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VOLATILE OILS OF MATURE AND JUVENILE LEAVES OF *JUNIPERUS HORIZONTALIS*: CHEMOSYSTEMATIC SIGNIFICANCE

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Abstract—Comparison of 39 terpenoids between young (juvenile) foliage and mature (adult) foliage from naturally growing plants of *Juniperus horizontalis* revealed no significant differences. Canonical variate analysis of the terpenoids of *J. scopulorum* and *J. virginiana* along with the mature foliage of *J. horizontalis* and co-plotting juvenile foliage showed a slight loosening of the *J. horizontalis* group but not enough to blur taxonomic distinctions. These results stand in sharp contrast with the previous work on *J. scopulorum* and appear to be due to the indeterminant growth pattern seen in *J. horizontalis*.

INTRODUCTION

In the course of sampling populations of *Juniperus horizontalis* Moench., it has been noted (R. P. Adams, unpublished work) that the juvenile leaves of this species are not regular in their occurrence as in other species of *Juniperus* from the section *Sabina* [1]. Juvenile leaves are formed in *Juniperus* from the time of the first cotyledons until the plants are several feet tall for the upright species (usually several years). The sharp-tipped, acicular juvenile leaves presumably offer resistance to herbivore browsing while the plants are small. Rarely one will find a tree where the genes for the production of juvenile leaf types have apparently mutated such that only juvenile leaves are produced on an otherwise mature tree (R. P. Adams, unpublished work). As junipers from the section *Sabina* mature they generally produce only scale-like leaves (mature foliage) except at the end of the growing tips (called terminal whips) where the juvenile leaves are found. There are three prostrate junipers in North America: *J. monticola* f. *compacta* Mart., *J. monticola* f. *orizabensis* Mart. and *J. horizontalis* in the section *Sabina*. Both forms of *monticola* tend toward prostrate shrubs and have the normal growth of juvenile leaves at the terminal whips on mature plants [1]. Growth in *J. horizontalis* is quite indeterminate with the prostrate branches sometimes rooting to form secondary clones. Growth is not only at the terminal whips but at secondary whips which may arise at almost any point along a branch.

The previous study of the terpenes of mature and young leaves in *J. scopulorum* revealed considerable differences [2]. This has also been reported in *Pinus* [3], *Sequoiadendron* [4], *Picea* [5], *Citrus* [6], and *Tanacetum* [7]. Since the separation of juvenile and mature leaves presents a problem in chemosystematic studies of *J. horizontalis*, we undertook this study to learn more about the possible variation involved and its effect on comparisons with closely related taxa.

RESULTS

Analysis of variance on the 39 terpenoids (each greater than 0.2% of the total oil) and per cent yield (g/g dry foliage wt) revealed no significant differences between the mature and juvenile foliage samples. Two compounds were nearly significant ($P = 0.05$). These were α-muurolene ($P = 0.064$) and δ-cadinene ($P = 0.088$).

von Rudloff [8] reported a very low concentration of oil in the juvenile leaves of one *J. virginiana* sample. We found the per cent yield of oil to be fairly similar (0.33 and 0.38% in juvenile and adult, $P = 0.25$) in *J. horizontalis*.

Canonical variate analysis (CVA) of the 30 most discriminating characters between *J. scopulorum*, *J. virginiana*, and *J. horizontalis* (mature foliage) yielded two eigenroots accounting for 94.15 and 5.85% of the variation among groups. Both roots were significant at levels above 0.0001 by Bartlett's test of sphericity [9–11]. The first canonical axis discriminated primarily between *J. scopulorum* and *J. virginiana/horizontalis* (Fig. 1). The second axis discriminated between *J. horizontalis* and *J. scopulorum/virginiana*. The effects of mixing adult and juvenile leaves in a chemosystematic study is shown in Fig. 1. The juvenile samples (triangles) cluster with the mature samples of *J. horizontalis*, although somewhat loosely. Samples 5A (adult) and 5J (juvenile) are from the same plant and both show differentiation toward *J. virginiana*. The maintenance of individual relationships with both adult and juvenile foliage has also been shown in *J. scopulorum* [2].

Analysis of variance omitting samples 5A and 5J resulted in slightly higher but still non-significant F ratios.

DISCUSSION

Several factors may account for the unexpected results which contrast with the 19 significant differences found in a previous study of the closely related taxon, *J. scopulorum*

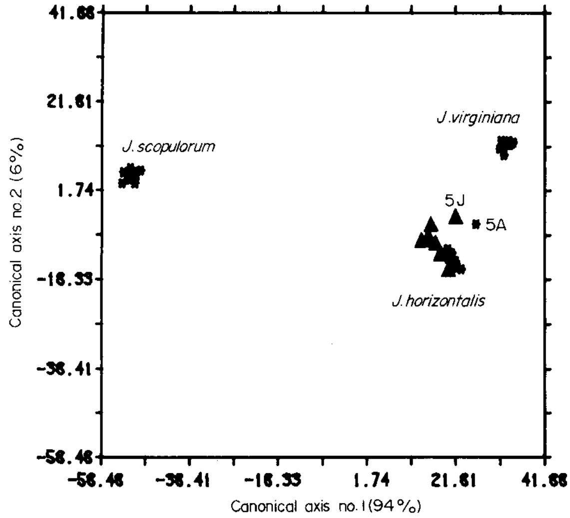

Fig. 1. Canonical variate analysis of the volatile oil of mature foliage with juvenile foliage samples (triangles) superimposed. Samples 5A and 5J are adult and juvenile foliage from plant No. 5 and show some differentiation toward *J. virginiana.*

[2]. In the previous study, all the plants were clonal and grown in a greenhouse. Environmental and genetic variability were therefore minimized [12–15]. Growth in *J. scopulorum* is strongly apical and determinant. It appears that the indeterminant and incomplete dominance of the scale-like (mature) leaf type over the acicular (juvenile) leaves in mature plants in *J. horizontalis* may not be strongly linked to the stability of the metabolic processes for the production and regulation of terpenoid synthesis.

Considering that no significant differences were found in ANOVA, it is somewhat surprising that the juvenile samples failed to cluster more closely with the mature samples (Fig. 1). It appears that mixing adult and juvenile foliage may not present too large a problem at the interspecific level and perhaps in the analysis of putative hybridization but would be significant in intraspecific studies of *J. horizontalis.*

EXPERIMENTAL

Fresh foliage was collected and frozen (until steam distilled) from 15 trees of *J. scopulorum* at Soda Springs, ID and 15 trees of *J. virginiana* at Washington, D.C. Samples of both mature and juvenile foliage were taken from seven plants of *J. horizontalis* and adult only foliage was taken from four additional plants of *J. horizontalis*; all 11 plants were collected near Mackinaw City, MI. All female cones were removed from plants to remove sexual bias [14]. Voucher specimens are filed at the Science Research Centre, Gruver. The volatile terpenoids were removed by steam distillation for 2 hr [12] and the extracts kept sealed at −20° until analysed by GLC. The GLC conditions used were: deactivated SP2100 glass capillary column (0.25 mm i.d. × 30 m): sample injections 0.1 μl of oil, split 1:20; N_2 14.3 cm/sec; injector 180°; FID 235°; temp programme 70° initial, 1.5°/min for 18 min, 2.5°/min for 25 min, 6°/min for 6 min, 4°/min for 6 min then isothermal at 217° for 12 min. Quantifications were by peak area integration with the solvent peaks later arithematically removed and the component values renormalized to 100%. The yield of oil %(w/v) was determined by drying the steamed foliage for 48 hr at 100°, weighing, and then using the corrected oil wt (i.e. without solvents) to obtain g oil/g dry foliage (× 100). Extraction for 2 hr removes about one-third of the total steam-extractable oil [8] and thus values should be multiplied by a factor of about 3 for comparisons with 24-hr distillations. Component identification follows the previously reported MS identifications for these species [16].

The data were coded, checked and then subjected to analysis of variance (one-way) with two populations: seven juvenile samples and 10 mature samples. Canonical variate analysis (CVA) follows the programs of refs. [9–11]. CVA was run using three *a priori* groups: *J. scopulorum* (15 samples, mature foliage); *J. virginiana* (15 samples, mature foliage); and *J. horizontalis* (nine samples, mature foliage). The seven juvenile foliage samples plus one odd appearing adult foliaged plant (No. 5A) were added as exemplars for ordination into the canonical axes to examine the effects of different foliage and to see if plant 5A would cluster with *J. horizontalis.*

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