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DIURNAL VARIATION IN THE TERPENOIDS OF JUNIPERUS SCOPULORUM (CUPRESSACEAE)—SUMMER VERSUS WINTER[1]

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ABSTRACT

Samples of *Juniperus scopulorum* taken in January, 1977, were compared to samples of July, 1975. Only one significant difference was found in the winter sample compared to thirteen in the summer, 1975 sample. Five compounds showed significant changes from day to day in the winter compared to eleven in the summer. Thirty-two compounds showed significant differences among trees in the winter in contrast to thirty-six significant differences in the summer. Diurnal variation can apparently be minimized by sampling during the most dormant period and is strongly encouraged for chemosystematic studies.

IN a previous study of *Juniperus scopulorum* (Adams and Hagerman, 1977), diurnal variation was found to be considerable during the summer but much less than genotype variation. Part of these daily variations is probably associated with growth of the leaves (Adams and Hagerman, 1976; Powell and Adams, 1973), but undoubtedly much of the diurnal variation is influenced by photosynthesis during the day (Fretz, 1976; Lincoln and Langenheim, 1977; Tatro et al., 1973; Hopfinger, Kumamoto, and Scora, 1979) and by catabolism of the terpenoids during the night (Adams and Hagerman, 1977). If this is a major factor then one would expect to find less diurnal variation during a season when the plants are not very active photosynthetically and are not growing. The literature has been recently reviewed (Adams and Hagerman, 1977; Hopfinger et al., 1979).

MATERIALS AND METHODS—Four trees of *J. scopulorum* were sampled on January 24, 25, 1977, on the campus of Colorado State University, at 3 pm, 6 pm, 10 pm, 2 am, and 6 am on each day. All procedures were the same as used previously in the study of summer diurnal variation (Adams and Hagerman, 1977). Analysis was by three-way ANOVA, 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; and 6 am) as in the previous study. SNK tests ($P = 0.05$) were used to determine if the means of the sample periods differed significantly (error variance = DPT residual, df = 18).

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RESULTS AND DISCUSSION—Table 1 shows the three-way ANOVA for eight compounds that had any significant differences for either days, periods, or days × periods in interactions. In addition, seven compounds in Table 1 showed highly significant differences among trees, as well as twenty-four (for a total of 31) compounds which showed highly significant differences among trees. One had a significant difference ($P = 0.05$) among trees. The pattern shown was general for all terpenoids analyzed, in that most (32 of 40) had F ratios for among trees many times greater than the Fs for the other factors. Only terpinolene had a significant F for among periods. This variation is shown in Fig. 1 along with the daily temperatures and the four major trends from the summer study. Terpinolene (dashed line, Fig. 1) showed an increase in the late afternoon (3 pm to 6 pm) then a decline through the night. This is somewhat similar to the summer pattern for γ-terpinene, linalool, 4-terpineol, etc. One might note that terpinolene is much less variable than the compounds shown in Fig. 1 from the summer samples.

No significant differences were found for terpinolene from 6 am to 3 pm, but the 6-pm sample was significantly larger ($P = 0.05$) than the samples from 6 am to 3 pm. In addition, the 10-pm sample was significantly larger than the 6-am sample.

The day to day variation in compound 88 (oxygenated sesquiterpene) was highly significant along with significant differences for para-cymene, 4 terpineol and beta-eudesmol. The latter three have only a small amount of the variance in the D (days) term compared to the T (trees) term (Table 1). In contrast, compound 88's days' variance is about 20% of the trees' variance term. Since compound 88 is rather small (0.21%), it is possible that some non-random error in our analysis procedure may have been responsible.

Figure 1 suggests that diurnal variation may be greater in the summer than winter, and this is confirmed in Table 2. Notice that the number of significant or highly significant differences for periods, days, and interaction terms is much less in the winter samples than the summer samples. The differences among trees is about the same. In the summer samples there were 37 compounds (each greater than 0.1% total oil) that showed one or more significant differences in one of the treatments compared to 35 compounds in the winter samples. Note the changes in average F ratio for each term (Table 2). The days (D) F has dropped by 34% with the trees (T) F showing a small increase. The F for the sampling periods (P) has dropped by 67%! There were 13 compounds with at least significant differences in the summer and only one in the winter. The same trend is seen in the interaction terms. It seems obvious that short term fluctuatuions in the oil content are strongly linked with active metabolism (Powell and Adams, 1973; von Rudloff, 1972). In conjunction with our previous studies of this species (Adams and Hagerman, 1976; Powell and Adams, 1973), I would conclude that even during times of no growth the terpenoids remain an active metabolic pool, at least useful for catabolism as an energy and carbon source.

In general, the amount of diurnal variation in the oils of *J. scopulorum* in the winter is less than day to day variation and both are much, much less than differences in the variation of the genotypes studied. Diurnal and daily variations were much smaller during the winter than sum-

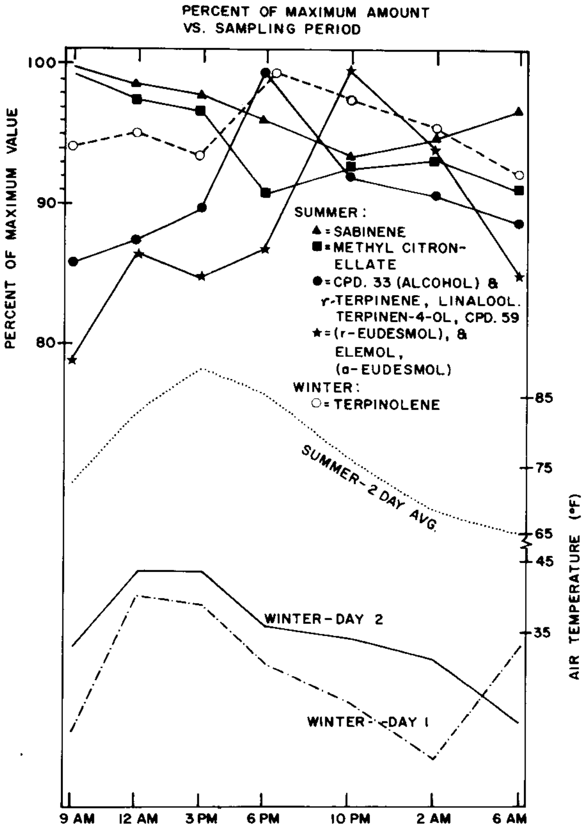

Fig. 1. Plot of the percent of maximum value observed versus sampling period and air temperatures versus time. Summer plots taken from Adams and Hagerman (1977). Terpinolene was the only compound that showed any significant diurnal differences in the winter. The same trees were sampled in both summer and winter.

TABLE 1. *Three-way analysis of variance, complete factorial design, with 2 days (D), 4 trees (T), and 7 sampling periods (P) for the eight compounds that showed any significance for either days, periods or days × periods interactions.* * *= significant at P = 0.05;* ** *= significant at P = 0.01*

Source of var.	df	Variance	F	Variance	F	Variance	F	Variance	F
		myrcene		p-cymene		terpinolene		4-terpineol	
D	1	.198	3.25	.0012	6.98*	.0002	.06	.523	5.58*
T	3	3.091	52.37**	.0317	187.99**	.1284	50.73**	9.658	103.09**
P	6	.256	1.26	.0008	.49	.0052	5.29**	.274	1.90
DT	3	.059	.29	.0002	.10	.0003	.26	.094	.65
DP	6	.252	.23	.0008	.47	.0015	1.54	.132	.91
TP	18	.147	.72	.0024	1.45	.0013	1.32	.059	.41
DTP	18	.203		.0016		.0010		.144	
		cpd. 61		citronellol		cpd. 88		β-eudesmol	
D	1	.00007	.003	.037	.57	.035	15.26**	.046	4.5*
T	3	.05503	2.154	1.919	29.76**	.173	75.35**	.714	70.73**
P	6	.01268	2.170	.013	.96	.004	.57	.004	.06
DT	3	.02555	4.373*	.064	4.88*	.002	.27	.010	.16
DP	6	.00530	.907	.009	.72	.010	1.15	.024	.38
TP	18	.0114	1.951	.015	1.16	.004	.48	.034	.54
DTP	18	.00584		.013		.009		.063	

TABLE 2. *Summary of the significant differences found in three-way ANOVA. Summer data from Adams and Hagerman (1977). The same four trees (genotypes) were used for the study in both summer and winter. Average F ratio is for those compounds significant at least at P = .05 and average value greater than 0.1%*

Source of variation	No. of significant differences (.05)		No. of highly significant differences (.01)		Avg. F ratio	
	summer	winter	summer	winter	summer	winter
Days (D)	7	2	4	3	5.00	3.29
Trees (T)	1	1	35	31	195.10	207.13
Periods (P)	3	0	10	1	2.75	0.90
D × T	1	2	4	0	1.71	0.90
D × P	2	0	2	0	1.46	0.84
T × P	0	0	0	0	1.15	0.83

mer. Diurnal variation could be minimized in chemosystematic studies by sampling when the plants are least active, metabolically.

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