

Trunk wood essential oil profile comparison of *Abies concolor* (Pinaceae) and *Abies grandis* (Pinaceae) from northern Idaho (USA)

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ABSTRACT

Abies concolor and *Abies grandis* are essential oil-bearing plants in the Pinaceae family. Essential oil produced through steam distillation of the trunk wood was examined to establish the essential oil profile from cultivated populations of both species in northern Idaho (USA). The resulting essential oils ($n = 6$) were analyzed by GC/MS and GC/FID. Prominent volatile compounds (averages) from *A. concolor* trunk wood include α -pinene (12.2%), camphene (8.5%), β -pinene (29.0%), δ -3-carene (9.7%), limonene (5.1%), and bornyl acetate (9.4%). Prominent volatile compounds (averages) from *A. grandis* trunk wood include tricyclene (2.4%), α -pinene (11.8%), camphene (23.4%), β -pinene (11.0%), δ -3-carene (2.3%), limonene (8.5%), and bornyl acetate (17.5%). Comparing the two species, trunk wood essential oil profiles are similar, with 6 prominent volatile compounds in common. However, key volatile markers differentiate each species and could be used for future chemotaxonomic investigations. *Published online www.phytologia.org Phytologia 104(4): 66-73 (December 21, 2022). ISSN 030319430.*

KEY WORDS: *Abies concolor*, *Abies grandis*, aromatic profile, chemotaxonomy, conifer, essential oil, trunk, Pinaceae.

Abies concolor (Gordon & Glend.) Lindl. Ex Hildebr. and *Abies grandis* (Douglas ex D. Don) Lindl. are aromatic fir trees in the Pinaceae family (The World Flora Online 2022).

Abies concolor has a native range that spans western North America, the southern Rocky Mountains, and south to northern Mexico (Auders and Spicer 1990; Cronquist et al. 1972; Laacke 1990). However, throughout its widespread distribution, there exist many isolated pockets, both geographically and genetically, of native populations (Flora of North America 1993). *Abies concolor* trees grow at elevations between approximately 600 to 4000 meters and reach about 60-70 meters in height, with a smooth bark with elongated markings. The 2-2.5 mm wide curved needles are bluish green in color on both adaxial and abaxial surfaces (Auders and Spicer 1990; Cronquist et al. 1972).

Abies grandis is the tallest of the fir species and grows to a height of approximately 80 meters. This species is native to moist forests of the northwestern United States and southwest Canada and is found anywhere from sea level to 1800 meters elevation (Auders and Spicer 1990; Flora of North America 1993). Populations are described as morphologically and chemically uniform (Flora of North America 1993). The 2 mm wide and 20-35 mm long needles are a glossy dark green color on the adaxial surface and a greenish white on the abaxial surface, with a sharp tip at the end (Auders and Spicer 1990).

Conifers have been used by native peoples of British Columbia as medicine for respiratory illnesses and dermatological ailments in the form of tonics and external poultices, respectively, as well as for bedding and ground cover in living quarters (Turner 1998; Turner and Hebda 1990). In south-central Colorado, native peoples used *A. concolor* resin on skin blemishes or would mix resin with sugar to create a drink to fight urinary tract infections (Bye and Linares 1986). Salishan elders (Vancouver Island) drank *A. grandis* bark infusions to treat several ailments including tuberculosis, ulcers, colds, and stomach issues (Turner and Hebda 1990). The Southern Kwakiutl Indians of British Columbia collected pitch from young trees to create tonics for coughs, tuberculosis and as a laxative. Southern Kwakiutl Indians also held pieces of the root in their mouths to remedy canker sores (Turner and Bell 1973). In more recent history, the culinary world refers to *A. grandis* as the “grapefruit pine” because of its aromatic profile and citrus flavor (Valerón et al. 2021). The wood of both species is considered light and nondurable, and is used for woodworking and pulp, rather than construction (Uphof 1968).

The essential oil profile of both *Abies spp.*, extracted from foliar portions, cones, and/or cortical specimens, has been previously analyzed and established. *Abies concolor* leaf and cone essential oils have been found to be primarily composed of α -pinene, camphene, β -pinene, limonene, and bornyl acetate (Adams et al. 2011; Swor et al. 2022; Wajs-Bonikowska et al. 2017). *Abies grandis* leaf essential oil has been found to be primarily composed of α -pinene, camphene, β -pinene, β -phellandrene, and bornyl acetate (Adams et al. 2015; Zavarin et al. 1977). To the best knowledge of the authors, the essential oil profiles of the trunk wood has not been previously established in full for either species. Previous research on evergreen species in the Caprifoliaceae and Pinaceae families has shown that trunk wood essential oil often has a different essential oil profile than other portions of the tree, and often contains unique compounds that can be used for chemotaxonomic investigations (Poulson et al. 2020, 2021; Wilson et al. 2019, 2021). The current study establishes essential oil profiles for samples extracted from the trunk wood of both *A. concolor* and *A. grandis*, and provides an integrative tool for chemotaxonomic investigations.

MATERIALS AND METHODS

Abies grandis and *Abies concolor* plant material was collected from privately owned cultivated tree farmland in Bonner County, Idaho, USA. *Abies grandis* plant material was collected December 14, 2021 (48°34'40.1" N 116°26'56.7" W; 680 m elevation). *Abies concolor* plant material was collected February 8, 2022 (48°28'13.0" N 116°27'36.1"W; 674 m elevation). Four trees of each species were cut approximately halfway up the trunk utilizing the stump culture technique (Wunderlich 2020). Only the trunk material was used for this research, which includes the inner and outer bark, cambium, sapwood, and heartwood sections. Representative voucher samples used for identification are held in the University of Idaho Stillinger Herbarium in Moscow, ID, USA, and the Consortium of Pacific Northwest Herbaria in Seattle, WA, USA.

The plant material was prepared for distillation as follows (Figure 1). The limbs were removed flush against the trunks, leaving only the main tree trunk with no needles or limb material. The plant material, which included four trees (average age of nine years – determined by dendrochronology) for each species, was chipped with a woodchipper, blended, and stored in an airtight container at -20 ± 2 °C until steam distilled. Three separate steam distillations were performed on the prepared chips for each species, resulting in a total of six distillations for this study. The distillations were conducted in a 12 L food grade stainless steel distillation chamber with approximately 2.5 liters of water added to the chamber. Steam was passed through suspended chips for two hours after pass-over and the essential oil was separated from hydrosol using a cooling condenser and collected in an analytical graduated cylinder. The essential oil was stored in a sealed amber glass bottle until analysis.

Essential oil samples were analyzed, and volatile compounds identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 0.25 mm

× 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in ethanol), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min., oven ramp rate of 4.5 °C per min. to 310 °C with a hold time of 5 min. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per sec., source temp. 230 °C, and quadrupole temp. 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams 2007) using Chemstation library search in conjunction with retention indices. Note that limonene/β-phellandrene/1,8-cineole, bornyl acetate/2-undecanone, β-cubebene/β-elemene, and fenchone/terpinolene elute as single peaks. Their amounts were determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene), 81 and 108 (1,8-cineole), 69 and 81 (fenchone), 93 and 121 (terpinolene), 95 and 121 (bornyl acetate), 58 and 71 (2-undecanone), 105 and 161 (β-cubebene), and 81 and 93 (β-elemene). Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B GC and Agilent J&W DB-5, 0.25 mm × 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in ethanol, 1% for reference compounds in ethanol, 0.1% soln. for C7–C30 alkanes in hexane), 25:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 2 min., oven ramp rate of 3.0 °C per min. to 250 °C with a hold time of 3 min. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were identified using retention indices coupled with retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

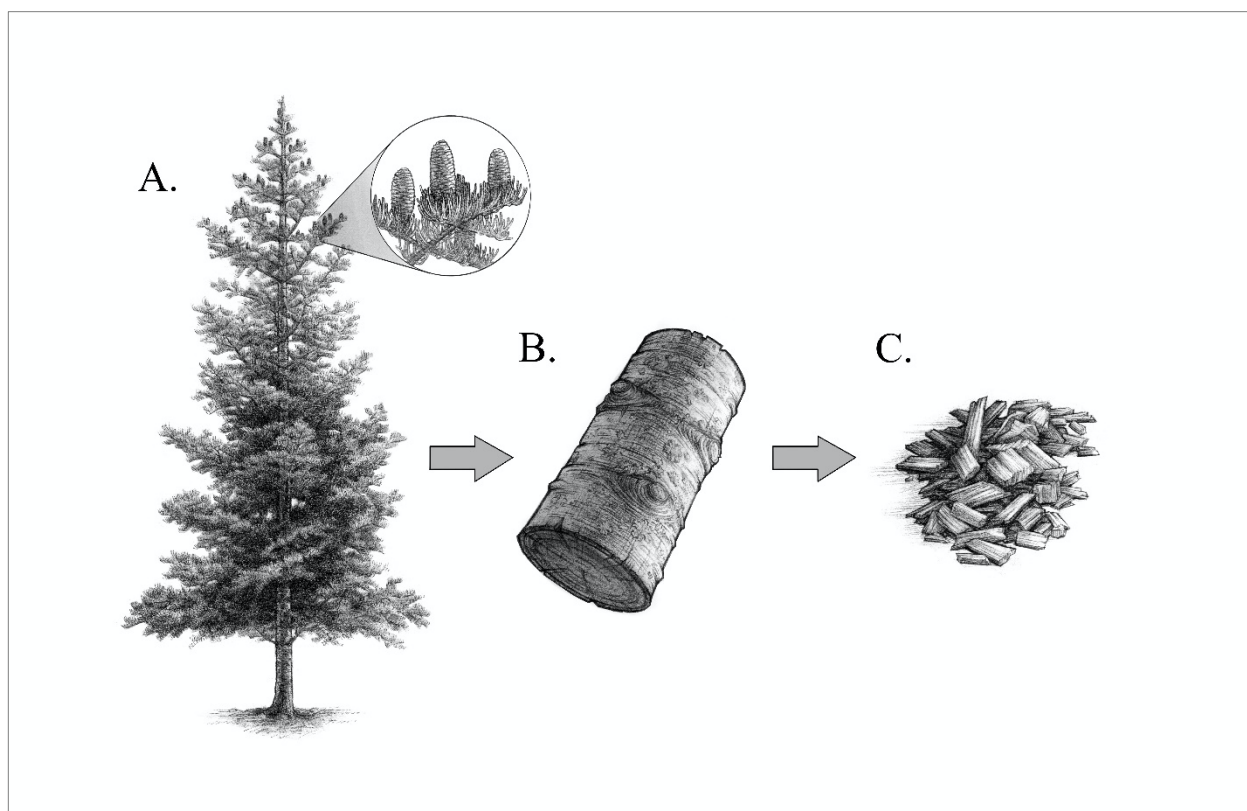


Figure 1. Botanical illustration of *Abies concolor* plant material collection and processing (plant material for both species was collected and processed identically). The tree (A) was felled according to the stump culture technique (Wunderlich 2020), all limbs removed flush against the trunk (B), trunk sections were chipped and blended (C), and stored at -20 ± 2 °C until steam distillation. Illustrated by Zach Nielsen, Utah Valley University (Orem, UT, USA).

RESULTS AND DISCUSSION

The aromatic profile of both *Abies concolor* and *Abies grandis* trunks were determined by GC/MS and GC/FID, and are detailed in Table 1. Prominent volatile compounds (averages) from *A. concolor* trunk wood include α -pinene (12.2%), camphene (8.5%), β -pinene (29.0%), δ -3-carene (9.7%), limonene (5.1%), and bornyl acetate (9.4%). Prominent volatile compounds (averages) from *A. grandis* trunk wood include tricyclene (2.4%), α -pinene (11.8%), camphene (23.4%), β -pinene (11.0%), δ -3-carene (2.3%), limonene (8.5%), and bornyl acetate (17.5%). The essential oil profile of both *Abies spp.*, extracted from foliar portions, cones, and/or cortical specimens, has been previously analyzed and established. However, to the best knowledge of the authors, the essential oil profiles of the trunk essential oils have not been previously established for either species. Previously studied *A. concolor* leaf and cone essential oils have been found to be similar to the trunk essential oil analyzed in this study, with all samples being primarily composed of α -pinene (leaf/cone 11.2-20.5%; trunk 12.2%), camphene (leaf/cone 7.5-25.9%; trunk 8.5%), β -pinene (leaf/cone 24.2-52.0%; trunk 29.0%), δ -3-carene (leaf/cone 5.5-6.5%; trunk 9.7%), limonene (leaf/cone 5.4-6.9%; trunk 5.1%), and bornyl acetate (leaf/cone 14.6-22.1%; trunk 9.4%) (Adams et al. 2011; Swor et al. 2022; Wajs-Bonikowska et al. 2017). Previously studied *A. grandis* leaf essential oil has also been found to be similar to the trunk essential oil analyzed in this study, with all samples being primarily composed of α -pinene (leaf 4.4-7.4%; trunk 11.8%), camphene (leaf 8.3-11.5%; trunk 23.4%), β -pinene (leaf 20.3-31.0%; trunk 11.0%), and bornyl acetate (leaf 12.7-26.2%; trunk 17.5%) (Adams et al. 2015; Zavarin et al. 1977). The differences are the prominence of limonene (leaf 0.8-2.5%; trunk 8.5%) and β -phellandrene (leaf 13.7-25.2%; trunk 1.1%) in foliar and trunk samples of *A. grandis* essential oil. The overall similarity in essential oil profiles, when comparing extracts from different plant parts of the same species, may be characteristic of plants in the Pinaceae family (Poulson et al. 2020).

Many of the minor compounds differentiate the trunk essential oil profiles of *A. concolor* and *A. grandis* from each other. While santene is only present in traces in *A. concolor* essential oil, it comprises 0.5% (avg.) of *A. grandis* essential oil. The opposite is found with linalool; which comprises 0.9% (avg.) of *A. concolor*, but is only detected in traces in *A. grandis* essential oil. Examining the entire essential oil profile, 28 compounds are detected in one species but not the other, and could be used for chemotaxonomy. Those found in *A. concolor* essential oil, but not in *A. grandis*, include fenchone, trans-pinocarveol, pinocamphone, myrtenol, thymol methyl ether, cumin aldehyde, cis-3-en-5-one, γ -muurolene, trans-nerolidol, β -calacorene, caryophyllene oxide, cedrol, cis-14-nor-muurol-5-en-4-one, and manool oxide. Those found in *A. grandis* essential oil, but not in *A. concolor*, include ethyl isovalerate, ethyl octanoate, 2-undecanone, citronellic acid, β -elemene, γ -elemene, 6,9-guaiadiene, cardina-3,5-diene, δ -selinene, α -selinene, trans-cadina-1,4-diene, germacrene B, intermedeol, and farnesol acetate. These key differences in trunk essential oil profile have been previously used to distinguish and identify plant species when traditional taxonomic methods cannot be used, such as when identifying trees burnt in wildfires (Wilson et al. 2021).

While many of the prominent compounds in the essential oils extracted from the trunk of these two species are the same, their relative abundance varies greatly. In *A. concolor* essential oil, α -pinene, β -pinene, and δ -3-carene were detected at higher relative percentages. In *A. grandis* essential oil, camphene, limonene and bornyl acetate were detected at higher relative percentages. The relative percent differences of the same compounds found in the two species varies greatly, being as low as 3.0 (α -pinene) and as high as 122.3 (δ -3-carene) (Table 2). These differences in compound abundance could also potentially be used for future chemotaxonomic investigations.

Table 1. Aromatic profile of *Abies grandis* and *Abies concolor* essential oil from trunk material only. Compounds not detected in a sample are denoted as not detected (ND) and those with values less than 0.1% are denoted as traces (tr). Compounds less than 1.0% that were unidentified are not included. KI is the Kovat's Index using a linear calculation on the DB-5 column (Adams 2007), those in bold font were calculated using an alkane standard. Relative area percent is determined by GC-FID. All essential oil samples were analyzed in triplicate to ensure repeatability (SD <1).

Compound Name	KI	<i>Abies concolor</i>			<i>Abies grandis</i>		
		1	2	3	1	2	3
ethyl isovalerate	849	ND	ND	ND	0.2	0.2	0.2
santene	884	tr	tr	tr	0.4	0.6	0.6
tricyclene	921	1.0	0.7	0.9	2.3	2.4	2.4
α -thujene	924	0.2	0.1	0.2	0.1	0.1	0.1
α -pinene	932	13.0	10.4	13.3	11.9	11.4	12.2
camphene	946	9.4	7.4	8.8	22.6	23.5	24.2
sabinene	969	0.3	0.2	0.3	0.2	0.2	0.2
β -pinene	974	32.2	25.3	29.5	11.1	10.6	11.3
myrcene	988	1.1	1.0	1.2	0.5	0.6	0.6
δ -3-carene	1008	11.3	8.6	9.2	2.0	2.4	2.6
o-cymene	1022	0.5	0.5	0.5	0.2	0.1	0.2
limonene	1024	6.4	3.9	4.9	7.1	9.6	8.8
β -phellandrene	1025	0.8	2.3	2.1	0.8	0.8	1.7
1,8-cineole	1026	tr	tr	tr	0.1	0.1	0.1
fenchone	1083	0.3	0.5	0.4	ND	ND	ND
terpinolene	1086	0.3	0.2	0.2	0.1	0.3	0.3
linalool	1095	0.7	1.2	0.9	tr	tr	tr
endo-fenchol	1114	0.2	0.2	0.2	tr	tr	tr
trans-pinocarveol	1135	0.2	0.6	0.2	ND	ND	ND
camphor	1141	0.3	0.4	0.3	0.7	0.6	0.6
camphene hydrate	1145	1.3	1.9	1.5	0.6	0.5	0.4
pinocarvone	1160	0.2	0.3	0.2	ND	ND	ND
borneol	1165	0.2	0.8	0.6	0.5	1.3	1.1
terpinen-4-ol	1174	0.2	0.5	0.4	0.1	0.1	0.1
ρ -cymen-8-ol	1179	0.2	0.2	0.1	0.1	tr	tr
cryptone	1183	0.5	1.0	0.6	0.4	0.1	0.2
α -terpineol	1186	0.3	0.5	0.4	0.1	0.1	0.1
myrtenol	1194	0.3	0.5	0.3	ND	ND	ND
ethyl octanoate	1196	ND	ND	ND	0.1	0.1	0.1
endo-fenchyl acetate	1218	0.1	0.2	0.2	tr	tr	tr
citronellol	1223	0.6	1.1	0.9	0.3	0.5	0.4
thymol methyl ether	1232	tr	0.1	0.1	ND	ND	ND
cumin aldehyde	1238	0.1	0.2	0.1	ND	ND	ND
piperitone	1249	0.1	0.1	0.1	tr	tr	tr
p-menth-8-en-3-ol acetate	1270	0.1	0.2	0.1	0.1	0.1	0.1

bornyl acetate	1284	7.8	11.5	8.9	19.8	17.0	15.7
2-undecanone	1293	ND	ND	ND	0.1	0.1	0.1
cis-3-en-5-one	1305	0.4	0.4	0.3	ND	ND	ND
citronellic acid	1312	ND	ND	ND	0.1	0.1	0.1
α -cubebene	1348	0.4	0.5	0.5	0.8	0.8	0.7
citronellyl acetate	1350	0.8	1.8	1.6	1.2	1.0	0.9
neryl acetate	1359	0.1	0.6	0.4	0.6	0.5	0.5
α -copaene	1374	0.3	0.4	0.4	0.6	0.5	0.5
β -cubebene	1387	0.2	0.1	0.2	0.5	0.4	0.4
β -elemene	1389	ND	ND	ND	0.1	0.1	0.1
(E)-caryophyllene	1417	0.1	0.1	0.2	0.4	0.4	0.3
γ -elemene	1434	ND	ND	ND	0.3	0.4	0.3
6,9-guaiadiene	1442	ND	ND	ND	0.1	0.1	0.1
cadina-3,5-diene	1449	ND	ND	ND	0.1	0.1	0.1
α -humulene	1452	0.0	0.2	0.0	0.1	0.1	0.1
cis-cadina-1(6)-4-diene	1461	ND	ND	ND	0.1	0.1	0.1
γ -muurolene	1478	0.1	0.5	0.1	ND	ND	ND
β -selinene	1489	tr	tr	tr	0.3	0.3	0.3
δ -selinene	1492	ND	ND	ND	tr	0.1	0.1
epi-cubebol	1492	0.6	0.4	0.5	1.2	1.1	1.1
α -selinene	1498	ND	ND	ND	0.1	0.1	0.1
α -muurolene	1500	0.3	0.4	0.4	0.5	0.4	0.4
cubebol	1514	1.1	0.6	0.7	1.8	1.7	1.9
δ -cadinene	1522	0.5	0.6	0.6	1.7	1.7	1.4
trans-cadina-1,4-diene	1533	ND	ND	ND	0.1	0.1	0.1
germacrene B	1559	ND	ND	ND	0.2	0.2	0.2
trans-nerolidol	1561	1.1	1.8	1.5	ND	ND	ND
β -calacorene	1564	0.1	0.5	0.1	ND	ND	ND
caryophyllene oxide	1582	0.1	0.2	0.1	ND	ND	ND
gleenol	1586	0.1	0.1	0.1	0.1	0.1	0.1
cedrol	1600	tr	0.1	0.1	ND	ND	ND
1,10-di-epi-cubenol	1618	0.4	0.6	0.5	0.9	0.9	0.9
1-epi-cubenol	1627	0.1	0.3	0.2	0.3	0.3	0.3
intermedeol	1665	ND	ND	ND	0.5	0.5	0.5
cis-14-nor-muurol-5-en-4-one	1688	0.1	0.1	0.1	ND	ND	ND
farnesol acetate	1830	ND	ND	ND	0.4	0.4	0.3
manool oxide	1987	0.1	tr	0.2	ND	ND	ND
Totals		97.2	93.2	96.1	95.9	95.8	96.3

Table 2. The relative area % of prominent compounds in *Abies concolor* and *Abies grandis* essential oil, averaged across all samples. The relative percent difference (RPD) is provided.

Shared Prominent Compounds	<i>Abies concolor</i> (avg.)	<i>Abies grandis</i> (avg.)	RPD
α -pinene	12.2	11.8	3.0
camphene	8.5	23.4	93.3
β -pinene	29.0	11.0	89.8
δ -3-carene	9.7	2.3	122.3
limonene	5.1	8.5	50.1
bornyl acetate	9.4	17.5	60.2

ACKNOWLEDGEMENTS

The authors would like to thank the following individuals and organizations for their assistance with the study: Michael Carter from the Young Living Highland Flats Tree Farm and Distillery for assisting with sample collection, Zach Nielsen from Utah Valley University for the botanical illustration, and the D. Gary Young Research Institute for providing support for this project.

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