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

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ABSTRACT

The volatile oils of four trees of *Juniperus scopulorum* were examined at 9 am, 12 noon, 3 pm, 6 pm, 10 pm, 2 am, and 6 am on consecutive days with a temperature range of 64 F and 88.5 F daily. Three-way analysis of variance of 37 compounds revealed 36 with significant differences among trees, 11 with significant differences between days, 13 compounds with significant diurnal variation and 9 compounds which showed some significant interaction term differences. Most of the variation occurred among trees. Oxygenated terpenes and sesquiterpenes tended to increase during the day while sabinene decreased until late evening and increased during the early morning. Correlations with temperature appeared to lag and did not match the pattern in three species of *Juniperus* reported by other investigators. The effect of diurnal variations on chemosystematic classifications was estimated by using numerical taxonomy and principal coordinate analysis. Diurnal effects were not found to be important sources of error for chemosystematics in *J. scopulorum* if character weighting were used to maximize the genotype differences. However, this may not be true for work involving population sampling of other species over large regions.

FLUCTUATIONS in our daily environment, including enormous changes in photosynthetic rates, present an interesting problem for physiologists and a critical problem for chemosystematists. When sampling a physiologically active pool of terpenoids in natural populations, the chemosystematist must be aware of the magnitude of diurnal and seasonal variations. Although conifers might be expected to be more buffered than herbaceous angiosperms, significant seasonal variation has been found in the foliage of *Picea glauca*, *P. pungens* (von Rudloff, 1962, 1972), *Juniperus ashei* (Adams, 1969), *J. pinchotii* (Adams, 1970), and *J. scopulorum* (Powell and Adams, 1973). However, Zavarin et al. (1971) concluded that major seasonal fluctuations in the volatile oil of *Pinus ponderosa* were restricted to the current year's foliage and Tatro et al. (1973) concluded that there were no significant seasonal changes in the leaf oil composition of *J. californica*, *J. occidentalis*, or *J. osteosperma* (in southern California). Differences between young and mature leaves may account for much of the seasonal variation reported. Adams and Hagerman (1976) have recently shown that 19 of 36 compounds analyzed from *J. scopulorum* cv. *platinum* differed significantly between young and mature foliage. Similar results have been shown in *Sequoiadendron* (Levinson, Lemoine, and Smart, 1971), *Picea* (von Rudloff, 1972) and *Pinus* (Zavarin et al., 1971).

Seasonal and diurnal changes in the terpenoids provide additional evidence that these compounds are metabolically active as has been shown repeatedly in *Mentha* species and other important essential oil crop plants. Of physiological interest are the implications about the metabolic roles of terpenoids as well as the paths of biosynthesis. As additional evidence is gathered concerning the biological and ecological significance of the terpenes, the taxonomic significance must be reappraised. In sampling for chemosystematic studies one has to take into consideration many factors including sample sizes, seasonal variation, sexual differences, populational variation, and diurnal variation.

The purpose of this paper is to report on diurnal variation in *J. scopulorum*, discuss the biosynthetic implications, and to evaluate the effects of diurnal variation on sampling for chemosystematic studies in *J. scopulorum*.

MATERIALS AND METHODS—Four trees of *J. scopulorum* were sampled on consecutive days, July 28, 29, 1975 on the campus of Colorado State University, Fort Collins, Colorado, 5,000 ft elevation. These trees were chosen to avoid the possibility of changes occurring in the volatile oils during transport as shown by Cheng and von Rudloff (1970). Samples of fresh foliage (200 g) were taken at 9 am, 12 noon, 3 pm, 6 pm, 10 pm, 2 am, and 6 am on consecutive days. Air temperatures were taken from a nearby CSU weather

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TABLE 1. *Thirty-seven compounds with the mean values (% total oil) from Juniperus scopulorum used in the three-way analysis of variance of sampling periods, days and trees*

| Cpd. | # identity | Mean | Cpd. | # identity | Mean |
|---|---|---|---|---|---|
| 3 | α-pinene/ α-thujene | 2.92 | 62 | citronellol | 0.34 |
| | | | 72 | | 0.10 |
| 7A | sabinene | 48.30 | 73 | | 0.16 |
| 10 | myrcene | 2.21 | 77 | | 0.14 |
| 12 | α-terpinene | 1.44 | 78 | | 0.06 |
| 14 | limonene | 2.43 | 79B | | 0.06 |
| 17 | γ-terpinene | 2.25 | 79A | | 0.20 |
| 20 | terpinolene | 1.02 | 80 | | 1.02 |
| 33 | (alcohol) | 1.40 | 82 | elemol | 3.93 |
| 38 | linalool | 1.62 | 82A | | 1.50 |
| 40B | methyl citronellate | 5.62 | 83 | (decomposition?) | 5.71 |
| 41 | (bornyl acetate) | 0.10 | 83B | | 0.45 |
| 42 | terpinen-4-ol | 6.42 | 86 | (γ-eudesmol) | 0.52 |
| 45 | | 0.26 | 87 | | 0.19 |
| 50 | (estragole) | 0.10 | 90 | α-eudesmol | 0.40 |
| 53 | | 0.43 | 91 | β-eudesmol | 0.99 |
| 57 | | 0.06 | 95B | acetate II | 5.18 |
| 58 | | 0.27 | 96 | | 0.05 |
| 59 | | 0.14 | | | |
| 61 | | 0.34 | | | |

station (300 m away). Female cones were removed and the foliage steam distilled. Separation was accomplished by capillary gas liquid chromatography (GLC) with quantification by an electronic digital integrator (see Powell and Adams, 1973 for detailed conditions).

Thirty-seven compounds (see Table 1 for percent of total oil and identities) were coded, checked, and analyzed by three-way analysis of variance, complete factorial design, with 2 days (D), 4 trees (T), and 7 sampling periods (P: 9 am; 12 noon; 3 pm; 6 pm; 10 pm; 2 am; 6 am). Student-Newman-Keul's (SNK) multiple range test ($P = 0.5$) was used to determine if the means of any of the sample periods differed significantly. Error variance used in the SNK tests was the DPT residual ($df = 18$). In order to evaluate the effects of diurnal variation on classification procedures, a one-way analysis of variance was made on four data sets of genotypes (i.e., 14 samples of each of the four trees). This procedure generates a set of F ratios for each character (terpenoid) which maximizes the variation among trees while minimizing the contribution of within trees variation (see Adams, 1975a for detailed studies of character weighting). Similarity measures were then calculated by the F-weighted Manhattan metric (absolute character differences divided by the range over all OTU's) as used by Adams (1975a). Similarity measures were computed between all pairs of the 56 samples taken in the study ($2D \times 4T \times 7P$). These individual samples were then clustered by using principal coordinate analysis (Gower, 1966).

RESULTS AND DISCUSSION—Table 2 shows the results of the three-way analysis of variance, complete factorial design. In general, very high F ratios were encountered among genotypes (trees) with several significant and highly significant differences between the consecutive days and also among sampling periods. The interaction terms (DT, DP, TP) were generally not significant with the exception of a few compounds for DT and DP. No significant interactions were found between tree and period of sampling (TP). The three-way analysis of variance is summarized in Table 3. Of the 37 compounds analyzed, all but one showed significant or highly significant differences among trees. Eleven compounds showed significant differences between the two days of sampling, whereas 13 compounds exhibited significant differences among sampling periods. The various interaction terms accounted for only small amounts of variance in most cases. The principal exception being compounds 82A and 83 (both of which may be decomposition products) which showed highly significant differences in the DT interaction term. The average F ratio for all 37 compounds is also shown in Table 3 and reveals that the variance due to genotype differences accounts for most of the variance encountered in the samples (analysis of the mean squares revealed the same pattern).

Student-Newman-Keuls (SNK) multiple range tests of 13 compounds which showed significant or highly significant F ratios in the 3-way analysis of variance showed a general trend of significant difference between the 9 am and 6 pm samples or between the 9 am and 10 pm samples (Table 4), that is, the differences in sampling in the morning (9 am) versus late afternoon (6 pm) or evening (10 pm). These differences can be seen more graphically in Fig. 1. Three compounds were not graphed due to their small concentrations (cpd. 57, max. of .079%; cpd. 78, max. of .093%; cpd. 96A, max. of .123%). The remaining 10 compounds showed four basic patterns. Sabinene (a $C_{10}$ hydrocarbon) decreased steadily from 9 am till 10 pm, then began to increase during the early morning. This corresponds somewhat with temperature with a slight lag (Fig. 1). Methyl citronellate shows a stronger negative correlation with temperature but behaved much as sabinene except for the samples at 6 am. Five compounds showed a very similar pattern (cpd. 33, an unknown $C_{10}$ alcohol; γ-terpinene, a $C_{10}$ hydrocarbon; linalool, a $C_{10}$ alcohol; terpinen-4-ol, a $C_{10}$ alcohol; and cpd. 59, probably a $C_{10}$ alcohol or ester). Compound 33 is graphed in Fig. 1 and shows a positive, lag correlation with temperature. Notice that these compounds reach a maximum at the 6 pm sampling, then decrease

TABLE 2. *Three-way analysis of variance, complete factorial design with 2 days (D), 4 trees (T), and 7 sampling periods (P). Degrees of freedom: D(1), T(3), P(6), D × T(3), D × P(6), T × P(18), error degrees of freedom (18). Significance levels of F ratios: df = 1, 18 ($F_{.05} = 4.41$, $F_{.01} = 8.29$); df = 6, 18 ($F_{.05} = 2.66$, $F_{.01} = 4.01$); df = 3, 18 ($F_{.05} = 3.16$, $F_{.01} = 5.09$); df = 18, 18 ($F_{.05} = 2.19$, $F_{.01} = 3.08$)*

| Source of var. | df | Variance | F | Variance | F | Variance | F |
|---|---|---|---|---|---|---|---|
| | | *α-pinene/α-thujene* | | *sabinene* | | *myrcene* | |
| D | 1 | .024 | 1.4 | .05 | 0.0 | .107 | 3.1 |
| T | 3 | 1.667 | 9.8** | 407.57 | 171.5** | 4.220 | 124.1** |
| P | 6 | .040 | 2.3 | 9.23 | 3.9* | .072 | 2.1 |
| DT | 3 | .018 | 1.1 | .25 | 0.1 | .010 | 0.3 |
| DP | 6 | .039 | 2.3 | 5.16 | 2.2 | .012 | 0.4 |
| TP | 18 | .016 | 1.0 | 1.47 | 0.6 | .026 | 0.8 |
| DTP | 18 | .017 | | 2.38 | | .034 | |
| | | *α-terpinene* | | *limonene* | | *γ-terpinene* | |
| D | 1 | .0103 | 0.8 | .0088 | 0.8 | .011 | 0.5 |
| T | 3 | 1.1027 | 81.1** | 5.7005 | 538.2** | 2.794 | 132.2** |
| P | 6 | .0249 | 1.8 | .0169 | 1.6 | .086 | 4.1** |
| DT | 3 | .0102 | 0.8 | .0137 | 1.3 | .011 | 0.5 |
| DP | 6 | .0139 | 1.0 | .0192 | 1.8 | .021 | 1.0 |
| TP | 18 | .0091 | 0.7 | .0061 | 0.6 | .023 | 1.1 |
| DTP | 18 | .0136 | | .0106 | | .021 | |
| | | *terpinolene* | | *(alcohol)* | | *linalool* | |
| D | 1 | .000001 | 0.001 | .0085 | 2.5 | .052 | 4.7* |
| T | 3 | .22363 | 86.0 ** | .3621 | 107.8** | 3.894 | 350.9** |
| P | 6 | .00501 | 1.9 | .0360 | 10.7** | .069 | 6.2** |
| DT | 3 | .00065 | 0.2 | .0050 | 1.5 | .091 | 8.2** |
| DP | 6 | .00216 | 0.8 | .0052 | 1.6 | .028 | 2.6 |
| TP | 18 | .00255 | 1.0 | .0096 | 2.9 | .019 | 1.7 |
| DTP | 18 | .00260 | | .0034 | | .011 | |
| | | *methyl citronellate* | | *(bornyl acetate)* | | *terpinen-4-ol* | |
| D | 1 | .847 | 12.9** | .0117 | 16.0** | .087 | .4 |
| T | 3 | 4.445 | 67.5** | .0327 | 44.6** | 23.058 | 100.0** |
| P | 6 | .314 | 4.8** | .0005 | .7 | .941 | 4.1** |
| DT | 3 | .324 | 4.9* | .0009 | 1.3 | .182 | .8 |
| DP | 6 | .088 | 1.3 | .0011 | 1.5 | .318 | 1.4 |
| TP | 18 | .071 | 1.1 | .0007 | 1.0 | .248 | 1.1 |
| DTP | 18 | .066 | | .0007 | | .230 | |
| | | *Cpd. 45* | | *(estragole)* | | *Cpd. 53* | |
| D | 1 | .0092 | 6.6* | .00020 | 1.9 | .0002 | .2 |
| T | 3 | .0390 | 27.8** | .00082 | 8.0** | .0121 | 13.1** |
| P | 6 | .0029 | 2.1 | .00017 | 1.6 | .0016 | 1.7 |
| DT | 3 | .0006 | .4 | .00001 | .1 | .0001 | .1 |
| DP | 6 | .0035 | 2.5 | .00009 | .9 | .0007 | .7 |
| TP | 18 | .0012 | .9 | .00007 | .7 | .0007 | .7 |
| DTP | 18 | .0014 | | .00010 | | .0009 | |
| | | *Cpd. 57* | | *Cpd. 58* | | *Cpd. 59* | |
| D | 1 | .00051 | 1.7 | .002 | 1.5 | .0016 | 5.0* |
| T | 3 | .00887 | 28.3** | 3.985 | 2979.7** | .0017 | 5.2** |
| P | 6 | .00124 | 4.0** | .003 | 2.2 | .0013 | 3.9* |
| DT | 3 | .00004 | .1 | .006 | 4.7** | .0004 | 1.2 |
| DP | 6 | .00026 | .8 | .001 | .8 | .0004 | 1.3 |
| TP | 18 | .00090 | 2.9 | .002 | 1.5 | .0005 | 1.6 |
| DTP | 18 | .00031 | | .001 | | .0003 | |
| | | *Cpd. 61* | | *citronellol* | | *Cpd. 72* | |
| D | 1 | .0111 | 1.9 | .0161 | 1.7 | .00013 | .2 |
| T | 3 | .2738 | 47.7** | 1.382 | 146.4** | .00268 | 4.0* |
| P | 6 | .0063 | 1.1 | .010 | 1.1 | .00018 | .3 |

TABLE 2. *Continued*

| Source of var. | df | Variance | F | Variance | F | Variance | F |
|---|---|---|---|---|---|---|---|
| DT | 3 | .0018 | .3 | .011 | 1.1 | .00060 | 1.1 |
| DP | 6 | .0087 | 1.5 | .010 | 1.0 | .00021 | .4 |
| TP | 18 | .0053 | .9 | .008 | .9 | .00076 | 1.4 |
| DTP | 18 | .0057 | | .009 | | .00055 | |
| | | *Cpd. 73* | | *Cpd. 77* | | *Cpd. 78* | |
| D | 1 | .00020 | 1.2 | .0026 | 3.8 | .0049 | 6.3* |
| T | 3 | .02648 | 162.6** | .0482 | 70.0** | .0275 | 35.1** |
| P | 6 | .00052 | 3.2 | .0014 | 2.0 | .0034 | 4.4** |
| DT | 3 | .00011 | .7 | .0042 | 6.0** | .0013 | 1.7 |
| DP | 6 | .00018 | 1.1 | .0028 | 4.1** | .0018 | 2.3 |
| TP | 18 | .00020 | 1.2 | .0008 | 1.2 | .0011 | 1.4 |
| DTP | 18 | .00016 | | .0007 | | .0008 | |
| | | *Cpd. 79B* | | *Cpd. 79A* | | *Cpd. 80* | |
| D | 1 | .0035 | 1.4 | .0011 | 1.3 | .035 | 1.7 |
| T | 3 | .0319 | 13.0** | .0295 | 36.2** | 12.619 | 605.0** |
| P | 6 | .0031 | 1.2 | .0015 | 1.9 | .028 | 1.3 |
| DT | 3 | .0010 | .4 | .0007 | .9 | .045 | 2.2 |
| DP | 6 | .0026 | 1.1 | .0019 | 2.3 | .027 | 1.3 |
| TP | 18 | .0026 | 1.1 | .0008 | .9 | .028 | 1.4 |
| DTP | 18 | .0024 | | .0008 | | .021 | |
| | | *elemol* | | *Cpd. 82A* | | *(decomposition)* | |
| D | 1 | .265 | 3.6 | 30.56 | 59.7** | 49.4 | 18.8** |
| T | 3 | 30.963 | 416.9** | 6.60 | 12.9** | 109.5 | 41.7** |
| P | 6 | .319 | 4.3** | .26 | .5 | 1.3 | .5 |
| DT | 3 | .055 | .7 | 4.32 | 8.4** | 11.5 | 4.4** |
| DP | 6 | .201 | 2.7* | .50 | 1.0 | .7 | .3 |
| TP | 18 | .076 | 1.0 | .78 | 1.5 | 1.6 | .7 |
| DTP | 18 | .074 | | .51 | | 2.6 | |
| | | *Cpd. 83B* | | *(γ-eudesmol)* | | *Cpd. 87* | |
| D | 1 | 1.67 | 1.9 | .013 | 4.6* | .0062 | 6.4* |
| T | 3 | 1.74 | 1.9 | .268 | 98.2** | .3536 | 367.0** |
| P | 6 | .55 | .6 | .013 | 4.7** | .0013 | 1.3 |
| DT | 3 | .49 | .5 | .003 | .9 | .0002 | .3 |
| DP | 6 | 1.34 | 1.5 | .003 | 1.1 | .0003 | .3 |
| TP | 18 | .57 | .6 | .003 | 1.0 | .0004 | .4 |
| DTP | 18 | .89 | | .003 | | .0010 | |
| | | *α-eudesmol* | | *β-eudesmol* | | *acetate II* | |
| D | 1 | .034 | 7.5* | .041 | 1.0 | .28 | .7 |
| T | 3 | .545 | 122.5** | 1.162 | 29.3** | 46.14 | 118.7** |
| P | 6 | .013 | 3.0* | .025 | .6 | .93 | 2.4 |
| DT | 3 | .013 | 2.9 | .058 | 1.5 | .32 | .8 |
| DP | 6 | .004 | .9 | .046 | 1.2 | 1.17 | 3.0* |
| TP | 18 | .007 | 1.6 | .034 | .9 | .28 | .7 |
| DTP | 18 | .004 | | .040 | | .39 | |
| | | *Cpd. 96A* | | | | | |
| D | 1 | .0014 | 1.2 | | | | |
| T | 3 | .0163 | 14.8** | | | | |
| P | 6 | .0084 | 7.6** | | | | |
| DT | 3 | .0010 | .9 | | | | |
| DP | 6 | .0024 | 2.1 | | | | |
| TP | 18 | .0020 | 1.8 | | | | |
| DTP | 18 | .0011 | | | | | |

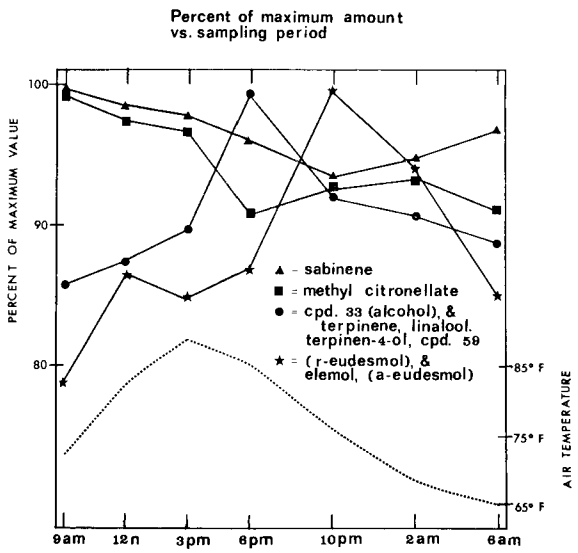


Fig. 1. Diurnal variation in the percent of maximum amount of 4 terpenoids representing the major trends versus sampling period in *J. scopulorum*. Compounds 57, 78, and 96A were omitted from consideration on this graph due to the small concentrations of those compounds. Notice the lag in production of the sesquiterpenes (elemol, γ-eudesmol, α-eudesmol) behind the terpene alcohol group (cpd. 33, etc.).

through the night. The fourth trend is seen in three sesquiterpene alcohols (elemol, γ-eudesmol, and α-eudesmol). These compounds increased until noon, then showed a small decrease (in all three compounds) during the hot afternoon, then increased until 10 pm. Gamma eudesmol decreased steadily through the night but both elemol and α-eudesmol (not shown in Fig. 1) registered a slight increase between the 2 am and 6 am samples. This pattern appears to be a lag pattern of the third trend involving the $C_{10}$ alcohols (cpd. 33 in Fig. 1).

Tatro et al. (1973), in a study of diurnal variation of *J. californica*, suggest that volatilization may be mostly responsible for the changes, with the more volatile terpenes (α-pinene, sabinene in their study) evaporating as temperatures increase. They found the concentration of α-pinene to be well correlated negatively with air temperatures, although sabinene changed quickly from 38.6% (7:30 am, 51 F) to 29.9% (10:30 am, 67 F) and this was about the same at 2 pm (84 F) but increasing to 39.7% by 5 pm (58 F). We have found no significant differences in α-pinene/α-thujene (these peaks could not be separated and thus were analyzed as one). Sabinene had an F ratio of 3.9 (significant at $P = .05$) and the SNK tests (Table 4) revealed significant differences between the 9 am and 10 pm samples and between the 9 am and 2 am samples. No significant differences were found during the daylight hours.

TABLE 3. *Summary of the significant differences found in the 3-way ANOVA (Table 2). The number of significant differences for each of the terms is summarized from Table 2 and is based on a total of 37 compounds analyzed*

| Source of variation | No. of significant differences (.05) | No. of highly significant differences (.01) | Average F ratio (37 cpds.) |
|---|---|---|---|
| Days (D) | 7 | 4 | 5.00 |
| Trees (T) | 1 | 35 | 195.10 |
| Periods (P) | 3 | 10 | 2.75 |
| D × T | 1 | 4 | 1.71 |
| D × P | 2 | 2 | 1.46 |
| T × P | 0 | 0 | 1.15 |

Nevertheless, considerable diurnal variation was found in the present study. The fact that terpene hydrocarbons, alcohols, and aldehydes all decrease before the sesquiterpenes (hydrocarbons and oxygenated) increase seems to agree with a possible synthesis pathway discussed by Adams and Hagerman (1976). A study of the terpenoids of *J. scopulorum* cv. *platinum* (Adams and Hagerman, 1976) showed that young and mature foliage taken from the same plant at the same time (within minutes) in a greenhouse, differed in the composition of the terpenoids. The terpene hydrocarbons and terpene alcohols (linalool and terpinen-4-ol) were largest in the new foliage whereas the sesquiterpenoids were larger in the mature leaves. This pattern has been generally observed in *Picea* (von Rudloff, 1972), *Pinus* (Zavarin et al., 1971), and *Sequoiadendron* (Levinson et al., 1971). Thus, it would seem that an alternative theory to volatilization might be that the precursors are formed early in the day with interconversions taking place later during the day. Since geraniol and nerol (terpene alcohols) are generally believed to be the precursors of mono- and sesquiterpenoids (Francis, 1971), it is possible that we are observing an increase of the terpene alcohols first during the day with interconversion to sesquiterpene alcohols later. This leaves the dominant compound (sabinene, 46 to 50%) decreasing during the day due to perhaps both volatility and synthesis of other compounds. Further work will be needed to elucidate these relationships.

Of particular concern to taxonomists and systematists who use these terpenoids as taxonomic characters is the effect of diurnal variation on classification. Although the differences between the four genotypes sampled in this study are slight, it seemed appropriate to test the effects of diurnal variation on these data when analyzed for taxonomic purposes. In order to estimate these effects, the samples were divided into four data sets of 13 samples each (i.e., 4 genotypes). To maximize the differences between genotypes and minimize the differences within genotypes a one-way ANOVA was performed on the four sets.

TABLE 4. *Student-Newman-Keuls multiple range tests of 13 compounds which showed significant or highly significant F ratios in the 3-way ANOVA (Table 2). Tests were made at the P = .05 level. Any means underlined by a common line are not significantly different*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sabinene (%) | 10 pm | 2 am | 6 pm | 6 am | 3 pm | 12 noon | 9 am |
| | 46.7 | 47.2 | 48.1 | 48.4 | 48.9 | 49.1 | 49.7 |
| γ-terpinene | 9 am | 6 am | 12 noon | 3 pm | 2 am | 10 pm | 6 pm |
| | 2.08 | 2.19 | 2.24 | 2.24 | 2.29 | 2.30 | 2.42 |
| cpd. 33 (alcohol) | 9 am | 12 noon | 6 am | 3 pm | 2 am | 10 pm | 6 pm |
| | 1.34 | 1.36 | 1.36 | 1.40 | 1.40 | 1.43 | 1.54 |
| linalool | 9 am | 12 noon | 3 pm | 6 am | 10 pm | 2 am | 6 pm |
| | 1.52 | 1.56 | 1.59 | 1.59 | 1.64 | 1.66 | 1.80 |
| methyl citronellate | 6 pm | 6 am | 10 pm | 2 am | 3 am | 12 noon | 9 am |
| | 5.39 | 5.41 | 5.54 | 5.55 | 5.72 | 5.81 | 5.90 |
| terpinen-4-ol | 9 am | 12 noon | 3 pm | 6 am | 10 pm | 2 am | 6 pm |
| | 5.85 | 6.26 | 6.30 | 6.34 | 6.67 | 6.68 | 6.87 |
| cpd. 57 | 6 pm | 6 am | 10 pm | 3 pm | 12 noon | 2 am | 9 am |
| | .043 | .058 | .059 | .060 | .065 | .077 | .079 |
| cpd. 59 | 9 am | 12 noon | 3 pm | 6 am | 2 am | 10 pm | 6 pm |
| | .127 | .134 | .140 | .140 | .144 | .146 | .168 |
| cpd. 78 | 3 pm | 6 pm | 12 noon | 2 am | 10 pm | 9 am | 6 am |
| | .033 | .040 | .050 | .060 | .069 | .074 | .093 |
| elemol | 9 am | 3 pm | 12 noon | 6 pm | 2 am | 6 am | 10 pm |
| | 3.64 | 3.80 | 3.82 | 3.90 | 4.00 | 4.06 | 4.25 |
| (γ-eudesmol) | 9 am | 6 am | 3 pm | 12 noon | 6 pm | 2 am | 10 pm |
| | .471 | .502 | .506 | .519 | .519 | .557 | .593 |
| (α-eudesmol) | 9 am | 3 pm | 6 pm | 12 noon | 2 am | 6 am | 10 pm |
| | .347 | .377 | .386 | .404 | .409 | .417 | .478 |
| cpd. 96A | 12 noon | 3 pm | 6 pm | 9 am | 2 am | 10 pm | 6 am |
| | .021 | .037 | .041 | .052 | .055 | .056 | .123 |

Similarity measures were calculated between all possible pairs of the 56 samples by using a F-1 weighted Manhattan metric (absolute differences divided by the range over all samples or OTU's, Adams, 1975a). Principal coordinate analysis (Gower, 1966) was then used to display the samples according to clusters within the similarity matrix. The first coordinate accounted for 33% of the variation of the similarity matrix; the second coordinate removed 27% and the third coordinate removed 19% of the variation from the similarity matrix. Additional coordinates removed only 1.7, 1.4, 1.2 and 1.1% of the variation and were judged to represent error variance. Figure 2 shows the samples plotted onto the first and second principal coordinates. Notice that these two coordinates (representing 60% of the variance of the similarity matrix) clearly split the 56 samples into 4 groups, each of which represents a single genotype. Figure 3 shows the 56 samples plotted onto coordinates one and three. In this case we see three groups in which samples of trees 1 and 4 cluster together. In summary, we see that coordinate one (33%) divided the samples into two groups (tree 3, and trees 1, 2, 4), whereas coordinate two (27%) divided the samples into three groups (tree 1, and trees 2, 3 and tree 4), then coordinate three (19%) divided the samples into two groups (trees 1, 3, 4, and tree 2). Analysis of the 4th through 6th coordinates revealed additional minor combinations of the genotypes, but no clustering by day or by sampling period.

CONCLUSION—The amount of diurnal variation in *J. scopulorum* trees sampled was much smaller than genotype variation when the trees were growing in a relatively uniform environment (CSU campus). Interestingly, the amount of day to day variation was comparable to the amount of diurnal

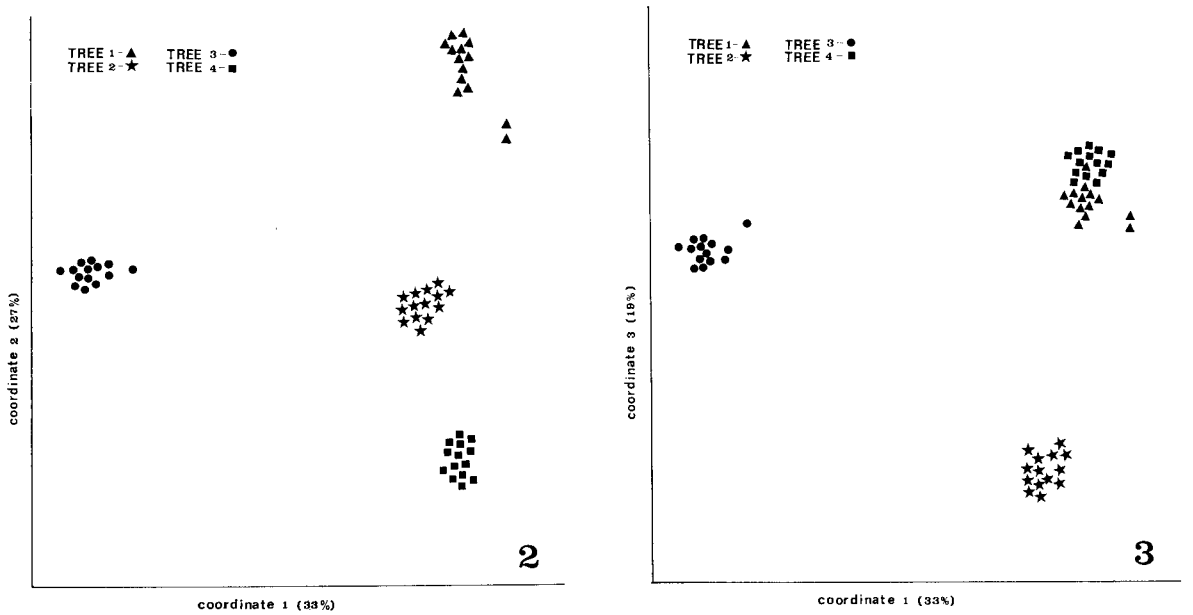


Fig. 2, 3. **2.** Principal coordinate analysis of the 56 OTU similarity matrix. Coordinate 1 extracted 33% of the variation in the similarity matrix and coordinate 2 accounted for 27% of the variation. The only trend apparent was the genotypes of the 4 trees. The two outlying triangles of tree 1 were sampled on day two at 2 and 6 am. **3.** Principal coordinate analysis of the 56 OTU similarity matrix. Coordinate 1 (33%) versus coordinate 3 (19% of the variation). The major trend of the third axis was to split the genotypes into trees 1, 3, 4 and tree 2. No evidence of clustering by day or sampling period was seen.

variation. The procedures used by numerical-chemosystematists to minimize random error within a population (weighting character matches by variance between/variance within populations) will of course not eliminate diurnal variation from the data. For example, if population 1 is sampled at 9 am, then population 2 is sampled at noon, the diurnal change from 9 am to noon will appear as a regional difference between populations 1 and 2. This difference would then be maximized by weighting. Fortunately, the diurnal changes in *J. scopulorum* appear to be small compared to genotype differences (which in turn are small compared to regional differences, Adams 1972, 1975b, c; Adams and Turner, 1970).

There is some evidence on the effects of diurnal variation on chemosystematics in other species of *Juniperus*. In populational studies (Adams and Turner, 1970; Adams, 1972, 1975b, c), the same populations of *J. ashei* and *J. pinchotii* were sampled in the fall of 1967 and 1970. The same pattern of population differentiation was reconfirmed in both cases. These differences were generally smaller than the diurnal changes reported in *J. californica* (Tatro et al., 1973). If large changes are occurring in these two taxa it seems rather unlikely that each population would have been resampled at the same time of day, 3 years apart. This same type of reconfirmation study has been done on geographical variation in the terpenes of *J. virginiana* (Flake, von Rudloff, and Turner, 1969, 1973). They also reconfirmed the same pattern of differentiation where the differences in the concentrations were very small. Again, it seems improbable that the original population (additional populations were added) were sampled at the same time of the day, one year apart.

The present study was conducted during a portion of the year when *J. scopulorum* is, metabolically, very active (Powell and Adams, 1973), whereas samples taken for chemosystematic purposes are deliberately taken either from the dormant season (as in the population studies cited above) of the year or from uniform garden materials (Adams, 1970; Powell and Adams, 1973; von Rudloff, 1975). Tatro et al. (1973) sampled *J. californica* from Redlands, California, on November 13 and found large differences during the day. The sizes of these differences were such that chemosystematic studies of that taxon on a regional basis would appear to be very difficult, if sampling was done during that portion of the year. Additional research will be needed to find a suitable time for sampling *J. californica*.

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