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SEASONAL VARIATION OF SEXUAL DIFFERENCES IN THE VOLATILE OIL OF *JUNIPERUS SCOPULORUM*

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Key Word Index *Juniperus scopulorum* Sarg.; Cupressaceae; seasonal variation; sexual differences; terpenoids.

Abstract Very few differences between male and female trees were found in the volatile oil composition of *Juniperus scopulorum* Sarg. Differences were more apparent when the compounds were calculated as percent of total oil than as weight per g dry matter. Almost all of the sexual differences occurred in the growing season (March May). Essentially no differences were observed during the rest of the year. The observed differences were very small and should not affect the choice of plant material used in revegetating efforts (when browse potential is important). These differences should not affect chemosystematic studies, particularly if sampling is done during the period of the year when the plants are dormant.

INTRODUCTION

The genus *Juniperus* is almost unique of the conifers in having some species which are monoecious, and other species which are dioecious. The control of this sexual expression is not understood but it may be associated with stress or hormonal balance. Differences in chemical composition between male and female plants would be of physiological and taxonomic significance. In addition, if chemical differences in the volatile terpenes did occur between the sexes, male or female plants could be selected for cloning and subsequent revegetation use to maximize deer browse, etc. Most clones of cultivated juniper are known to be invariant in sexual expression although Vasek [1] reported that sexual expression in *Juniperus osteosperma* varied from year to year (i.e. male to female, female to male, male to neuter, female to neuter). In *Juniperus scopulorum* sexual differences appear to be closely regulated and we have not yet observed a monoecious tree.

Reports on the chemistry on different sexes are not frequent but a recent report [2] on the dioecious genus *Cannabis*, showed considerable differences in the concentration of cannabinoids in some strains. *Cannabis* from countries south of 30° latitude failed to mature (in Ottawa, Canada) and also failed to show sexual differences in the cannabinoid content, whereas those plants that did mature (from countries north of 30° latitude) showed sexual differences in the cannabinoids. Thus, we felt that if sexual differences do occur in the volatile oils of *Juniperus scopulorum*, these differences might vary seasonally. If these seasonal sexual differences occur, they need to be investigated for chemosystematic and physiological reasons. The literature on seasonal variation of terpenoids has been recently reviewed [3,4]. The purpose of this paper is to report on seasonal variations of the sexual differences in the volatile oils of *Juniperus scopulorum*

RESULTS

Table 1 shows the results of the statistical analysis. The most obvious result is that very few differences exist at any time of the year. The second result is that sexual differences are more noticeable in the data using percent than the weight based data (13 compounds per year versus 2 compounds per year, respectively). Both data sets show most of the differences during the growing season (March May). This is during the pollinating season (late April and May). Only one compound showed a significant difference in the dormant period of the year (compound 65 in November).

DISCUSSION

Very few sexual differences in the volatile oils occur in *Juniperus scopulorum*. Most of the differences are associated with the growing season (March–May). Since pollination of *Juniperus scopulorum* is in late April and May, some of the young female cones (before and just after pollination) were probably too small to recognize and remove from the foliage before distillation. The oil of the female cones is known to be different from the foliage [5] in *Juniperus*; this might account for some of the observed differences. The volume of cones at this stage is quite small compared to the volume of foliage, so this factor alone may not account for the differences.

For chemosystematic purposes it appears that sampling during the dormant season (fall and winter) will

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Table 1. Seasonal variation in sexual differences in *Juniperus scopulorum* using % and wt data. *t* = trace, less than 0·05%. See Vinutha and von Rudloff [11] for discussion of the sesquiterpene, acetate II.

	% data			Weight data		
		% in			g/g dry wt	
Sampling date	Cpds significantly diff. between male and female plants	male	female	Cpds diff	male	female
May 1, 1971	α-terpinene	1·91	1·49	acetate II	0·011	0·015
	γ-terpinene	3·30	2·63			
	4-terpinenol	6·68	5·31			
	cpd 27-unknown	0·33	0·20			
	cpd 59-unknown	0·56	0·23			
	cpd 68A-unknown	0·06	0·20			
May 29, 1971	none			none		
June 26, 1971	none			none		
July 24, 1971	methyl eugenol	0·65	0·51	none		
Aug. 21, 1971	cpd 65-unknown	0·17	t	none		
Sept. 18, 1971	none			none		
Oct. 16, 1971	none			none		
Nov. 13, 1971	cpd 65-unknown	0·15	t	none		
Dec. 11, 1971	none			none		
Jan. 8, 1972	none			none		
Feb. 5, 1972	none			none		
Mar. 3, 1972	cpd 54-unknown	1·84	2·46	none		
Apr. 1, 1972	linalool	0·87	0·57	4-terpinenol	0·0043	0·0033
	4-terpinenol	5·99	4·63			
Apr. 28, 1972	elemol acetate	2·78	3·43	none		

minimize the differences just as it minimizes the seasonal variation as suggested previously by Powell and Adams [3].

In a previous study [3] we recommend using percent data rather than weight data as the former is less affected by seasonal variation. We feel that the one compound difference in the fall of the percent data is not sufficient reason to use the weight calculated data. At present we have no satisfactory hypothesis to explain why the weight data shows fewer differences than the percent data. Another curious fact is that on May 1, 5 of 6 compounds with significant differences were in larger amount in the male trees. This trend is true all year except for the sample of March 3, 1972. Apparently a few compounds are increased in the male and the deficit distributed over many compounds which are not significant.

In general, sexual differences in the volatile oils of this species are not great enough to warrant their consideration in choosing plants for revegetation of browse species. Our experience indicates that regional variations are far larger than these sexual differences [5–10]. These sexual differences are also of little significance to chemosystematics, particularly if dormant season sampling is utilized.

EXPERIMENTAL

Six male and nine female trees of *Juniperus scopulorum* were permanently tagged near Masonville, Colorado. The foliage of these trees were sampled every four weeks throughout the year (14 sample sets) and the volatile compounds removed by steam distillation as previously described [3]. Female cones were removed before distillation to eliminate that variable. The volatile compounds were separated by capillary GLC (WCOT) and quantified by a digital integrator (see Powell and Adams [3] for GLC conditions, etc.). The composition of the volatile oil was computed as % total oil and wt/g dry wt [3]. These kinds of data (% and wt) were arranged in two sets (6 male and 9 female trees) for each of the 14 samplings. Analysis of variance and test of significant differences were performed on each 4 week sample (male vs female) for each of the 100 terpenoid compounds used. The peak ID's are the same as previously used [3], and their identities have been previously reported [11].

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