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Analyses of the Volatile Leaf Oils of *Juniperus deppeana* and its Infraspecific Taxa: Chemosystematic Implications

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Key Word Index – *Juniperus deppeana*; Cupressaceae; infraspecific taxa; leaf oils; monoterpenes; sesquiterpenes.

Abstract – Eight OTUs of *Juniperus deppeana* were collected and their volatile leaf oils analysed by GC/GCMS. The volatile oils are dominated by oxygenated terpenes such as camphor, linalool, *cis*-verbenol, 4-terpineol, verbenone, borneol and *trans*-sabinene hydrate. The major monoterpene found was α-pinene. Sesquiterpenoids were minor components of the volatile leaf oils. The six named taxa were only distinguished by combinations of terpenoid characters. The differentiation of the Arizona *J. deppeana* (var. *pachyphlaea* ?) and *J.d.* forma *sperryi* from the other taxa in Mexico was the major subdivision found. *Juniperus deppeana* forma *sperryi* was clearly distinguished as were *J.d.* var. *robusta* and *J.d.* var. *patoniana*.

Introduction

Juniperus deppeana and *J. gamboana* are the only juniper species in the Western Hemisphere with trunk bark that exfoliate in rectangular blocks instead of strips [1]. The distinctive bark-pattern of *Juniperus deppeana* undoubtedly gave rise to its common name, alligator juniper. However, one infraspecific taxon, *J. deppeana* forma *sperryi* (Correll) R. P. Adams, has bark that exfoliates in strips as well as flaccid foliage [2] and another taxon (*J.d.* var. *patoniana*) has bark that exfoliates in longitudinal strips [1].

The latest treatment of *Juniperus* [3] recognized the following infraspecific taxa: *Juniperus deppeana* Steudel var. *deppeana*; *J.d.* var. *deppeana* forma *sperryi* (Correll) R. P. Adams; *J.d.* var. *pachyphlaea* (Torrey) Martinez; *J.d.* var. *patoniana* (Martinez) T. A. Zanoni; *J.d.* var. *robusta* Martinez and *J.d.* var. *zacatecensis* Martinez.

Previously we have examined *Juniperus deppeana* and its varieties using 63 morphological characters [4]. That analysis showed *J. deppeana* var. *robusta* to be most similar to *J.d.* var. *patoniana* followed by successive clustering with *J.d.* var. *deppeana* and then with *J.d.* var. *zacatecensis*. Subsequent analysis using the volatile leaf oils clearly differentiated the deppeanan junipers from the other junipers of Mexico and Guatemala [5]. The similarities among the varieties based on morphology [4] were somewhat different from similarities based on volatile oils [5]. An enormous amount of intra-populational variation was found in both morphology [4] and volatile oils [5]. In fact, it now appears that no juniper species in the Western Hemisphere has as much infraspecific variation as *J. deppeana* [3–5, 8].

In the previous studies [4, 5] we did not have the facilities to completely identify the volatile oils of *J. deppeana*. The recent advent of glass and fused quartz capillary gas chromatography [6], coupled with computer-aided mass spectral identification [7] now makes possible a rather complete analysis of the volatile leaf oils of the infraspecific taxa of *J. deppeana*. We were also able to obtain specimens from Arizona, referable to *J.d.* var. *pachyphlaea* (Torrey) Martinez, which were not analysed in previous studies. In addition, we discovered a few *J. deppeana* trees south of Bavispe, Sonora, Mexico that had the bark exfoliating in strips (furrowed) as well as some degree of branch flaccidness. It thus seemed prudent to compare their volatile leaf oils with that of *J.d.* forma *sperryi*.

Results

The composition of the volatile leaf oils of the infraspecific taxa of *J. deppeana* as well as population samples from Arizona (BA, SA) and Bavispe, Mexico (BV) are shown in Table 1. In

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TABLE 1. COMPOSITION OF THE VOLATILE LEAF OILS OF *JUNIPERUS DEPPEANA* VAR. *PATONIANA* (DP); *J.D.* VAR. *ZACATECENSIS* (DZ); *J.D.* VAR. *DEPPEANA* (DD); *J.D.* VAR. *ROBUSTA* (DR); *J. DEPPEANA*, SEDONA, ARIZONA (SA); *J. DEPPEANA*, BISBEE, ARIZONA (BA); *J. DEPPEANA* F. *SPERRYI* (SP) AND *J. DEPPEANA*, BAVISPE, SON. (BV).

Component*	% Total oil								
	DP	DZ	DD	DR	SA	BA	SP	BV	*F* ratio
Tricyclene	t	t	0.6	t	1.0	0.9	t	0.8	10.5
α-Pinene	5.0	10.1	3.9	10.7	16.0	13.9	21.8	2.9	5.7
α-Fenchene	–	–	–	t	t	1.0	–	–	59.7
Camphene	t	t	0.7	t	0.6	t	t	t	4.2
Sabinene	2.4	1.8	1.3	1.6	14.6	3.5	6.6	9.1	12.4
β-Pinene	t	0.6	t	0.5	0.9	1.1	2.1	t	11.5
Myrcene	0.8	1.1	0.6	1.3	3.2	3.2	3.3	1.4	20.3
4-Carene	–	0.9	t	0.6	1.7	0.7	0.8	t	2.8
3-Carene	t	2.5	t	0.9	7.0	5.4	–	1.7	4.5
α-Terpinene	0.8	–	t	–	0.9	0.6	1.3	1.3	28.4
o-Cymene	1.6	0.6	0.7	1.3	1.4	3.9	3.8	1.1	7.3
β-Phellandrene	2.2	–	–	3.6	11.4	14.2	–	–	30.5
Limonene	–	2.9	2.5	–	0.7	–	8.3	4.1	17.1
γ-Terpinene	1.5	t	t	0.6	1.6	1.0	3.0	2.5	8.3
(o-Menth-1(7),3-diene)	0.8	t	0.9	0.6	0.6	t	3.1	1.1	4.4
DRX2, RRT = 0.305	1.1	1.0	0.6	0.9	t	–	t	–	7.2
FFX1, RRT = 0.306	–	–	0.6	–	t	–	1.3	1.0	20.9
o-Cymenene	t	–	–	1.3	t	t	–	–	17.8
Terpinolene	1.1	1.2	–	t	1.5	0.8	–	–	21.1
Linalool	8.5	6.3	1.5	8.4	1.3	1.1	3.6	2.7	7.6
(*trans*)-Rose oxide	0.9	0.9	0.8	0.7	t	t	t	t	5.1
BSX1, RRT = 0.339	1.6	2.1	1.1	1.5	t	0.6	t	t	4.7
cis-Sabinene hydrate	0.9	0.8	0.5	0.6	0.7	0.6	1.6	0.7	1.5
Camphor	11.2	10.2	43.9	19.0	7.8	9.8	6.2	8.9	6.5
trans-Pinocarveol	7.3	6.6	0.8	4.4	t	–	1.5	0.5	16.2
trans-Sabinene hydrate	–	–	–	–	0.7	1.5	–	–	79.4
Camphene hydrate	–	t	4.1	0.7	t	0.6	0.6	0.6	13.1
cis-Verbenol	12.6	14.4	8.9	10.7	1.0	2.2	0.9	t	5.1
DDX2, RRT = 0.381	t	0.6	t	t	t	0.7	0.6	t	1.1
Borneol	1.4	1.5	8.8	3.6	t	t	t	–	5.9
DDX3, RRT = 0.387	4.1	3.6	–	–	t	1.0	t	7.4	69.0
4-Terpineol	11.8	2.3	1.5	2.8	6.1	5.6	9.0	9.9	4.4
Myrtenal	1.9	1.8	1.0	1.2	t	t	t	t	7.8
o-Terpineol	1.0	1.3	tt	1.2	1.4	1.1	1.0	0.8	2.3
Verbenone	7.2	6.5	3.5	5.7	1.1	3.7	0.5	0.5	4.7
Myrtenol	1.9	2.0	1.0	1.1	t	t	–	–	4.8
trans-Piperitol	–	–	t	–	0.7	1.2	t	7.0	11.3
DRX5, RRT = 0.423	0.7	–	–	t	–	–	–	–	3.2
DZX5, RRT = 0.437	–	3.1	2.9	–	t	–	0.8	t	26.9
DRX6, RRT = 0.437	3.1	–	–	2.0	–	–	–	–	25.8
Myrtenyl acetate	–	–	–	–	t	0.9	–	–	13.0
Piperitone	t	1.8	t	1.5	t	1.3	1.2	4.6	1.3
Linalyl acetate	–	t	t	t	t	0.6	t	t	5.0
trans-Citral	t	t	t	–	t	0.7	t	t	13.4
Bornyl acetate	t	t	1.9	1.5	1.1	1.4	1.2	1.0	1.6
DDX5, RRT = 0.530	1.4	t	–	–	t	t	–	–	14.1
α-Terpineol acetate	–	t	t	t	2.7	6.7	t	t	12.3
DDX6, RRT = 0.658	–	–	–	t	t	t	–	t	3.8
α-Muurolene	t	t	t	t	t	t	0.6	0.7	4.2
γ-Cadinene	t	t	t	0.6	0.9	0.6	1.8	1.4	2.9
δ-Cadinene	t	t	t	t	0.8	0.7	0.8	1.5	5.6
Elemol	–	t	t	t	t	t	1.8	3.5	23.5
COX1, RRT = 0.715	t	t	t	–	t	t	0.6	t	5.3
α-Cadinol isomer 1	–	t	t	t	t	t	1.0	t	9.0
DDX7, RRT = 0.733	t	t	t	t	t	0.7	0.7	0.7	4.3
Cubenol	t	t	t	t	0.7	0.8	–	2.2	7.9

TABLE 1 – *CONTINUED*

Component*	% Total oil								
	DP	DZ	DD	DR	SA	BA	SP	BV	*F* ratio
γ-Eudesmol	–	t	t	–	t	–	0.6	1.6	13.2
α-Cadinol isomer 2	t	0.6	t	t	t	t	0.7	1.7	1.6
β-Eudesmol	–	t	t	–	t	t	t	3.7	15.0
α-Eudesmol	t	t	t	–	t	t	0.6	2.4	11.9
"Acetate II"	t	0.7	t	–	t	–	–	2.2	2.6
Manoyloxide	t	t	t	t	t	t	0.5	t	3.5

*Compound names in parenthesis are tentatively identified. Compositional values in parenthesis indicate that a component elutes at that retention time but no spectrum was obtained. Trace, t, indicates that the compound was less than 0.5% of the total oil. Components are listed in order of their retention on OV1. Relative retention times are relative to hexadecyl acetate.

general, the oils are dominated by the oxygenated monoterpenes such as camphor, linalool, *cis*-verbenol, 4-terpineol, verbenone, borneol and *trans*-sabinene hydrate. The sesquiterpenoids are generally rather minor components. Alpha pinene usually dominates the monoterpene hydrocarbons; however, sabinene and β-phellandrene are major components in the Sedona, Arizona (SA) population. The divergence of the deppeanan junipers from the other junipers is probably indicated by the relatively large number of compounds, 12, that could not be identified. Mass spectra for the structurally unknown (greater than traces) compounds are: DRX2, RRT = 0.305, *m/z* (%) MW 152, 97 (100), 43 (92), 81 (76), 109 (68), 95 (66), 41 (58), 53 (43), 56 (28), a terpene alcohol; FFX1, RRT = 0.306, *m/z* (%) MW ?, 41 (100), 43 (64), 109 (30), 67 (27), 91 (27), 93 (25), 95 (25); BSX1, RRT = 0.339, *m/z* (%) MW 152, 108 (100), 93 (68), 41 (56), 67 (28), 95 (26), 91 (18), 81 (17), aromatic terpene alcohol (?); DDX2, RRT = 0.381, *m/z* (%) MW 152, 59 (100), 94 (66), 49 (79), 93 (31), 91 (30), 77 (24), 43 (22), terpene alcohol; DDX3, RRT = 0.387, *m/z* (%) MW 152, 59 (100), 94 (68), 79 (57), 91 (33), 43 (33), 93 (23), 77 (20), isomeric to DDX2 above; DRX5, RRT = 0.423, *m/z* (%) MW 154, 93 (100), 91 (47), 77 (34), 79 (17), 136 (15), 41 (15), 92 (15), terpene alcohol; DZX5, RRT = 0.437, *m/z* (%) MW 152, 109 (100), 84 (63), 41 (61), 91 (53), 55 (41), 119 (34), 43 (33), terpene alcohol; DRX6, RRT = 0.437, *m/z* (%) MW 152, 91 (100), 119 (74), 109 (49), 134 (43), 77 (39), 92 (33), 84 (32), aromatic terpene alcohol; DDX5, RRT = 0.530, *m/z* (%) MW 152, 79 (100), 43 (83), 91 (48), 41 (32), 77 (29), 107 (24), 92 (22), 119 (14), terpene alcohol; DDX6, RRT = 0.658, *m/z* (%) MW 204, 161 (100), 105 (75), 119 (66), 43 (57), 41 (57), 91 (42), 81 (39), sesquiterpene; COX1, RRT = 0.715, *m/z* (%) MW 222, 41 (100), 43 (58), 79 (60), 93 (54), 69 (48), 91 (45), 81 (45), sesquiterpene alcohol; DDX7, RRT = 0.733, *m/z* (%) MW ?, 43 (100), 41 (65), 109 (51), 93 (57), 67 (30), 119 (22), 55 (20), 96 (18), sesquiterpene alcohol?

In order to visualize the chemical relationships among the taxa, similarities were computed and a principal coordinate analysis (PCO) was performed. The PCO resulted in seven eigenroots that accounted for 29.7, 21.3, 15.3, 11.6, 8.3, 7.5 and 6.3% of the variation among the eight OTUs. The first coordinate axis (Fig. 1) splits the Arizona populations (BA, SA), the Bavispe population, BV (just south of BA), and *J.d.* f. *sperryi* (Davis Mtns., Texas) from all of the deppeanas in Mexico. The second axis (Fig. 1) splits the furrowed bark populations (SP, BV) from the other junipers. It also shows some differentiation between the two Arizona populations (BA, SA). The third axis (Fig. 1) primarily shows the differences between *J.d.* var. *patoniana* (DP) and the other junipers. Four major groups are seen in Fig. 1: the Arizona populations (BA, SA = *J.d.* var. *pachyphlaea*?); *J.d.* forma *sperryi* (SP, BV); *J.d.* var. *patoniana* (DP); and *J.d.* var. *deppeana* (DD, eastern Mexico), *J.d.* var. *robusta* (DR, western Mexico) and *J.d.* var. *zacatecensis* (DZ, western Mexico). The minimum spanning tree has been superimposed (Fig. 1) and it is noticeable that the linkage between groups is relatively low, indicating that the custers are not well defined.

The fourth coordinate axis accounted for only 11.6% of the variation among OTUs and primarily indicated differentiation between BV and SP (Fig. 2). The fifth axis (8.3%) clearly distinguishes *J.d.*

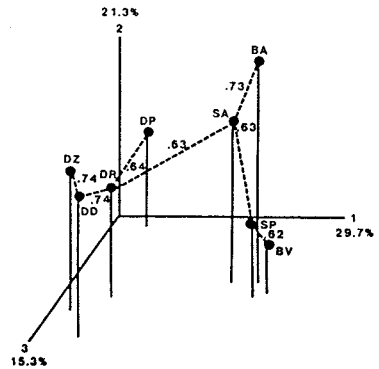

FIG. 1. PRINCIPAL COORDINATE ANALYSIS OF THE EIGHT OTUs WITH A MINIMUM SPANNING TREE (DASHED LINES) SUPERIMPOSED. The percent variation among taxa explained by each principal coordinate is indicated on each axis. OTUs are: DD = *J. deppeana* var. *deppeana* (eastern Mexico); DR = *J.d.* var. *robusta*; DP = *J.d.* var. *patoniana*; DZ = *J.d.* var. *zacatecensis*; BA = *J. deppeana* var. (*pachyphlaea*?), Bisbee, Arizona; SA = *J. deppeana* var. (*pachyphlaea*?), Sedona, Arizona; SP = *J.d.* forma *sperryi* (Davis Mtns, Texas); BV = *J.d.* forma (*sperryi*?), Bavispe, Son., Mexico. Similarities are shown next to the dashed lines. Axis one separates the Arizona (*pachyphlaea*?) junipers and the *sperryi* forms from the deppeana varieties from Mexico (DD, DP, DR, DZ). Axis 2 separates the *sperryi* form (SP, BV) from the other junipers. Axis 3 distinguishes *J.d.* var. *patoniana* (DP).

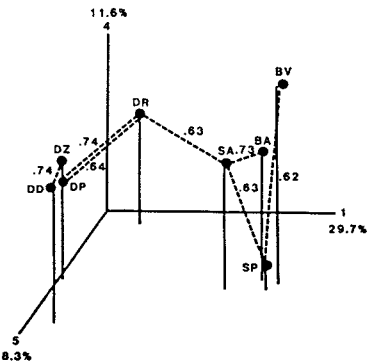

FIG. 2. PRINCIPAL COORDINATE ANALYSIS OF THE 6 TAXA. DEFINITIONS ARE AS IN FIG. 1. Axis 4 separates the SP from BV and axis 5 distinguishes *J.d.* var. *robusta*.

var. *robusta* (DR) from the complex (Fig. 2). The differentiation of *J.d.* var. *zacatecensis* (DZ) from *J.d.* var. *deppeana* (DD) is not seen in Figs 1 or 2, but these taxa were resolved on the sixth coordinate axis which accounted for 7.5% of the variation among the OTUs.

These results generally agree with the previous reports [4, 5] in showing the deppeanan junipers from Mexico to be closely related. We have previously reported that populations of both *J.d.* var. *robusta* and *J.d.* var. *zacatecensis* failed to cluster on the basis of the leaf volatile oils [5]. Infraspecific variation was not examined in this study but the lack of structure among the deppeanas from Mexico (DD, DR, DP, DZ), as depicted in Figs 1 and 2, may be a result of this variation.

The infraspecific taxa of *J. deppeana* can be placed into corresponding chemical groups as follows: *J. deppeana* var. *deppeana* (DD, Mexico); *J.d.* forma *sperryi* (SP, BV?); *J. deppeana* (Arizona, BA SA, possibly var. *pachyphlaea*); *J.d.* var. *patoniana* (DP); *J.d.* var. *robusta* (DR) and *J.d.* var. *zacatecensis* (DZ). The study presents a fairly complete identification of the leaf volatile oils and should lay the foundation for a comprehensive infraspecific and populational analysis which must be done before these taxa can be adequately understood.

Experimental

Samples consisted of 10–12 branchlets, 12–15 cm long from the following: *J. deppeana* var. *deppeana*, Rio Frio, Puebla, Mexico, *Zanoni 2619–2623*, Pachuca, Hidalgo, Mexico, *Zanoni 2696–2700*; Los Lirios, Coahuila, Mexico, *Zanoni 2825–2829*; *J.d.* var. *patoniana*, El Salto, Durango, Mexico, *Zanoni 2744, 2752, 2753, 2764, 2765*; *J.d.* var. *robusta*, 67 km W. of Durango, Durango, Mexico, *Zanoni 2755–2761, 2763*, Cuauhtemoc, Chihuahua, Mexico, *Zanoni 2832–2838*; *J.d.* var. *zacatecensis*, Sambrerete, Zacatecas, Mexico, *Zanoni 2726–2740*; *J.d.* var. (*pachyphlaea*?), Bisbee, Arizona, *Adams 1005–1009*; Sedona, Arizona, *Adams 1016, 2112–2116*; *J.d.* forma *sperryi*, Davis Mtns., Texas, type locality, isotype, *Adams 352*; S. Bavispe, Sonora, Mexico, *Zanoni 2871–2873, 2879*. Fresh foliage was frozen until steam distilled. The volatile terpenoids were removed by steam distillation for 2 h [9] for quantification analyses and 24 h for yield calculations. The extracts were kept at −20° until analysed to minimize chemical degradation. Voucher specimens are on deposit at TEX.

GC/MS analyses were run with a Finnigan Model 4000 Quadrapole Gas Chromatograph-Mass Spectrometer (Finnigan Corp., Sunnyvale, CA). MS scans were taken repetitively from mass 40 to mass 300 every second [10].

Chromatographic separation was achieved using a J & W fused quartz capillary column 0.32 mm i.d. × 30 m coated with OV1 (= DB1). All MS analyses were made in the split mode

(30:1 split ratio) using He carrier with an average linear velocity through the column of 21 cm/s. The column temperature was held at 55° for 6 min after injection and then programmed at 3° per min to 220°, 2 μl of the sample oils were injected after diluting with diethyl ether (1:30). Butyl acetate and hexadecyl acetate were added as internal standards. These compounds were chosen as standards because butyl acetate elutes before the most volatile terpenes and the hexadecyl acetate elutes after most terpenes found in these oils. Quantifications were made by peak area integration and summation using FID.

Identifications were made by comparisons of the MS of each component in the oils with MS of known terpenes and searches of spectra from the Finnigan Library (Finn Lib) of the U.S. National Bureau of Standards (NBS). Relative retention times (RR_t hexadecyl acetate = 1.00) were also compared to the RR_t of known terpenoids run under the same conditions.

Similarities between taxa were computed as *F*-1 weighted (see Table 1 for *F* values from ANOVA) Gower metrics [11, 12] with the chemical matches divided by the range of the character encountered in the study. Terpenoids with a range less than 0.5% among the 6 taxa were not used in the calculation of similarities. The similarity matrix was factored by principal coordinate analysis (PCO) following the programs of Gower [12] and Blackrith and Reyment [13].

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