Phytochemistry, Vol. 28, No. 3, pp. 765–770, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00
Pergamon Press plc.

MATURATIONAL VARIATION IN NEEDLE ESSENTIAL OILS FROM *PSEUDOTSUGA MENZIESII*, *ABIES CONCOLOR* AND *PICEA ENGELMANNII*

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(*Received in revised form* 9 *August* 1988)

Key Word Index—*Pseudotsuga menziesii*, *Abies concolor*, *Picea engelmannii*; Pinaceae; terpene; essential oils; needle maturation.

Abstract—The maturational variation in current year needle essential oils of Douglas fir, *Pseudotsuga menziesii*, white fir, *Abies concolor* and Engelmann spruce, *Picea engelmannii*, was determined. Strong seasonal and between-species differences were found. Most compounds increased in concentration through the season. White fir foliage had the highest essential oil concentration and Engelmann spruce foliage had the lowest concentration. Our results are compared with previously reported essential oil compositions for Douglas fir and Engelmann spruce. This is the first detailed report of the needle essential oil composition of white fir.

INTRODUCTION

Mixed conifer forests are widespread in the western United States and are a major source of wood products. Three species of trees are particularly abundant in the mixed conifer forests of the southwestern U.S. These include: Douglas fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco; white fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.; and Engelmann spruce, *Picea engelmannii* Parry ex Engelm.

The terpene composition of these species has been used for taxonomic classification of species based on cortical essential oils [1–3] and leaf essential oils [4–10]. Terpenes have also been used as indicators of tree growth potential [11], implicated in host plant resistance to feeding by insects [12–15], and associated with resistance to browsing [16]. The objective of this study was to determine the seasonal and between-species variation in leaf essential oils in three conifer species that serve as principal hosts of the western spruce budworm, *Choristoneura occidentalis* Freeman. Through the sample period, foliage goes from acceptable to unacceptable for feeding by the western spruce budworm [13]. These chemical analyses will serve as baseline information for future research on the role of essential oils in resistance to insect feeding.

RESULTS AND DISCUSSION

Species patterns

As expected, there were strong between-species terpene differences. Terpenes in the foliage of dormant conifers provide excellent evidence for species and subspecies taxonomic determination [6]. We found similar strong patterns in the young, physiologically active foliage of conifers. Twelve of the specific terpenes we evaluated were different in relative concentration between species across the three sample periods ($P \leq 0.05$) (Table 1).

Douglas fir foliage from Arizona was characterized by high levels of α-pinene, camphene, β-pinene, limonene, and unknown **3** (Table 2). The terpene patterns of Douglas fir in Arizona were strikingly different from those reported by von Rudloff [4, 5, 9]; Arizona Douglas fir was much higher in limonene and lower in *l*-borneol. Camphene, α-pinene, and β-pinene levels in Douglas fir foliage resembled those of populations in the Rocky Mountains of British Columbia [9] and in New Mexico (R. G. Cates, unpublished). New Mexico populations had detectable levels of bornyl acetate, which was absent in the Arizona samples. The sesquiterpenes caryophyllene and longifolene were present as reported previously [17].

Engelmann spruce foliage had generally lower levels of most of the low-M_r terpenes than reported by others [3, 6, 7] (Table 3). However, we found much lower between-tree variation in terpenes (Table 3). Von Rudloff [6] suggested that between-tree variation of Engelmann spruce terpenes was extremely high and that many samples were required to observe differences. We report relatively low variation in Engelmann spruce, perhaps because our sample trees were of uniform age and vigour. We report similar levels of *d*-camphor, slightly lower levels of β-pinene and myrcene, and much higher levels of terpinen-4-ol than von Rudloff [7].

White fir foliage was characterized by extremely high levels (compared to the other species) of α- and β-pinene and significant amounts of camphene and limonene (Table 4). High levels of α- and β-pinene were also found in the cortex of white fir [1]. This is the first detailed reported of needle essential oils in this species.

Table 1. Results from repeated measures multivariate analysis of variance tests on foliage concentrations of monoterpenes, oxygenated monoterpenes and sesquiterpenes in current-year foliage of Douglas fir, white fir and Engelmann spruce*

Terpene	Probability level(s) for F-value(s) from repeated measures multivariate analysis of variance		
	Species differences†	Sample time differences‡	Sample time × species interaction§
All monoterpenes¶	<0.001	<0.001	<0.001
Tricyclene	<0.001	<0.001	NS
α-Pinene	<0.001	NS	NS
Camphene	<0.001	<0.001	<0.001
β-Pinene	<0.001	NS	0.069
Myrcene	0.005	NS	NS
3-Carene	NS	NS	NS
p-Cymene	NS	NS	NS
Limonene	<0.001	NS	NS
γ-Terpinene	NS	NS	NS
trans-β-Ocimene	NS	NS	0.015
Terpinolene	<0.001	<0.001	<0.001
All oxygenated monoterpenes	<0.001	<0.001	<0.001
Fenchone	0.038	NS	NS
Linalool	<0.001	<0.001	<0.001
d-Camphor	NS	NS	NS
l-Borneol	NS	NS	NS
Terpinen-4-ol	0.039	<0.001	0.091
Citronellol	NS	0.016	NS
l-Carvone	0.022	0.070	NS
l-Piperitone	NS	NS	NS
All sesquiterpenes**	NS	0.040	NS
Carophyllene	NS	NS	NS
Longifolene	NS	0.035	NS
Other			
Unknown **1**	NS	NS	NS
Unknown **2**	0.055	NS	NS
Methyl chavicol/α-terpineol	NS	0.004	NS
Unknown **3**	<0.001	<0.001	<0.001

*Analyses conducted on data transformed to log (value + 1) to stabilize variance. Sample sizes were $n = 20$ trees for each species. The same trees were sampled at each sample time. NS = $P > 0.10$.

†Host species were Douglas fir, white fir, and Engelmann spruce.

‡Sample times were 6 June, 20 June and 25 July 1984, which corresponds with western spruce budworm early-instar-feeding, late-instar-feeding, and post-feeding periods, respectively.

§A significant interaction implies the pattern of change in concentration over time varied for different host species.

¶All monoterpenes considered simultaneously as a vector of dependent variables. All compounds below the detection limit of 5.0 ppm were excluded from the analysis.

‖All oxygenated monoterpenes considered simultaneously as a vector of dependent variables.

**All sesquiterpenes considered simultaneously as a vector of dependent variables.

Table 2. Mean terpene levels (ppm) in Douglas fir foliage at three sample periods during the growing season*

Specific terpenes	Sampling period		
	6 June	20 June	25 July
Monoterpenes			
Tricyclene	44.2 (12.56)†	65.2 (15.44)	89.9 (10.51)
α-Pinene	272.2 (29.56)	373.9 (65.43)	366.7 (63.43)
Camphene	216.9 (34.76)	464.6 (98.42)	663.2 (122.50)
β-Pinene	477.3 (69.70)	522.8 (98.84)	336.2 (50.73)
Myrcene	32.0 (10.92)	42.9 (17.86)	27.9 (08.52)
α-Phellandrene	6.0 (03.48)	nd§	nd
3-Carene	3.2 (00.73)‡	13.4 (10.88)	15.3 (12.83)
α-Terpinene	9.8 (07.33)	4.5 (02.03)	nd
p-Cymene	17.3 (14.78)	nd	3.2 (00.68)
Limonene	256.4 (47.80)	382.0 (68.96)	283.8 (55.06)
cis-β-Ocimene	27.7 (16.23)	nd	33.2 (21.40)
γ-Terpinene	2.7 (00.23)	11.9 (09.43)	nd
trans-β-Ocimene	19.6 (17.08)	nd	nd
Terpinolene	11.6 (05.98)	5.0 (02.53)	38.6 (08.36)
Oxygenated monoterpenes			
Fenchone	3.2 (00.51)	8.3 (02.54)	6.6 (02.68)
Linalool	3.6 (00.57)	5.3 (01.52)	48.0 (07.81)
d-Camphor	3.6 (00.70)	12.7 (06.39)	7.8 (03.69)
l-Borneol	9.2 (02.62)	16.2 (04.72)	10.7 (02.45)
Terpinen-4-ol	4.4 (01.05)	16.7 (04.71)	6.9 (01.46)
Citronellol	33.0 (10.51)	29.8 (06.47)	13.4 (04.53)
l-Carvone	7.4 (03.04)	25.5 (10.36)	17.5 (07.22)
l-Piperitone	8.7 (05.72)	5.2 (02.00)	4.6 (01.63)
Sesquiterpenes			
Caryophyllene	15.5 (05.30)	10.3 (03.93)	16.1 (12.96)
Longifolene	9.4 (02.44)	15.9 (02.88)	48.3 (27.26)
Other			
Unknown **1**	7.9 (03.67)	nd	17.6 (15.08)
Unknown **2**	13.1 (09.26)	3.1 (00.63)	5.7 (01.52)
Methyl chavicol/α-terpineol	4.6 (01.07)	12.5 (02.47)	9.9 (02.25)
Unknown **3**	30.7 (18.65)	102.4 (25.83)	239.7 (55.74)
Total	1556.0 (145.1)	2168.0 (356.0)	2326.0 (322.9)

*$n = 20$.

†Numbers in parentheses are standard errors of the means.

‡Mean values below detection limit of 5 ppm indicate compound detected in some samples.

§nd = not detectable in any sample.

The proportion of monoterpenes, oxygenated monoterpenes, and sesquiterpenes was distinct for Engelmann spruce when compared to Douglas fir and white fir (Fig. 1). Engelmann spruce terpenes were composed of roughly equal proportions of monoterpenes and oxygenated monoterpenes (40–50% each); the remaining 10–15% of the essential oils were sesquiterpenes. Conversely, the essential oils of white fir and Douglas fir foliage were dominated by monoterpenes (*ca* 90%), had low proportions of oxygenated monoterpenes (5–10%), and had very low percentages of sesquiterpenes (<5%). This pattern was generally consistent throughout the growing season.

Maturation patterns

Changes during needle maturation of nine terpene compounds were detected ($P \leq 0.05$) (Table 1). Four of the nine compounds (tricyclene, methyl chavicol/α-terpineol, citronellol and longifolene) had consistent seasonal changes across all species (season × species interactions had P values ≥ 0.05) (Table 1). The seasonal pattern for the remaining five compounds was species-dependent.

In Douglas fir (Table 2), tricyclene, camphene, linalool, unknown **3** and longifolene were major components that steadily rose during the season. Douglas fir from California has a similar pattern for camphene [18]. Citronellol was the only major terpene that consistently declined during the season. Several minor components declined steadily.

Our results indicate that there were few strong seasonal patterns in foliage chemical composition for Engelmann spruce (Table 3). *d*-Camphor, terpinen-4-ol, and methyl chavicol/α-terpineol increased slightly while α-terpinene, *p*-cymene, terpenolene, *l*-piperitone, and unknown **3** declined slightly. β-Pinene, limonene, *l*-carvone, and un-

Table 3. Mean terpene levels (ppm) in Engelmann spruce foliage at three sample periods during the growing season*

Specific terpenes	Sampling period		
	6 June	20 June	25 July
Monoterpenes			
Tricyclene	6.6 (03.16)†	4.1 (00.71)	7.2 (01.83)
α-Pinene	23.2 (08.79)	7.4 (02.23)	12.6 (04.35)
Camphene	9.1 (03.35)	18.0 (11.29)	5.5 (01.36)
β-Pinene	4.3 (01.78)‡	27.2 (19.70)	6.7 (02.16)
Myrcene	5.3 (02.10)	5.6 (02.22)	3.5 (00.65)
α-Phellandrene	5.3 (01.59)	nd§	3.5 (06.98)
α-Terpinene	4.6 (02.08)	4.4 (01.93)	nd
p-Cymene	8.2 (05.68)	3.6 (01.08)	nd
Limonene	11.2 (05.77)	8.9 (02.21)	9.1 (03.74)
cis-β-Ocimene	3.7 (00.94)	nd	3.4 (00.93)
γ-Terpinene	4.2 (01.22)	6.7 (03.20)	4.4 (01.28)
trans-β-Ocimene	nd	10.8 (06.46)	4.0 (01.53)
Terpinolene	5.1 (01.70)	4.6 (01.17)	3.8 (00.72)
Oxygenated monoterpenes			
Fenchone	nd	3.5 (00.98)	nd
Linalool	4.2 (01.22)	nd	4.6 (01.26)
d-Camphor	6.0 (01.64)	8.9 (02.92)	10.7 (02.21)
l-Borneol	7.2 (02.53)	9.3 (02.89)	8.7 (01.82)
Terpinen-4-ol	5.3 (01.18)	6.4 (01.48)	14.0 (02.64)
Citronellol	7.8 (03.03)	20.3 (04.54)	13.7 (01.82)
l-Carvone	7.1 (02.60)	3.2 (00.45)	5.4 (01.56)
l-Piperitone	15.8 (08.70)	6.3 (01.44)	5.6 (01.29)
Sesquiterpenes			
Caryophyllene	13.4 (05.13)	4.9 (02.43)	6.3 (01.76)
Longifolene	9.9 (02.21)	32.1 (16.08)	15.7 (02.17)
Other			
Unknown **1**	3.0 (00.53)	2.8 (00.32)	3.4 (00.59)
Unknown **2**	4.0 (01.08)	3.7 (00.89)	5.5 (01.33)
Methyl chavicol/α-terpineol	7.0 (01.91)	9.0 (01.80)	12.2 (02.49)
Unknown **3**	8.9 (02.58)	8.0 (01.80)	4.5 (01.20)
Total	216.9 (25.23)	237.5 (55.98)	183.4 (16.02)

*$n = 20$.

†Numbers in parentheses are standard errors of the means.

‡Mean values below detection limit of 5 ppm indicate compound detected in some samples.

§nd = not detectable in any sample.

known **3** were major components in white fir that steadily increased during the season (Table 4). Longifolene is the only compound in white fir that steadily declined with season.

White fir foliage had the highest total essential oil concentration, followed by Douglas fir and Engelmann spruce (Tables 2–4). Total needle essential oils increased with season in white fir and Douglas fir (Tables 2, 4) and increased, then decreased, in Engelmann spruce (Table 3).

EXPERIMENTAL

Plant material. Foliage used in this study was collected from 20 trees of each conifer species. The trees were of similar age, height, and general vigour and were located on the Kaibab National Forest, North Kaibab Ranger District, in Arizona. Trees were located in an area that had a history of western spruce budworm infestation. Current year foliage was collected at three times during the 1984 growing season to correspond with *C. occidentalis* feeding: early-instar-feeding (June 6), late-instar-feeding (June 20), and post-feeding (July 25). Early season foliage is the preferred food of *C. occidentalis* and late season foliage is resistant to feeding [13]. Foliage-bearing branches were clipped at random from the mid-crown of study trees. The current year foliage was immediately removed and placed on dry ice. Samples were transported on dry ice and stored at −90° until analysed. Samples were prepared for GC by grinding 100 mg of foliage with liquid N_2 in a mortar and pestle. The ground powder was treated with 1 ml aliquots of Et_2O. Extracts were transferred to 5 ml sample vials, an int. standard (fenchyl acetate) was added, and the extracts dild to vol.

Analysis. Analysis was done by GC using a FID and an on-column injector. A fused silica open tubular capillary column was used for the analysis (30 m × 0.25 mm coated with silicone SE-30 to a thickness of 1.0 microns). Column temp. was programmed to increase 4°/min. to a final temp. of 230° for 12 min.

Table 4. Mean terpene levels (ppm) in white fir foliage at three sample periods during the growing season*

Specific terpenes	Sampling period		
	6 June	20 June	25 July
Monoterpenes			
Tricyclene	57.4 (54.35)†	7.5 (03.30)	14.7 (03.62)
α-Pinene	770.5 (161.90)	644.5 (156.80)	923.8 (198.60)
Camphene	172.3 (91.40)	28.8 (07.88)	189.1 (37.14)
β-Pinene	1364.0 (309.40)	1752.0 (328.00)	2317.0 (434.10)
Myrcene	72.2 (27.99)	23.7 (14.58)	41.9 (17.72)
α-Phellandrene	nd§	nd	nd
3-Carene	4.6 (01.79)‡	nd	4.8 (01.73)
α-Terpinene	nd	3.2 (06.68)	nd
p-Cymene	2.7 (00.16)	nd	5.7 (03.23)
Limonene	384.8 (77.87)	413.4 (98.84)	468.1 (105.30)
cis-β-Ocimene	9.4 (06.93)	nd	11.6 (09.13)
γ-Terpinene	nd	4.8 (02.28)	6.3 (02.38)
trans-β-Ocimene	nd	nd	4.1 (00.96)
Terpinolene	nd	5.3 (02.78)	12.1 (03.50)
Oxygenated monoterpenes			
Fenchone	5.4 (02.93)	nd	5.6 (01.95)
Linalool	nd	7.6 (03.50)	11.1 (04.90)
d-Camphor	9.2 (03.29)	4.4 (01.31)	6.2 (01.87)
l-Borneol	9.4 (03.76)	7.3 (01.86)	8.8 (02.54)
Terpinen-4-ol	3.8 (01.28)	7.8 (02.69)	7.3 (02.03)
Citronellol	21.2 (09.45)	24.2 (05.55)	23.6 (11.03)
l-Carvone	8.8 (04.32)	15.2 (07.38)	58.2 (24.55)
l-Piperitone	12.3 (05.06)	3.3 (00.47)	6.4 (02.02)
Sesquiterpenes			
Caryophyllene	24.1 (13.70)	31.2 (19.50)	3.7 (01.03)
Longifolene	17.9 (05.01)	15.5 (04.85)	10.5 (01.64)
Other			
Unknown **1**	5.1 (02.58)	3.7 (01.18)	4.0 (01.48)
Unknown **2**	3.2 (00.68)	nd	nd
Methyl chavicol/α-terpineol	6.9 (01.97)	11.2 (03.32)	12.8 (05.05)
Unknown **3**	7.0 (01.99)	16.7 (05.48)	223.6 (48.54)
Total	2992.0 (486.3)	3053.0 (557.1)	4394.0 (641.1)

*$n = 20$.

†Numbers in parentheses are standard errors of the means.

‡Mean values below detection limit of 5 ppm indicate compound detected in some samples.

§nd = not detectable in any sample.

Identification and quantification. Individual terpene components were identified by comparing retention for elutant peaks with authentic standards. R_ts of authentic samples were determined for the GC conditions listed above. RR_ts were calculated for synthetic mixtures of 10–12 authentic samples. Terpenes not previously reported for conifers are listed as unknown.

Terpenes were quantified by adding fenchyl acetate to the foliage extract soln and using this peak as an internal standard of known response. Fenchyl acetate (99 + % pure) was chosen as the internal standard because it had a similar RR_t and did not interfere with other compounds in the extract. Data from the chromatograph were recorded directly on an integrator programmed to yield relative concentrations at the completion of each chromatogram. Terpene concentration was determined by the following equation: terpene concentration = [(absolute area for terpene/absolute area for fenchyl acetate) × mass of fenchyl acetate in each sample]/dry wt of sample.

Statistical analysis. Data were analysed using a repeated-measures model (tree species and season are assumed to be fixed effects and individual trees are the only random effect). Data were transformed [log (value + 1)] to meet assumptions of homogeneity of variance. The analyses were accomplished using Biomedical Computer Programs (BMDP) routine 4V [19]. For statistical analysis, compounds below the detection limit of 5 ppm were treated as 2.5 ppm instead of 0 to meet linear dependence requirements of the repeated-measures model. If all tree samples were below the detection limit, chemicals are reported as not detectable.

Acknowledgements—We thank Dr Duane F. Zinkel (Forest Products Laboratory, Madison, Wisconsin, U.S.A.) for terpene standards, technical advice, and review of the manuscript. Dr Richard Foust (Bilby Research Center, Northern Arizona University, Flagstaff, Arizona, U.S.A.) provided advice on analytical techniques and reviewed the manuscript.

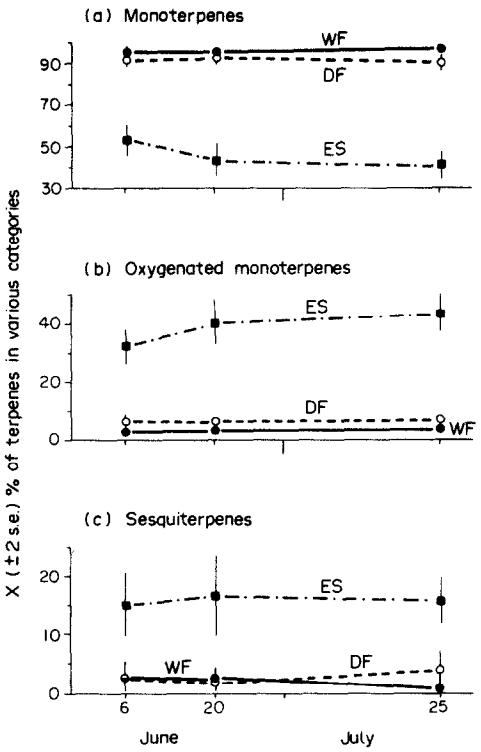

Fig. 1. Percent of essential oils that are monoterpenes, oxygenated monoterpenes and sesquiterpenes for Douglas fir (DF), white fir (WF) and Engelmann spruce (ES).

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