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# VARIATION IN LEAF OIL TERPENE COMPOSITION OF SITKA SPRUCE*

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**Key Word Index**—*Picea sitchensis*; Pinaceae; monoterpenes; isoamyl- and isopentenylisovalerate; intra- and inter-population variability; hybrids; chemotaxonomy.

**Abstract**—The composition of the leaf oil of Sitka spruce was determined and the quantitative variation within trees, within populations and amongst 3 southern, 5 central and 2 northern populations was recorded. No regional or geographic differences were found. In addition to the relatively large amounts of myrcene, piperitone, 1:8-cineole, and relatively low camphene and bornyl acetate percentages, the apparently unique isoamyl and isopentenyl isovalerate may serve to distinguish hybrids in areas of introgression with white spruce.

## INTRODUCTION

The Sitka spruce, *Picea sitchensis* (Bong.) Carr., differs somewhat from the other northwestern spruce species of North America in that its natural range extends along a narrow coastal strip from northern California to the Kenai peninsula in Alaska [1]. Although the range extends over 3000 km, it is confined mainly to a fairly narrow ecological regime (lowland coastal conditions). Only in the north is this spruce found also at higher elevations (up to 1000 m), which is unusual [2]. Wright [3] considers it to be a member of the northwestern spruce complex together with white spruce, *P. glauca* (Moench) Voss, Engelmann spruce, *P. engelmannii* Parry, and blue spruce, *P. pungens* Engelm., and found no serious genetic barriers between them in artificial hybridization. Sitka spruce is reported to hybridize or introgress extensively with white spruce in the Skeena and Nass River basins in northwestern British Columbia [2, 4, 5]. Other areas have been described [6] or are suspected [2], but the putative introgression with Engelmann spruce [5] could not be confirmed [2, 4]. Identification of hybrids, or the parent species in the areas of overlap, by means of morphological characters is problematic [2, 4, 5]. A major complicating factor appears to be that the cone scales of Sitka and white spruce intermediates are almost identical with those of Engelmann spruce [4].

In our chemosystematic studies of North American conifers utilizing leaf oil terpene compositions [7], we have found that botanically closely related species frequently have characteristic quantitative and sometimes also qualitative differences. Such differences can be useful in detecting hybridization and delineating introgression. Earlier, we analyzed the volatile oil of the whole foliage (leaves, twigs and buds) of Sitka, Engelmann [8] and white [9] spruce and found in addition to characteristic quantitative differences [7] two unidentified aliphatic esters which appeared to be unique in Sitka spruce. Considerable differences in composition between leaf, twig, bud and cortical oils exist in many conifer species [7]. Hrutfiord *et al.* [10] have found such differences in Sitka spruce, as well as variation in the cortical oil with age and location of the branchlets within a tree. We have now analyzed the leaf oil in more detail to establish if it can be used further in studies of hybridization and introgression between Sitka spruce and white spruce. The variation within trees, between trees of the same stand, and between southern, central and northern populations was determined to obtain a measure of the inherent variability and establish which terpenes can serve as characteristic biochemical markers.

* Part 5 in the series 'Chemosystematic studies in the genus *Picea*'.
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## RESULTS AND DISCUSSION

The overall composition of the volatile oil of the leaves of Sitka spruce is shown in Table 1. For comparison, the mean percentages of that of the twigs (branchlets without leaves and buds) from one southern, one central and one northern population are also shown. Because of the high within-tree variability of the latter [10] this oil was not further investigated.

The major leaf oil terpenes, myrcene, piperitone and camphor, are the same as those found in the whole foliage oil (leaves, twigs and buds) [8]. The two major unidentified aliphatic esters (peaks 10 and 12 [8]) were found to be isoamyl and isopentenyl isovalerate; traces of isoamyl alcohol and up to 0.3% of another isovalerate were also recorded. These esters have also the isoprene skeleton, but unlike the monoterpenes in which 2 isoprene units are linked head-to-tail, here 2 hemiterpenoid moieties are esterified with one another. Esters of isoamyl alcohol or isovaleric acid are common constituents of essential oils [11], but the above $C_{10}$ esters have only been found in the leaf oil of the yellow (Alaska) cedar, *Chamaecyparis nootkatensis* (D. Don) Spach (Cupressaceae) [12]. They were not found in the leaf or foliage oils of white, Engelmann, blue, black (*P. mariana* (Mill.) B.S.P.) or red (*P. rubens* Sarg.) spruce [7–9].

In addition to the above compounds, smaller amounts of α-pinene, camphene, β-pinene, α-phellandrene, car-3-

Table 1. Mean relative percentage composition of the volatile oil of the leaves and twigs of Sitka spruce

| Compound | Leaf* | Twig† | Compound | Leaf* | Twig† |
|---|---|---|---|---|---|
| Isoamyl alcohol | trace | — | Unidentified isovalerate | 0.1 | — |
| 2-Hexenal | 0.5 | — | Isoamyl isovalerate | 7.7 | — |
| Santene | trace | — | Isopentenyl isovalerate | 4.7 | — |
| Tricyclene | 0.1 | trace | Linalol | 0.4 | 0.8 |
| α-Pinene | 1.6 | 9 6 | Camphor | 11.4 | 1.5 |
| Camphene | 2.1 | 0.2 | Borneol | 1.7 | 1.5 |
| β-Pinene | 0 7 | 8.0 | Terpinen-4-ol | 0.8 | 0.6 |
| Sabinene | 0 1 | 0.6 | α-Terpineol | 2.4 | 0 2 |
| Myrcene | 27.3 | 7.8 | Citronellol | 0.5 | 0.1 |
| α-Phellandrene | 1.2 | 0.2 | *cis*-Piperitol | 0.4 | — |
| Car-3-ene | 0.6 | 9 8 | Piperitone | 19.3 | 0.5 |
| α-Terpinene | 0.1 | — | Bornyl acetate | 0.7 | 0.1 |
| Limonene | 3.6 | 5.5 | Citronellyl acetate | 0.2 | — |
| β-Phellandrene | 4 7 | 24.3 | Geranyl acetate | 0.5 | — |
| *cis*- and *trans*-Ocimene | 0.8 | ? | Sesquiterpene hydrocarbons | 0.2 | 0.5 |
| γ-Terpinene | 0.1 | 0.4 | Alcohols | 0.8 | 5.4 |
| Terpinolene | 0.5 | 1.9 | Unidentified (higher boiling) | — | 4 5 |
| 1:4-Cineole | 0.5 | — | | | |
| 1:8-Cineole | 3.2 | ? | | | |

* Means from 10 trees each of 3 southern, 5 central and 2 northern populations (see table 3 for locations).
† Means from 10 trees each of 1 southern (Brookings), 1 central (Vancouver) and 1 northern (Queen Charlotte Islands) populations.

ene, limonene, β-phellandrene, terpinolene, *cis*- and *trans*-ocimene, 1:4-cineole, 1:8-cineole, linalol, borneol, terpinen-4-ol, α-terpineol, citronellol, *cis*-piperitol, bornyl-, citronellyl- and geranyl acetate, as well as trace amounts of santene, tricyclene, sabinene, α- and γ-terpinene were identified. The smallamounts of sesquiterpenes were similar to the cadinene–muurolene isomers and their corresponding alcohols that we have found in spruce leaf oils before [7–9]. The artefact 2-hexenal, produced in cutting conifer leaves [7, 13] was recorded in 0.5–4% relative amounts. With the exception of 1:4-cineole and *cis*-piperitol, the above terpenes are all common spruce leaf oil components [7]. Unfortunately the latter two terpenes, as well as the unidentified isovalerate, are difficult to resolve from more prominent constituents and hence they cannot serve readily as distinctive biochemical markers. In contrast, the two major isovalerate esters are eluted after terpinolene but before camphor on low to medium polar GLC columns and hence they can be detected in small relative amounts as well.

Quantitative GLC analysis of the leaf oil from branchlets collected at different heights of individual trees (one each of a southern, a central and a northern population) showed a small degree of variation with height that is within the normal experimental error (e.g. Table 2, columns 1–4). This contrasts with large variations of the twig or cortical oils from different branches [10]. Tree-to-tree variation of the leaf oil within the same stand (Table 2, columns 4–8) was high, which is in agreement with previous findings [7]. The means from 5 trees and another set of 5 trees from the same population (Table 2, columns 9 and 10) differed. Thus, at least 10 trees per population must be sampled for representative means. In this aspect Sitka spruce resembles the Engelmann spruce [7].

The means of the major terpenes from 10 trees each of 2

Table 2 Within-tree and tree-to-tree variation of the major terpene percentages of the leaf oil of Sitka Spruce (Sooke district, Vancouver Island)

| | Tree 1* | | | | | Tree | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Terpene | A | B | C | D | Mean | 2 | 3 | 4 | 5 | Mean† | 5 Trees‡ |
| α-Pinene | 2.3 | 2.5 | 2 6 | 2.9 | 2.6 | 2.2 | 2 0 | 2 3 | 3.4 | 2.5 | 2.5 |
| Camphene | 3.8 | 3.5 | 3 7 | 3.8 | 3.7 | 1.4 | 2.2 | 3.9 | 5.6 | 3.4 | 2.1 |
| β-Pinene | 0.8 | 0.7 | 0.8 | 1.0 | 0.8 | 1.0 | 1.1 | 0.6 | 0.8 | 0.9 | 1.2 |
| Myrcene | 20.8 | 19.3 | 19.1 | 21.4 | 20.1 | 32.8 | 20 8 | 21.5 | 34.5 | 25.9 | 29.5 |
| Limonene | 1 4 | 1.3 | 1.6 | 1.3 | 1.4 | 2.6 | 2.8 | 2.8 | 1.9 | 2.3 | 3 4 |
| β-Phellandrene | 11.8 | 11.8 | 12.5 | 14.3 | 12.5 | 13.8 | 6.0 | 4.8 | 4 2 | 8 5 | 9.1 |
| 1:8-Cineole | 1.4 | 0.8 | 0.7 | 0.9 | 1.0 | 0.4 | 5.5 | 1.5 | 1 8 | 2.0 | 3.8 |
| Isoamyl isovalerate | 4.0 | 3.6 | 3.8 | 3.8 | 3.7 | 4.2 | 5.2 | 5.8 | 3.4 | 5.5 | 5 6 |
| Isopentenyl isovalerate | 2.4 | 2.0 | 2.0 | 1.9 | 2.1 | 2.6 | 4 4 | 4.8 | 1.7 | 3.1 | 4.7 |
| Camphor | 10.6 | 12.6 | 13.8 | 11 2 | 12.0 | 6.3 | 10.8 | 16.2 | 18.0 | 12.7 | 9.4 |
| Borneol | 6.3 | 7.1 | 6.9 | 6.0 | 6.5 | 4.9 | 3.7 | 1.0 | 0.4 | 3.3 | 2.7 |
| α-Terpineol | 2.3 | 3.0 | 2.4 | 2.0 | 2.4 | 0 9 | 2.9 | 1 0 | 1 8 | 2.1 | 1.6 |
| Bornyl acetate | 0.5 | 0.4 | 0.5 | 0.3 | 0.4 | 0 1 | 0 6 | 6.3 | 1.9 | 1.9 | 0.5 |
| Piperitone | 24.0 | 25.3 | 24.7 | 24.9 | 24.8 | 19.4 | 23.0 | 17.5 | 11.5 | 19.2 | 19.2 |

* Branchlets collected from 1–1 5 (A), 2–2.5 (B), 3–3.5 (C) and above 4 m (D).
† 5 trees Sooke Road, 20–50 m apart.
‡ 5 additional trees, Sooke to Metchosin.

Table 3. Mean percentages of the major leaf oil terpenes of different Sitka spruce populations (10 trees each)

| | Location* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| °North: | 53°37′ | 54°18′ | 49°09′ | 48°20′ | 49°15′ | 47°05′ | 46°33′ | 45°52′ | 44°44′ | 42°08′ |
| °West: | 132°00′ | 130°10′ | 125°55′ | 126°45′ | 123°08′ | 124°10′ | 123°58′ | 123°50′ | 124°02′ | 124°12′ |
| α-Pinene | 1.5 | 1.4 | 1.1 | 2.5 | 1.3 | 1.3 | 1.3 | 1.2 | 1.9 | 1.7 |
| Camphene | 2.2 | 1.7 | 1.7 | 2.7 | 2.1 | 1.5 | 1.6 | 1.9 | 1.6 | 3.3 |
| β-Pinene | 1.0 | 0.8 | 0.6 | 1.1 | 0.8 | 0.8 | 0.7 | 0.5 | 1.1 | 0.5 |
| Myrcene | 27.6 | 25.5 | 26.8 | 27.7 | 24.0 | 23.2 | 30.1 | 27.6 | 32.9 | 32.4 |
| α-Phellandrene | 1.1 | 1.2 | 1.8 | 1.2 | 1.5 | 1.4 | 1.7 | 1.3 | 1.9 | 1.6 |
| Limonene | 1.8 | 1.7 | 3.4 | 2.9 | 2.0 | 2.1 | 3.6 | 2.6 | 3.7 | 6.2 |
| β-Phellandrene | 5.5 | 4.3 | 6.1 | 8.8 | 2.8 | 4.8 | 6.2 | 4.3 | 6.0 | 3.5 |
| 1:8-Cineole | 3.4 | 2.8 | 4.7 | 2.9 | 2.7 | 5.5 | 3.3 | 4.1 | 2.1 | 3.4 |
| Isoamyl isovalerate | 7.8 | 5.8 | 8.2 | 5.6 | 7.0 | 6.5 | 9.5 | 8.6 | 7.5 | 5.5 |
| Isopentenyl isovalerate | 5.2 | 5.3 | 5.4 | 3.9 | 4.1 | 6.2 | 6.1 | 5.4 | 4.3 | 3.5 |
| Camphor | 8.8 | 11.2 | 7.5 | 11.1 | 4.1 | 6.8 | 6.3 | 10.1 | 6.2 | 17.2 |
| Borneol | 3.2 | 2.1 | 2.6 | 3.0 | 2.8 | 2.5 | 1.8 | 2.7 | 1.7 | 2.0 |
| α-Terpineol | 1.3 | 1.5 | 2.6 | 1.9 | 2.6 | 3.5 | 1.8 | 2.2 | 1.8 | 1.8 |
| Bornyl acetate | 2.6 | 1.8 | 0.7 | 1.2 | 2.9 | 0.4 | 0.6 | 0.6 | 0.3 | 0.9 |
| Piperitone | 16.0 | 23.2 | 26.0 | 19.2 | 21.7 | 21.5 | 17.9 | 16.7 | 17.8 | 13.2 |

* Nearest town or landmark (1) Alliford Bay, Queen Charlotte Islands (2) Prince Rupert, British Columbia (3) Tofino, Vancouver Island (4) Sooke, Vancouver Island (5) University of British Columbia, Vancouver (6) Ocean City, Washington (7) Bay Center, Washington (8) Canon Beach, Oregon (9) Otter Rock, Oregon (10) Brookings, Oregon.

northern, 5 central and 3 southern populations are shown in Table 3. There is remarkable overall similarity and although β-phellandrene and camphor means are more variable than the others, no geographic trend is discernible. This is noteworthy in view of the suggested northern and southern refugia of Sitka spruce and subsequent migration after the retreat of the ice shield [2]. Thus the leaf oil terpene composition will not be useful in determining provenance, as appears to be possible with e.g. some of the leaf polyphenols of Sitka spruce [14], or monoterpenes of the cortical oils of some members of the Pinaceae [15, 16]. The large tree-to-tree variability within stands indicates large individual genetic differences and hence clone identification and family characterization may be possible. More important from the point of view of hybridization and introgression is that the two highly characteristic isovalerate esters were present in each tree. Also, the relatively high myrcene and piperitone and relatively low α-pinene, camphene and bornyl acetate percentages (in relation to other North American spruce species) were consistent in all populations. These terpenes appear, therefore, suitable as biochemical markers, especially with respect to introgression and hybridization with white spruce.

Table 4. Relative percentages of major leaf oil terpenes of 3 spruce trees from the Haines Highway* (near mile 73), Yukon Territory and typical western white spruce†

| | Tree 1 | Tree 2 | Tree 3 | White spruce |
|---|---|---|---|---|
| Tricyclene | 0 | 0.2 | 0.1 | 0.3 |
| α-Pinene | 0.3 | 2.8 | 2.4 | 6.5 |
| Camphene | 0.1 | 4.0 | 4.7 | 10.2 |
| Myrcene | 24.7 | 8.7 | 3.5 | 5.6 |
| Limonene | 4.1 | 2.9 | 21.2 | 9.9 |
| β-Phellandrene | 8.2 | 7.4 | 0.5 | 0.3 |
| 1:8-Cineole | 3.5 | 4.8 | 1.8 | 0.4 |
| Isoamyl isovalerate | 0.9 | 0.5 | trace | — |
| Isopentenyl isovalerate | 0.8 | 0.2 | trace | — |
| Camphor | 2.4 | 4.5 | 34.0 | 43.5 |
| Borneol | 0.5 | 7.3 | 2.2 | 1.8 |
| Piperitone | 34.5 | 23.5 | 0.5 | 0.5 |
| Bornyl acetate | 0.7 | 9.8 | 14.2 | 12.0 |
| Citronellyl acetate | 4.1 | 0.5 | 0.3 | trace |
| Sesquiterpenes | 1.1 | 2.0 | 5.4 | 2.8 |

* Elevation 1000–1300 m. Cone scales and needles similar to Engelmann spruce or white × Engelmann spruce.

† Northwestern Alberta [7].

To test whether intermediates or hybrids can be recognized by leaf oil analysis 3 trees along the Haines Highway in southwestern Yukon Territory, which is in an area of possible introgression of Sitka and white spruce [17], were sampled. The cone scale and needle characteristics of these trees were similar to Engelmann spruce or white × Engelmann spruce intermediates, just as reported by Roche [4]. The relative percentages of their major leaf oil components are shown in Table 4, which also shows the mean values for typical white spruce from western populations which are distant from the natural ranges of either Sitka or Engelmann spruce. As can be seen from the relative percentages of myrcene, piperitone, α-pinene, limonene, 1:8-cineole, camphene and camphor, and bornyl acetate trees 1 and 2 are clearly intermediates, with those of tree 1 leaning more towards Sitka spruce than those of tree 2. Tree 3 has terpene percentages that are similar to those of western white spruce, although that of limonene is unusually high. Most significant, perhaps, is the presence of small percentages of the two isovalerate esters in trees 1 and 2, and traces in tree 3, indicting that gene exchange with Sitka spruce must have taken place. Hence, it is concluded that hybrids and the various intermediates in areas of introgression between Sitka and white spruce can be detected by leaf oil terpene analysis. The same may prove possible for intermediates between Sitka and Engelmann spruce, but unfortunately leaf samples of authentic hybrids have not become available to test whether intermediate terpene patterns are also found in these.

## EXPERIMENTAL

Initially, branchlets (50–100 g) were taken at 4 different heights (1–1.5 m, 2–2.5 m, 3–3.5 and above 4 m) from individual trees from a northern (Prince Rupert), a central (Vancouver) and southern (Brookings) population. Subsequently, branchlets were taken at random from different heights and sides of each tree to give a combined sample of about 200 g. All samples were collected in the fall or winter and were kept below 5° during transport and stored at −20°. The leaves were separated from the twigs by dipping the branchlets into liquid nitrogen and stripping the frozen needles off. These were steam-distilled for 6 hr and the volatile oil recovered as described before [18] GLC on 4 analytical and 2 preparative columns was carried out as reported earlier [18, 19] Peak integration and calculation of the relative percentages was done with a Hewlett-Packard Model 3352 data system. GC–MS was carried out with a Finnigan Model 3300 MS, using a 3 m × 0.03 cm glass column with polyethylene glycol 20M (2%) and OV-17 polysiloxane (1%) on high performance Chromosorb G (80–100 mesh). The mass spectra were recorded both at the front and back of each peak to determine whether a single compound or a mixture was eluted. Compounds which failed to give a parent ion with the electron impact (70 eV) method were analyzed by the chemical ionization method (methane as carrier-reagent gas). All major and most minor components were isolated by preparative GLC and their PMR and IR spectra were recorded. The identities of the compounds shown in Table 1 were confirmed by comparison of $RR_t$ and spectra with those of the known terpenes. Isoamyl, isopentenyl and isobutyl isovalerate were prepared by esterification of the respective alcohols with isovaleric acid and *cis*- and *trans*-piperitol by $LiAlH_4$ reduction of piperitone. The trees from the area of introgression with white spruce along the Haines Highway were situated near mile 73 (trees 1 and 2), elevation 1300 m, and near mile 84 (tree 3), elevation 1100 m.

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