Effect of Photoperiod and Nitrogen on the Composition of Foliar Monoterpenes of Juniperus horizontalis Moench. cv. Plumosa

Thomas A. Fretz
Department of Horticulture, Ohio Agricultural Research and Development Center, Wooster, OH 44691

Additional index words. essential oils, volatile oils, Juniper

Abstract. Monoterpenes from the foliage of Juniperus horizontalis 'Plumosa' (Andorra Juniper) grown under varying nitrogen levels and supplemental night lighting were measured using gas chromatography. Increasing photoperiod significantly increased the quantity of α-pinene and γ-limonene, while camphene, β-myrcene and linalool decreased. Other monoterpenes, including β-pinene, sabinene, terpinolene and α-terpineol were not significantly affected by photoperiod. Only α-terpineol showed a significant increase in relation to increasing level of N fertilization. All other monoterpenes decreased or remained unchanged as a result of increasing N levels.

There are indications that the biosynthesis and metabolism of the monoterpenes (leaf oils, volatile oils or essential oils) are influenced by environmental factors. Peppermint (Mentha piperita L.) oil, of acceptable commercial quality, can be produced only in certain geographical areas (7). There are numerous reports (2, 4, 7, 9, 15) that suggest that the monoterpenes of peppermint vary by growing region, but also by factors such as light intensity, fertilization, water balance and date of harvest. Peppermint plants grown on photoperiods of 14 hr or less produced only traces of essential oils indicating that individual monoterpenes are influenced by daylength (8). Temperature (3) and nutrition (11) also influence the production of monoterpenes hydrocarbons in peppermint.

Adams (1) observed larger variations in the monoterpene levels from tree to tree of Juniperus pinchottii Sudw., in the summer than in the winter. Similarly, Von Rudloff (14) concluded that collections of Picea glauca (Moench.) Voss., (white spruce), made during the early fall and winter were not subject to wide quantitative variations in monoterpenes yield.

Monoterpenes might be useful as a taxonomic aid for the identification of juniper cultivars. This study, preliminary to this objective, was to investigate the effects of daylength and nitrogen level on the quantity and quality of the monoterpenes of Juniperus horizontalis 'Plumosa' (Andorra juniper).

Materials and Methods

Ninety, 2-year old plants of Juniperus horizontalis 'Plumosa' grown in 4.5 liter containers in a media of 2 peat/v/v were selected for uniformity of growth and habit. All plants were tip-pruned and then placed in the greenhouses from July 1 through August 27. Using a 3 x 3 x 4 factorial design, the plants were supplied nitrogen as NH4NO3 at rates of 100, 200 or 400 ppm, daily or as required by Hefendahl (6) for a period of 15 min. The distilled oils were transferred to 1 ml sealed vials and stored at 0°C under N2 until chromatographic analysis.

For analysis, a Packard 409 Gas Chromatograph, equipped with a Varian 1200 digital integrator was employed. Separation of the monoterpenes was accomplished using 3.05 m x 3 mm O.D. stainless steel column packed with 20% LAC-446 on 80-100 mesh Chromosorb W HP. Injection and detection temp were maintained at 210°C. Carrier gas (N2) flow rate was 20 ml/min. Linear temp programming was employed from 80–190°C at a rate of 2.5°C/min with an upper temp hold for 20 min. In all chromatographic analyses, a 1 μl aliquot of the monoterpenes was used.

The area under each chromatographic peak was calculated and expressed as a % of the total ion count for each respective oil sample. Major peaks, i.e., those constituting greater than 0.5% of the total oil fractions, were identified by retention time, peak enrichment, and mass spectrometry. A DuPont mass spectrometer interfaced with a Varian Aerograph Model 2440 gas chromatograph was used for the GC-MS analysis. Leaf oil samples analyzed by mass spectrometer were chromatographed using the 20% LAC-446 on 80-100 mesh Chromosorb W HP column using the conditions previous described.

Results and Discussion

The detailed mass spectra used in the identification of the major leaf oils are available from the author upon request.

Table 1. Relative retention time of the major monoterpenes in Juniperus horizontalis 'Plumosa'.

<table>
<thead>
<tr>
<th>Monoterpene</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>0.46 – 0.47</td>
</tr>
<tr>
<td>Camphene</td>
<td>0.58 – 0.59</td>
</tr>
<tr>
<td>β-pinene</td>
<td>0.68 – 0.69</td>
</tr>
<tr>
<td>Sabinene</td>
<td>0.74 – 0.75</td>
</tr>
<tr>
<td>β-myrcene</td>
<td>0.83 – 0.84</td>
</tr>
<tr>
<td>γ-limonene</td>
<td>1.00</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>1.34 – 1.36</td>
</tr>
<tr>
<td>Linalool</td>
<td>2.68 – 2.73</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>3.64 – 3.73</td>
</tr>
</tbody>
</table>

1 Received for publication February 10, 1976. Approved for publication as Journal Article No. 10-76 of the Ohio Agricultural Research and Development Center. This investigation was supported in part by a grant from the Horticultural Research Institute, Washington, D. C. The author also wishes to express his appreciation to Ms. Marjorie R. Cobbs for her technical assistance during the course of this investigation.

2 Mailing address: Department of Horticulture, 2001 Fyffe Court, Columbus, Ohio 43210.

Relative retention times and identification of the major monoterpenes are given in Table 1.

The major monoterpene hydrocarbons observed in Andorra juniper were α-pinene, β-myrcene, γ-limonene and terpinolene. Variable, but present in smaller amounts, were camphene, β-pinene, sabinene, linalool and α-terpineol. These compounds comprised approx 80% of the total monoterpene composition present in Andorra juniper (Fig. 1).

While the effect (Table 2) of increasing the nitrogen level appears to decrease the levels of all monoterpenes, except γ-limonene and α-terpineol, care must be exercised in the interpretation of these data. It is possible that the differences may not be great enough to be truly biologically significant. These results are in general disagreement with those of Steward and co-workers (11) who noted a sharp response not only in the quantity of total monoterpene production in peppermint but also that the quality of particular monoterpenes was influenced by the fertility level. Our results, however, are in agreement...
with those of Thorin and Nommik (13) who noted that the monoterpene composition of *Pinus sylvestris* L., (Scots pine), was not affected by annual fertilizer applications. The minimal variation they observed was attributed to genetic differences between clones.

Similar results (Table 3) as those noted for the effects of increasing nitrogen level on the monoterpene composition, were also observed for the overall effects of increasing the hours of supplemental night light. All of the monoterpene declines in quantity as photoperiod increased, with the exception of α-pinene and γ-limonene. These results are in general disagreement with the data of Langston and Leopold (8) who noted that the quantity and quality of the monoterpenes in *Juniperus* were photoperiodically responsive.

Based upon the literature, these data agree with that of Hanover (5) and Smith (10) who’s results have shown that neither the quantity or the quality of the monoterpenes in the genus *Pinus*, are greatly influenced by changing environmental factors. They suggest that genetic composition plays a dominant role in the production of monoterpenes. In addition, it might be possible that the test plant, *Juniperus horizontalis* ‘Plumosa’ may not have been as particularly suited to the testing of this hypothesis as some herbaceous perennials, i.e. peppermint, as noted in this study between varying levels of supplemental night light and nitrogen levels. Further study is warranted to examine in more detail if environmental factors influence the quantity and quality of the monoterpenes in *Juniperus*. In addition, there is enough evidence to suggest the use of standard growing conditions when attempting to use monoterpenes as a taxonomic aid in the identification of *Juniperus horizontalis* cultivars.

### Literature Cited