sampling period, five twigs bearing terminal whips were selected on each tree and marked with India ink. The ink markings served as points of reference from which linear growth increments could be ascertained. Each twig was measured with a clear metric ruler at 4-week intervals throughout the study. The twig length recorded for the previous sampling period was subtracted from the current measurement to determine the growth increment for each twig. The mean growth increment for five twigs was used as an estimate of tree growth during the intersampling period.

Ambient air and soil temperatures were continuously recorded with thermographs. From these data the following variables were calculated: mean air and soil temperatures for the 4-week sampling periods; average maximum air and soil temperatures for the 4-week sampling periods; average minimum air and soil temperatures for the 4-week sampling periods; and average air and soil temperatures for the week prior to sampling. Precipitation for the sampling period and for the week prior to sampling was measured in the field and supplemented in the winter by data from the Colorado State University weather station.

Within 5 days after freezing, foliate samples were thawed, and excessive woody tissue and the female cones were discarded. Using a modified Clevenger type circulatory condenser, a 50 gram (green weight) portion of each foliage sample was steam distilled[2] as described by Adams (1969). After 24 hr of active distillation, the ether-terpenoid layer was removed and stored at –20 C in a tightly capped, preweighed glass vial. Prior to analysis on the gas/liquid chromatograph, the ether solvent was evaporated to approximately 20% of the sample by use of a jet of nitrogen. The oil samples were then weighed.

The foliage samples were removed from the distillation apparatus and dried for 48 hr in a chromatography oven maintained at 90 C. This time period is sufficient to establish a constant weight. The dried foliage samples were then weighed.

The volatile oils from the 210 samples (15 trees, sampled 14 times) were run in random order on a gas/liquid chromatograph with flame ionization detection. Separation was accomplished by a series of linear and isothermal temperature programs (see Powell, 1973 for detailed column conditions) on a capillary (200′ × .02″ I.D.)

[2] A 100 gram portion was steam distilled from each foliage sample for the first four sampling periods.

column coated with an 8% carbowax solution (PEG, 20 M). Due to the extraction of several high boiling compounds when 24-hr distillations were used, a 2-hr isothermal period (T = 215 C) was necessary to elute all of the components. Thus the total time of analysis was approximately 3 hr per sample. Identification of the major terpenoids composing the volatile oil of *J. scopulorum* had been previously accomplished by von Rudloff and Couchman (1964) and further substantiated by Adams (1969). The terpenoids were identified by comparison of their retention times with those of known compounds. A listing of the compounds encountered throughout the study is provided in Table 1. Of the 100 volatile oil constituents listed in Table 1, only 47 were consistently found in amounts greater than traces (.05%). The inability to consistently resolve tricyclene, α-pinene, and α-thujene into three distinct peaks prompted their consideration as a unit for further numerical analyses. Compounds 23 and 24 were also evaluated as a single entity.

Individual compounds were quantified with an electronic digitial integrator, coded (Adams, 1969), and key punched for computer analysis.

The percent yield of volatile oil from each foliage sample was calculated as follows:

$$\% \text{ oil yield} = 100.0 \times \text{oil wt.}/(\text{dry wt.} + \text{oil wt.})$$

The following formula was used to calculate the percent extracted dry weight for each foliage sample:

$$\% \text{ dry wt.} = 100.0 \times \text{dry wt.}/\text{fresh wt.}$$

The relative percentages of individual compounds were determined from the printed output supplied by the digital integrator. The weight of a compound (cpd.) was expressed as the number of grams of that compound per 100 grams of organic matter (= dry foliage wt. + wt. of the oil removed from the foliage sample before drying) and was computed as follows:

$$\text{wt. cpd. (i, tree j)} = \% \text{ cpd. (i, tree j)} \times \text{oil wt. (per 100 gms organic matter)}.$$

An analysis of variance (ANOVA) was performed on the percent yield, percent extracted dry weight, relative percentage of each compound, weight of each compound, and growth to determine which characters differed significantly from one sampling period to the next. Each character was further analyzed using the Student-Newman-Keul's multiple range test (Steel and Torrie, 1960) to determine which sample set means were significantly different.

Pooled sample correlation coefficients (r matrix) were calculated for the environmental data, growth data, percent extracted dry weight data, and all chemical data with F-ratios ≥ 1. The intercorrelation matrix allowed further insight into possible correlations between terpenoids, environmental variables, and growth activity. In addition, factor analysis was utilized to determine groups of mutually highly correlated variables.

To obtain a better understanding of the time of the year that the relative percentages of volatile oil constituents were least variable, the average annual percentage was calculated for each compound with an SNK test significant at the P = .05 level. For each of the 14 sampling periods an absolute deviation from its average annual percentage was then determined for each compound. The individual absolute deviations calculated for a particular sampling period were then summed;

TABLE 1. *100 compounds composing the volatile oil of foliage*[b]

Cpd. #	*Identity*	*Cpd. #*	*Identity*	*Cpd. #*	*Identity*
1	tricyclene	27		52*	
2	α-pinene/	28*		53	elemol
	α-thujene	29A*		54	
3*	(camphene)	29*		55	elemol
4*		29B*			acetate
5B*		30A*		56A*	
6A*	β-pinene	30		56*	
6	sabinene	31	(estragole)	57	γ-eudesmol
7A*		32		58	
7	myrcene	33A*		59	
7B*		33		60/	
8	α-terpinene	34		61	α-eudesmol/
9	limonene	35			β-eudesmol
10*	β-phel-	36	citronellol	62	
	landrene	37*		63*	
11	γ-terpinene	38A*		64*	
12	ρ-cymene	38*		65	
13	terpinolene	38B*		66A*	
14*		39		66*	
15A*		39B*		66B*	
15*		40		67*	
16*		40B*		67A*	
17	C_{10} alcohol	41*		68A	
18*		42		68	
19*		43		69A*	
20*		44*		69	acetate II[a]
21*	(camphor)	45A*		70	
22	linalool	45	methyl	70A	
23*			eugenol	70B*	
24	(methyl	46		70C*	
	citronellate)	47*		70D*	
25*	boryl	48		70E*	
	acetate	49		70F*	
26A*		50		70X*	
26	4-terpinenol	51		71	

[a] See discussion by von Rudloff and Couchman, 1964, and Vinutha and von Rudloff, 1968.

[b] Compounds tentatively identified are enclosed in parentheses. Those compounds which were generally present in amounts less than .05% (traces) are marked with an asterisk (*) following the compound number.

TABLE 2. *SNK tests for growth, percent yield, and percent extracted dry weight data. Any means not underscored by a common line are significantly different.*

Character = growth (P = .05)

Date	6/26	7/24	5/29	4/1	4/29	8/21	10/16	9/18	3/3	1/8	12/11	11/13	2/5
Mean	4.0	1.8	1.7	.89	.85	.60	.37	.33	.28	.24	.00	−.13	−.16
SNK													
TEST													

Character = percent yield (P = .05)

Date	11/13	10/16	1/8	9/18	12/11	3/3	2/5	4/1	4/29	8/21	7/24	6/26	5/1	5/29
Mean	8.2	8.2	8.1	7.9	7.8	7.7	7.3	7.2	7.0	6.8	6.5	6.4	6.0	5.9
SNK														
TEST														

Character = percent extracted dry weight (P = .05)

Date	8/21	4/1	4/29	5/1	5/29	2/5	7/24	12/11	6/26	3/3	11/13	1/8	8/18	10/16
Mean	42.4	42.0	41.1	40.1	40.1	40.1	39.6	39.4	39.0	38.9	38.7	38.7	38.0	37.5
SNK														
TEST														

an average absolute deviation was thus obtained. The same method was utilized to estimate at what time of the year the weights of volatile oil constituents were least variable. Computations for the weight data were based upon average annual weights for those compounds with significant SNK tests.

RESULTS—*Changes in linear growth (shoot elongation)*—Analysis of variance (ANOVA) revealed significant differences in linear growth activity during the year. The F-ratio (variance among sample means/variance within samples) for growth was 17.34 ($F_{.05} = 1.83$). The SNK test is illustrated in Table 2. Mean values not underscored with a common line are significantly different (P = .05).

Major growth activity was observed during May, June, and July; some twig elongation likely occurred prior to the first sampling period. Growth increments calculated for the early spring suggest the initiation of growth during March (Fig. 1). Changing air and soil temperatures were correlated with fluctuations in growth activity, but a direct correlation with precipitation was not in evidence (Table 3).

Changes in the yield of volatile oil—An F ratio of 9.80 from the ANOVA indicated significant differences were occurring in the percent yield of volatile oil during the year. The SNK test is shown in Table 2. Mean percent yields of volatile oil for several sampling periods were significantly different. The percent oil yield increased gradually throughout the summer and early fall, attaining a maximum value in mid-November (see Fig. 1). A decline was observed during the winter. When comparing percent yields of oil within a season, statistically significant differences were absent. Considerable variation was encountered, however, when spring and summer oil yields were compared with those calculated for the fall and

TABLE 3. *Intercorrelation matrix for growth, percent oil yield, and percent extracted dry weight with several environmental variables*

	PPT	MAT	AAH	AAL	MST	SAH	SAL	APW	SPW	RPW
GROWTH	.003	.666	.623	.628	.609	.584	.621	.383	.698	.044
% YIELD	−.116	−.586	−.556	−.570	−.551	−.533	−.553	−.257	−.615	.005
% DRY WT.	−.003	.123	.154	.087	.146	.152	.137	.065	.144	−.017

PPT, precipitation; MAT, average air temperature; AAH, average maximum air temperature; AAL, average minimum air temperature; MST, average soil temperature; SAH, average maximum soil temperature; SAL, average minimum soil temperature; APW, average air temperature for week prior to collection; SPW, average soil temperature for week prior to collection; RPW, precipitation for week prior to collection.

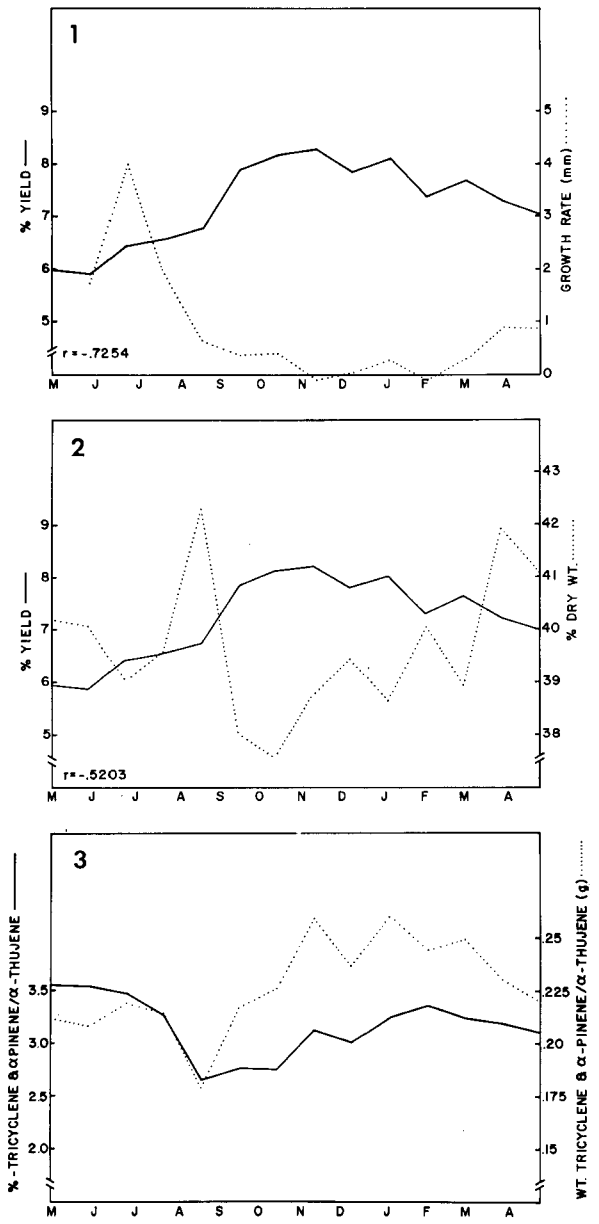

Fig. 1–3. **1.** Seasonal variations in the percent yield of volatile oil and the growth rate of *J. scopulorum*. See text for discussion. **2.** Graphical representation of the correlation between the percent yield of volatile oil and the percent extracted dry weight. **3.** Seasonal fluctuations in the relative percentage and the weight of compound 2, tricyclene + α-pinene/α-thujene. The relative percent of compound 2 exemplifies the first trend in the percent data.

winter sampling periods. Fluctuations in the yield of oil were negatively correlated with linear growth activity (Fig. 1). The correlations between percent oil yield and several environmental variables were low (Table 3).

TABLE 4. *Intercorrelation matrix for growth, percent oil yield, and percent extracted dry weight*

	GROWTH	% YIELD	% DRY WT.
GROWTH	1.00	–.725	.060
% YIELD	–.725	1.00	–.520
% DRY WT.	.060	–.520	1.00

Changes in the percent extracted dry weight—The percent extracted dry weight varied significantly during the year (Table 2), which was expected from the ANOVA ($F = 8.32$). The maximum dry weight was observed during August. Values observed during the fall and winter were low. Fluctuations in the dry weight could not be directly attributed to the environmental parameters measured (Table 3). The percent extracted dry weights were slightly correlated with the percent yield of volatile oil (Fig. 2). The correlation with growth was very small (Table 4).

Seasonal variations in the volatile oil components (computed as relative percents of the total oil)—Analysis of variance of the 47 nontrace components (see Table 1) revealed 19 compounds with significant F ratios. When these were subjected to the more conservative SNK test, 11 compounds were found to vary signficantly ($P = .05$). Examination of the SNK tests revealed three major trends among these 11 compounds. These major trends will be exemplified by a compound chosen to represent each trend.

The first trend is shown by compound 2 (tricyclene + α-pinene/α-thujene) in Table 5. The maximum percent was observed in May (Fig. 3). A decline was noted during the warm summer months and a minimum value was reached in mid-August. The percent of compound 2 increased gradually during the fall and winter months. This trend shows a small negative correlation with temperature, rainfall, and percent yield of volatile oil and a slight positive correlation with growth (see Table 6). Low percentages of compound 2 observed in the late summer and early fall may be the result of volatilization and/or resinification, but it is possible that these fluctuations merely reflect other compositional changes in the volatile oil.

The second trend is shown by compound 8 (α-terpinene), compound 13 (terpinolene), compound 26 (4-terpinenol), compound 32, and compound 35 is exemplified by compound 8 (α-terpinene). The SNK test is shown in Table 5. Maximum percentages were observed in June; a decline was noted in the summer and early fall with a second decline in March (Fig. 4).

Evidence suggests the possible synthesis of these compounds during periods of intensive growth activity (Table 6, Fig. 4). One may ex-

TABLE 5. *SNK tests for three compounds with significant seasonal variations in the percent composition of the volatile oil of J. scopulorum (P = .05). These compounds exemplify the three major trends encountered in the relative percent data*

Character = 2, tricylene + α-pinene/α-thujene														
Date	5/1	5/29	6/26	2/5	7/24	3/3	1/8	4/1	11/13	4/29	12/11	9/18	10/16	8/21
Mean	3.55	3.54	3.48	3.36	3.26	3.25	3.25	3.20	3.14	3.11	3.02	2.76	2.75	2.66
SNK														
TEST														
Character = 8, α-terpinene, (similar patterns were found for terpinolene, 4-terpinenol, compound 32, and compound 35)														
Date	6/26	7/24	5/1	2/5	8/21	5/29	9/18	4/1	1/8	4/29	12/11	11/13	3/3	10/16
Mean	1.83	1.77	1.67	1.57	1.56	1.54	1.50	1.50	1.49	1.46	1.44	1.41	1.36	1.25
SNK														
TEST														
Character = 31, (estragole), (similar patterns were found for compounds 39, 50, 70, and 71)														
Date	3/3	10/16	12/11	2/5	11/13	1/8	5/29	4/29	5/1	9/18	4/1	7/24	8/21	6/26
Mean	.22	.19	.19	.18	.18	.17	.17	.16	.15	.15	.15	.12	.11	.10
SNK														
TEST														

pect some loss of these rather volatile trepenoids during the warm summer months (Fluck, 1963), but it is unlikely that volatilization adequately explains the losses observed during the fall. Positive correlations with temperature (Table 6) further indicate that volatilization alone cannot account for the observed variations. Similar results were reported by Adams (1970) in a study of seasonal variation in the volatile oil constituents of *J. pinchotii*.

The third trend is exemplified by compound 31 (estragole?) and is shared by compounds 39, 50, 70, and 71. These compounds are all either highly oxidized terpenes or sesquiterpenes. They are rather nonvolatile and somewhat polar. The SNK test for compound 31 is shown in Table 5. The percent of compound 31 remained fairly constant during the winter months then increased to a maximum value in early March (Fig. 5). This trend was negatively correlated with temperature (Fig. 5) and growth and positively correlated with the percent yield of volatile oil (see Table 6).

Intercorrelation analyses between the compounds of trends one, two, and three showed (data available on request) that trend one had low correlations with trends two and three and that trends two and three were highly negatively correlated (–.76 to –.94). The positive correlation of trend two with growth suggests that those compounds may be precursors to those of trend three.

During November, December, and January the relative percentages of the eleven compounds with significant SNK tests deviated little from the mean percent (Fig. 6). Fluctuations during the summer and early fall were considerable.

Seasonal variations in the volatile components (computed as grams per 100 grams of dry matter) —When the components were quantified on a weight basis (grams per 100 grams dry matter), 17 compounds were found to have significant F ratios from the ANOVA. The more conservative SNK multiple range test showed 15 of the 17 to have significant differences during the year (P = .05). Analyses of these 15 compounds revealed four major trends.

The first trend is exemplified by compound 6 (sabinene) and is shared by component 2 (tricyclene + α-pinene/α-thujene), compound 17 (C_{10}

TABLE 6. *Correlations between compound 2 (trend one), compound 8 (trend two), and compound 31 (trend three), and growth, percent yield of volatile oil, percent dry weight, precipitation, average air temperature, and average soil temperature*

Cpd.	Growth	% yield	% dry weight	ppt.	Air temp.	Soil temp.
2	.469	–.475	.020	–.365	–.149	–.245
8	.757	–.689	.237	.154	.566	.571
31	–.697	.606	–.403	–.399	–.774	–.816

terpene alcohol), and compound 22 (linalool). The SNK test for compound 6 (sabinene) is shown in Table 7. In general the compounds of this trend were postively correlated with the percent yield of volatile oil and negatively correlated with temperature (see Table 8). Figure 7 shows the high positive correlation between sabinene (compound 6) and the percent yield of volatile oil (acetate II will be discussed under trend four). The decrease in weight for these compounds was typically in August, with increases in the fall.

The second trend is the major trend of the weight data and includes 7 compounds: compound 31 (estragole?), compound 33, compound 39, compound 50, compound 55 (elemol acetate), compound 70 (a sesquiterpene), and compound 71 (a sequi- or di-terepene). Compound 31 (estragole?) will be used to exemplify this trend and its SNK test is shown in Table 7. Minimum weight values occurred during the spring and summer sampling periods (Fig. 8). Weight increases during the October sampling period were followed by a slight decline. Late fall and winter values fluctuated little.

In general, the compounds of this trend followed closely that of trend one (see Table 8, cpds. 6 and 31) except for the earlier decline in June (as opposed to August in trend one) and the greater stability during the winter (compare sabinene in Fig. 7 with estragole in Fig. 8).

The third trend in the compounds computed on a weight basis is shown by a single compound, 32. The SNK test (Table 7) revealed several significant differences during the year. The weight increased during the early summer, attaining a maximum value in the July sampling period. Some fluctuations were observed during the fall and winter; these variations, however, were not statistically significant.

This trend is very similar to trend two of the percent data as shown in Fig. 4 and as exemplified by compound (α-terpinene) of the percent data.

Although seasonal variations in the weight of compound 32 followed closely the variation pattern of the percent of compound 32, fewer significant differences are encountered in the weight data. The weight of compound 32 is slightly correlated with several environmental variables and with growth, percent oil yield, and percent extracted dry weight data (Table 8). The weight of compound 32 (trend three) is negatively correlated with trends one, two, and four. The increase of this compound before the general increase of the precent yield of volatile oil and just after the major growth suggests that compound 32 may be a precursor to the other terpenoids.

The fourth trend is exemplified by compound 69 (acetate II) along with compounds 49 and 54. Although the relative percentages of these

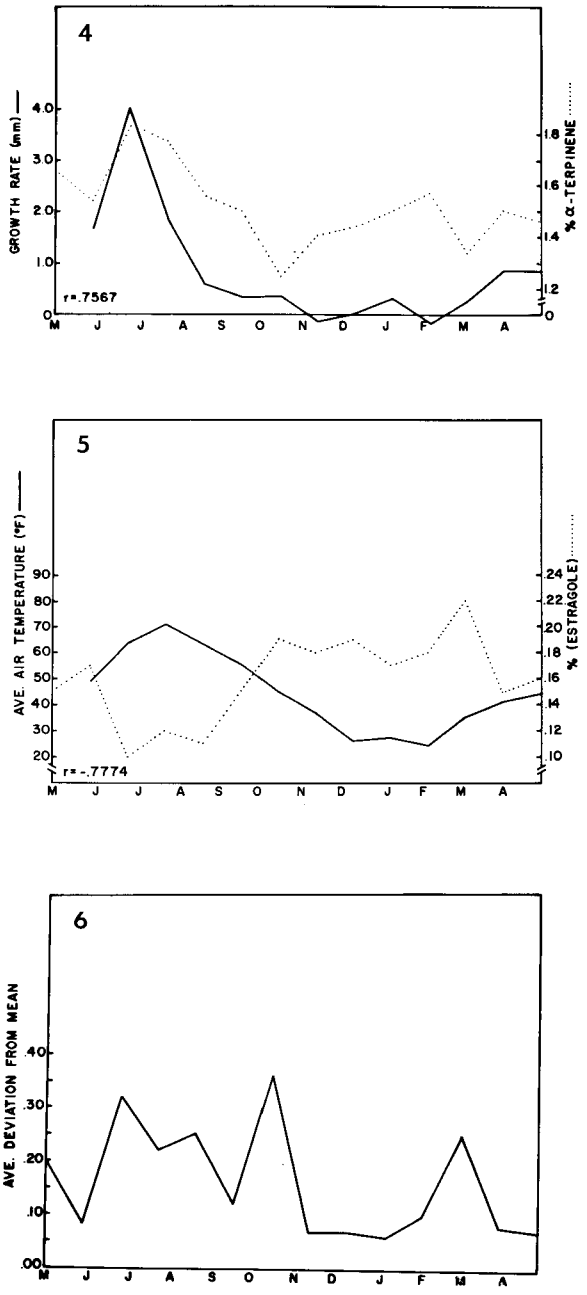

Fig. 4–6. **4.** Graphical representation of the correlation between seasonal variations in the relative percentage of compound 8 (α-terpinene) and the growth rate of *J. scopulorum*. Compound 8 represents the second major trend in the percent data. **5.** Seasonal variation in the relative percentage of compound 31 (estragole) and the average air temperature. Compound 31 exemplifies the third major trend in the percent data. **6.** Average absolute deviations from the average annual percentages of 11 compounds which had significant SNK tests. Note the period from November to February when the deviations are fairly constant. This appears to be the most suitable time to collect samples of foliage for chemosystematic studies.

TABLE 7. *SNK tests for four compounds of J. scopulorum with significant weight variations during the year. (P = .05) These four compounds represent the four major trends in the changes in composition when components were calculated as grams per 100 grams of organic matter*

Character = 6, sabinene, (similar patterns were found for cpds. 2, 17, and 22)

Date	1/8	11/13	3/3	12/11	10/16	2/5	9/18	4/1	4/29	6/26	5/1	5/29	7/24	8/21
Mean	1.77	1.72	1.68	1.64	1.59	1.57	1.50	1.43	1.38	1.32	1.30	1.30	1.28	1.16
SNK														
TEST														

Character = 31, (estragole), (similar patterns were found for compounds 33, 39, 50, 55, 70, and 71)

Date	3/3	10/16	11/13	12/11	1/8	2/5	9/18	4/29	4/1	5/29	5/1	8/21	7/24	6/26
Mean	.017	.016	.015	.015	.014	.013	.012	.011	.011	.010	.009	.008	.007	.006
SNK														
TEST														

Character = 32

Date	7/24	6/26	8/21	4/29	9/18	4/1	12/11	11/13	2/5	5/1	1/8	3/3	10/16	5/29
Mean	.018	.017	.016	.016	.016	.015	.014	.014	.014	.013	.012	.012	.011	.011
SNK														
TEST														

Character = 69, acetate II, (similar patterns were found for compounds 49 and 54)

Date	10/16	11/13	9/18	4/1	12/11	1/8	4/29	3/3	8/21	2/5	7/24	6/26	5/29	5/1
Mean	2.29	2.24	2.14	2.06	2.05	2.02	1.92	1.90	1.90	1.83	1.64	1.45	1.37	1.26
SNK														
TEST														

compounds failed to show any significant seasonal differences, the weight data for these compounds are quite variable when spring and summer values are compared (see Table 7). During the summer, weight increases were observed, as illustrated by compound 69 (acetate II) in Fig. 7. Compounds of trend four show high positive correlations with the percent yield of volatile oil and negative correlations with growth (Table 8). In these respects, this trend differs little from trends one and two.

A majority of the compounds exhibiting significant seasonal weight variations were rather highly correlated with the percent yield of volatile oil. The percent yield exhibits considerable seasonal variation, and one may expect that this variation will be reflected in the weight values calculated for individual compounds. Figure 9 shows that the averages of compounds possessing significant SNK tests deviated little from the mean weights during the February and April sampling periods. However, the fluctuations are sporadic throughout the year and stable periods are not apparent.

DISCUSSION—Significant seasonal variations were observed in the quantitative volatile oil composition of *J. scopulorum* foliage. Although some loss of the terpenoids was expected from volatilization and resinification during the warm

TABLE 8. *Correlations between compound 6 (trend one), compound 31 (trend two), compound 32 (trend three), and compound 69 (trend four), and growth, percent yield of volatile oil, percent dry weight, precipitation, average air temperature, and average soil temperature*

Cpd.	Growth	% yield	% dry weight	ppt.	Air temp.	Soil temp.
6	–.603	.855	–.607	–.380	–.796	–.807
31	–.775	.818	–.466	–.326	–.780	–.794
32	.423	–.338	–.345	.426	.599	.651
69	–.752	.912	–.520	.055	–.414	–.365

summer months, as had been suggested by Fluck (1963), volatilization and resinification alone do not adequately explain fluctuations observed in the relative percentage of volatile oil constitutents. The relative percentages of α-terpinene, terpinolene, 4-terpinenol, and two unidentified compounds were positively correlated with growth. Yet during periods of intensive growth activity these rather volatile terpenoids were being synthesized more rapidly than they were being lost. Similar results have been reported for *J. pinchotii* (Adams, 1970).

Several studies have shown that juvenile foliage is a dominant source of seasonal variation in the volatile oil composition (von Rudloff, 1962, 1972a, b; Zavarin et al., 1971). Since the foliage samples of *J. scopulorum* consisted of several years' growth, it was expected that the volatile oil composition would be quite variable during the spring and summer when juvenile needles constituted a significant portion of the foliage samples. Since the relative percentage of each compound varied considerably during the spring and summer (Fig. 6), much of the variability in sampling for systematic studies could be eliminated by restricting collections to the late fall or winter, as had been previously suggested by Adams (1969, 1970).

The weights of individual compounds were more variable than were their relative percentages. Figure 9 shows that, unlike the relative percentages, the weights of volatile oil constituents were quite variable throughout the entire study. Seasonal fluctuations in the weights of individual compounds are correlated with changes in the yield of volatile oil. Von Rudloff (1972b) suggested that the oil yields, and thus the weight of each terpenoid, were subject to ecological as well as genetic factors. The yield of volatile oil from the foliage of *J. scopulorum* was quite variable throughout the year and is negatively correlated with seasonal fluctuations in growth and temperature and positively correlated with dry weight. The correlation with dry weight was not unexpected. The volatile oil yields were determined on a dry weight basis and thus reflect fluctuations in the kinds and the amounts of the nonvolatile foliage constituents from one time of the year to the next (von Rudloff, 1972b). Problems are also encountered if one attempts to calculate yields of volatile oil on a fresh weight basis since changes in the foliage moisture content will exert a profound influence upon the results (von Rudloff, 1972b).

The negative correlations with growth and temperature may indicate that not only was some of the volatile oil lost by evaporation during the warm summer months but that accumulation of the volatile oil was largely accomplished after major growth activity had subsided. These re-

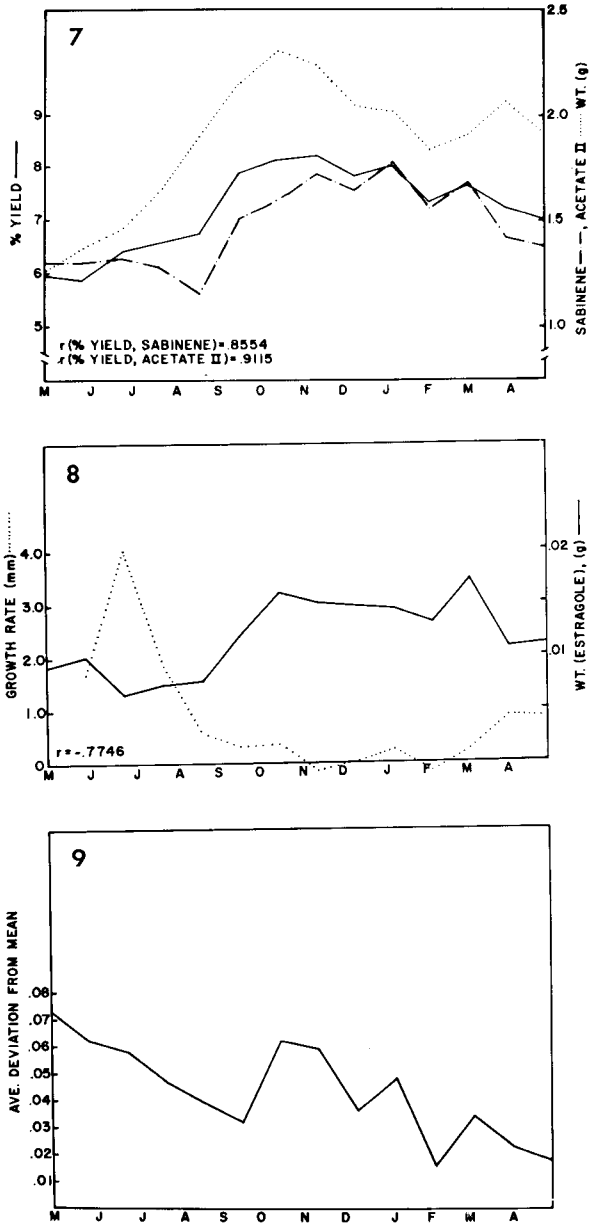

Fig. 7–9. **7.** Graphical representation of the correlation between the weights of compound 6 (sabinene) and compound 69 (acetate II) and the percent yield of volatile oil. The weight of sabinene exemplifies the first major trend in the weight data and the weight of compound 69 shows the fourth major trend in the weight data. Both of the trends are correlated with the percent yield of volatile oil. **8.** Seasonal variation in the weight of compound 31 (estragole) and the growth rate. The weight of compound 31 represents the third major trend of the weight data. **9.** Average absolute deviations from the average annual weights of 15 compounds with significant SNK tests. Notice that there is no season of the year when these deviations are rather constant, thus it is difficult to find a good sampling period if the weights of compounds are to be utilized in chemosystematic studies.

sults contradict those reported for ponderosa pine (Zavarin et al., 1971), black spruce (von Rudloff, 1962), white spruce (von Rudloff, 1972b), and Douglas fir (von Rudloff, 1972a). It is not possible at this time to ascertain if these contradictory results reflect specific differences or merely differences in the methods utilized to determine the oil yields. A decrease in the yield of volatile oil during the winter months is of interest for it may reflect the metabolic breakdown of terpenoids to provide energy during periods of stress. Although documentation of the involvement of terpenoids in metabolic activity is scanty, Burbott and Loomis (1967, 1969) suggested that the monoterpenes of peppermint may provide an additional source of energy as more suitable substrates become depleted.

Finally, the chemotaxonomist must be aware of the conflicting results that have been reported in studies of seasonal variation in the volatile oil composition. Fluctuations in the volatile oil composition appear to vary from one plant group to another. Although it seems that the best time to sample is when plants are not rapidly growing and are not in periods of stress, some preliminary investigation of seasonal variation should precede chemosystematic studies, particularly those studies on the infraspecific level.

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