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SEASONAL VARIATION IN THE TERPENES OF THE FOLIAGE OF BLACK SPRUCE*

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Key Word Index—*Picea mariana*; Pinaceae; black spruce; foliage; mono- and sesquiterpenes; seasonal variation.

Abstract—The changes in the relative amounts of the mono- and sesquiterpenes of the volatile oil of the leaves, buds and twigs of black spruce were determined at various intervals throughout the year. Major changes take place in the young leaves just after bud burst until mid-summer. Upon maturing the same quantitative composition as that of the mature leaves is reached. The composition of the latter, as well as that of the twigs, shows only minor changes from bud burst to mid-summer and none during the rest of the year. In contrast, large relative quantitative changes take place in the bud oil throughout the year. The relative changes of santene, tricyclene, camphene, bornyl acetate and limonene in the new growth follow similar curves, but those of α-pinene, β-pinene, myrcene, car-3-ene and the sesquiterpenes differ considerably. The sesquiterpenes, and possibly myrcene, appear to be metabolized at least in part as the young leaves mature.

INTRODUCTION

Previously, we reported on the seasonal variation of the terpenes found in the volatile oil of the foliage (leaves, twigs and buds) of the white spruce, *Picea glauca* (Moench) Voss [1]. During the early summer there was a large biosynthetic activity in the new growth affecting the relative amounts of all terpenes. In the previous year's growth, only minor changes were recorded during this period. The terpene composition of the new leaves approached that of the older leaves by July and it remained unchanged in both throughout the fall and winter. In contrast, quantitative changes were recorded in the volatile oil of the buds throughout the fall, winter and spring. In the leaves, the actual amounts of sesquiterpenes decreased after reaching a maximum in May, indicating that these were further metabolized. Recently, Hrutfiord *et al.* [2] reported on seasonal variations in the major monoterpenes of Sitka spruce, *P. sitchensis* (Bong.) Carr, and obtained similar results.

To obtain further insight into terpene formation in spruce species, the relative changes in the foliage of the black spruce, *P. mariana* (Mill.) B.S.P., have been studied. Initially (1970–1971) we followed the same procedures as used for white spruce [1]. However, since the composition of the leaf oil differs significantly from that of the twigs [3], the different parts of the foliage were steam-distilled separately and the changes in the oil of the young and older leaves, the twigs and the buds were studied separately (1971–1972). The chemical compositions of the foliage oil (combined leaves, twigs and buds) was described previously by us [4].

Whereas transportation of fall and winter samples does not cause measurable changes provided they are kept cold and dark [1, 3], it is not known whether this is true during the main growing season. The rapid changes recorded in the young leaves of white spruce [1] indicate that transportation and storage time should then be kept to a minimum. Hence, the major part of this study was carried out with foliage from a transplanted tree. Since the yield of volatile oil in black

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Table 1. Relative percentages of the terpenes of the volatile oils of the leaves, buds and twigs of black spruce (winter composition)*

Terpene	Leaves	Buds	Twigs
Santene	5·5		0·1
Tricyclene	1·3		trace
α-Pinene	8·8	14·5	18·4
Camphene	19·1	0·8	0·9
β-Pinene	1·3	11·9	9·8
Sabinene	0·1	2·5	2·0
Myrcene	2·2	4·0	5·3
Car-3-ene	0·2	50·5	50·5
Limonene	3·3	0·9	1·6
β-Phellandrene	0·4	0·7	1·8
Terpinolene	0·6	4·7	4·7
1:8-Cineole	0·6		
Camphene hydrate	1·7	trace	trace
Terpinen-4-ol	0·1	0·3	0·8
Borneol	0·9	trace	trace
α-Terpineol	0·5	0·3	0·5
Bornyl acetate	46·5	6·4	1·3
C_{15}-hydrocarbons†	1·0	0·7	1·1
C_{15}-alcohols†	2·7	0·4	0·8
% Yield of oil	1·3	0·2	0·3
% Fr wt of total foliage	79	4	17
% Moisture	48·4	32·4	29·2

* 8.3.1972; 15-yr-old tree transplanted from Candle Lake area in 1966.

† Cadinene–muurolene isomers and their corresponding alcohols.

spruce foliage is much larger (1–2%) than that in white spruce and branch-to-branch variation is relatively small [4, 5], the foliage of a single tree sufficed for a full year's study.

RESULTS AND DISCUSSION

The winter compositions of the volatile oils collected from the transplanted tree are listed in Table 1. These are in excellent agreement with those from trees sampled in the Candle Lake area, i.e. transplanting did not affect the composition of the volatile oils. As was found with the volatile oils of the white spruce, the leaf oil composition differs greatly from that of the buds and twigs. At the time of collection (March 1972) the volatile oils of the twigs and buds were similar although in the latter the percentage of bornyl acetate was significantly larger. When twig and bud samples from other trees were analyzed, larger differences were also recorded for some of the other terpenes. This was found to be a function of the time of collection (see below).

As shown previously [4, 5], the volatile oil of black spruce foliage differs from that of white spruce in that bornyl acetate is the main component (40–50% of the oil) and that it contains only traces of camphor. Also, the relative amounts of camphene and 1 : 8-cineole are larger, whereas those of myrcene and limonene are smaller. Table 1 also shows the yields of oil as based on fresh weight of the plant material, the percentage of the fresh weight of leaves, twigs and buds in a given foliage sample, as well as the percentage of moisture. Whereas the leaves are about 80% of the fr. wt of the foliage, the leaf oil is almost 95% of the foliage oil. Hence, except for the content of car-3-ene, the leaf oil composition is almost identical with that of the foliage [4].

There appear to be major differences in the biosynthesis of the monoterpenes of the leaves as compared with those of the twigs and buds. In the former, the dominant feature is the presence of relatively large amounts of the closely related santene, tricyclene, camphene, camphene hydrate, borneol and bornyl acetate. These are present only in relatively small amounts in the twigs and buds. In the latter, car-3-ene predominates and the relative amounts of β-pinene, sabinene, terpinolene and terpinen-4-ol are significant. Whereas one can postulate a single gene difference [6–8] to account for the shift from one terpene type to another when dealing with different species or varieties, a different mechanism must be proposed for such a shift from one organ to another within the same plant. The sesquiterpenes present are a mixture of cadinene and muurolene isomers and their corresponding alcohols [3] and they are the same in the leaves, buds and twigs.

The seasonal variations in the relative amounts of the major terpenes of the mature leaves, buds and young leaves (after flushing of the buds) are shown in Figs 1–3. The variation is plotted from the time the new vegetative buds have been formed (July–August) through the fall and winter and the main growing season (May–July in Saskatchewan) to the time of new bud formation. This gives rise to more continuous curves than by plotting it on a calender year basis [1]. As was found in white spruce, the relative percentages of the terpenes change only little in the mature leaves throughout the year. Only a slight decrease in the percentage of camphene and a corresponding increase in that of bornyl acetate

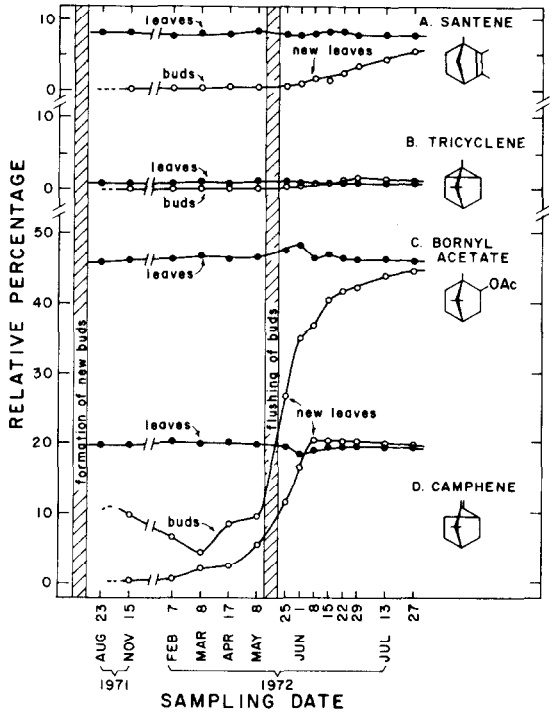

Fig. 1. Change in the relative percentage of (A) santene, (B) tricyclene, (C) bornyl acetate, (D) camphene in the volatile oil of the mature leaves (●), buds and young leaves (○) during the course of a single year (August to July).

(Fig. 1) was recorded during May in one to 2 yr-old leaves of black spruce. Since there appears to be some loss of the total amount of volatile oil from the fall through the winter [1, 4, 5], this small biosynthetic activity may reflect replenishment of such losses during May and June. There are major changes in the relative amounts of the major terpenes in the oil of the buds: all members of the camphene–borneol group, except bornyl acetate, show a rise from zero or trace amounts to maxima that are practically the same as the corresponding percentages from the older leaves (Fig. 1). Thus, as in white spruce [1], the young growth produces an oil composition that approaches that of the mature leaves by midsummer. Bornyl acetate (Fig. 1C) appears to be present in relatively large amounts in young buds but decreases to a minimum in winter before increasing again. The minimum appears to be the result of the formation of α-pinene, β-pinene (Fig. 2A and B) and especially car-3-ene (Fig. 3A) in the bud oil during winter. The rates of increase of camphene hydrate, borneol and 1:8-cineole (not shown) were similar to those of santene and tricyclene. At no time did the relative amount of camphor (the main component of white spruce leaf oil [1]) rise above 0·2%, i.e. no accumulation of this related terpene takes place. Of the other terpenes, only limonene (Fig. 2C) showed a similar curve of variation, indicating that it may have close biosynthetic relationship with the camphene group.

Entirely different rates of change were recorded for α- and β-pinene, myrcene (Fig. 2), car-3-ene, and the sesquiterpenes (Fig. 3). The largest relative changes were recorded for myrcene (Fig. 2D) and car-3-ene (Fig. 3A), but whereas a maximum in the relative amount of myrcene occurs just after flushing of the buds, that for car-3-ene occurred during late winter before flushing. The latter holds also to a lesser degree for β-pinene (Fig. 2B), but the curve for α-pinene (Fig. 2A) is closer to that of the sesquiterpene hydrocarbons (Fig. 3C). The oxygenated sesquiterpenes (Fig. 3B) go through a more pronounced minimum in late winter. The relative amounts of sesquiterpenes

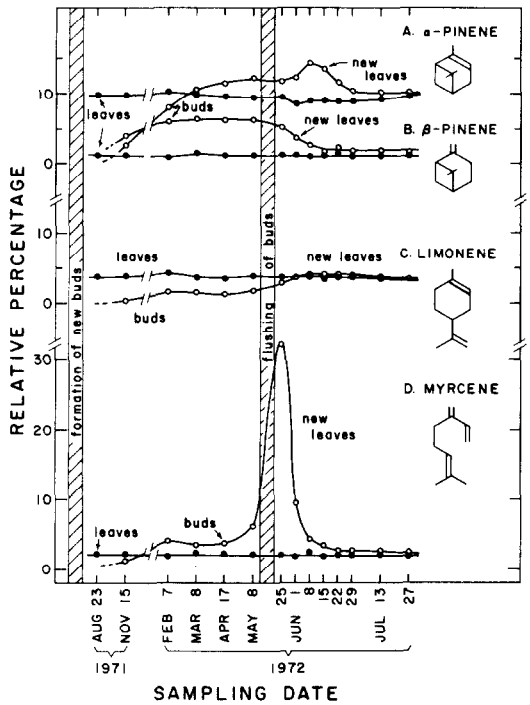

Fig. 2. Change in the relative percentage of (A) α-pinene, (B) β-pinene, (C) limonene, and D) myrcene in the volatile oil of the mature leaves (●), buds and young leaves (○) during the course of a single year (August to July).

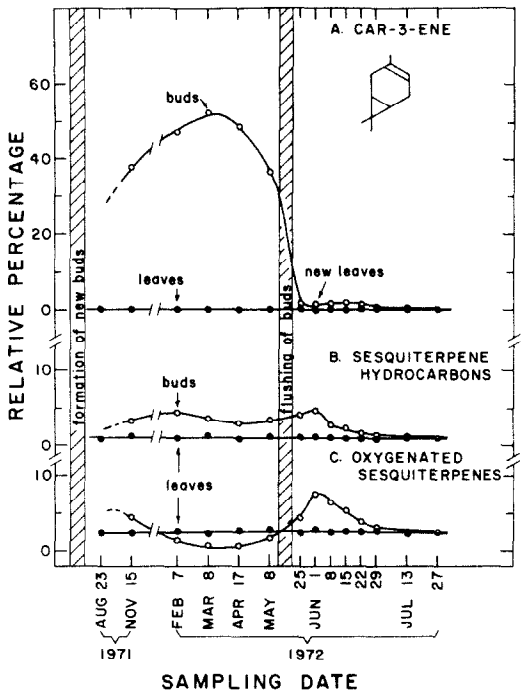

Fig. 3. Change in the relative percentage of (A) car-3-ene, (B) sesquiterpene hydrocarbons and (C) oxygenated sesquiterpenes in the volatile oil of the mature leaves (●), buds and young leaves (○) during the course of a single year (August to July).

found in the buds throughout the fall and winter were larger than in those of the white spruce, but as in that species, the actual amounts of sesquiterpenes decreased as the young leaves matured. Hence, these components must be metabolized further [1].

Conclusions as to which monoterpene is the precursor of others cannot be drawn by the method of determining changes in their relative amounts [see 1], but it is obvious that myrcene must be an intermediate. In contrast, car-3-ene may only be synthesized in the buds and simply decreases in relative amount owing to the synthesis of other terpenes later in winter or early spring. A similar high rate of change was not recorded in white spruce, and somewhat different conditions prevail also in Sitka spruce [2]. Thus, black spruce not only has a significantly different leaf oil composition in comparison with white, Sitka, Engelmann and Colorado spruce [3], but appears to differ also in the sequence in which the terpenes are formed in buds and young leaves. However, the occurrence of the main biosynthetic activity just before and after flushing of the buds, and the capability of producing the same overall terpene pattern of the older leaves as the young leaves mature is similar.

The changes in relative amounts of major terpenes of the twigs follow a different pattern than either that of the leaves or buds. All percentages fluctuated only within 10–20% of the relative amounts of the winter oil (see Table 1). That of car-3-ene rose from about 47% before flushing of the buds to 52% in June, whereas that of α-pinene decreased from 21 to 18% during the same time. This is about the same variation as that found from one twig to another at different locations of a tree. Not one of the major or minor components showed the marked maxima or minima found in the oils of the young leaves and buds. The minor variations recorded in the twig oil need not necessarily be seasonal since Hrutfiord *et al.* [2] have shown that the cortical oil of Sitka spruce varies by such amounts at different locations of a single tree. Juvonen [9] has found much larger ones in the twigs of *Pinus sylvestris* L. Thus, one may conclude that the seasonal variation of the terpenes of the twigs is insignificant in relation to the within-tree variation, which is in good agreement with the findings of Zavarin [10] for the cortical oils of some *Abies* species.

EXPERIMENTAL

The same general procedures as those described before were used [1, 3–5]. A 9-yr-old black spruce tree from the Candle Lake area was transplanted in 1966. After 2 yr the foliage oil was analyzed and compared with that of other trees from the same area. In the initial experiments (1970–1971) the foliage from 3 black spruce trees from the Candle Lake area was steam-distilled and volatile oil of the total foliage analyzed during the course of the year. From August 1971 to the end of July 1972 foliage samples (100–200 g) were taken randomly from all sides of the transplanted tree. These were separated immediately into leaves, twigs and buds by dipping the branchlets into liquid N_2 and stripping the leaves off. The buds were detached thereafter. Each sample of separated plant material was steam-distilled for 6 hr and volatile oil was recovered as reported previously [1, 5]. Aliquots (0·2–1 μl) of the oil were analyzed on the 2% PEG + 1% QF-1, 2% PEG + 1% OV-17, 5% SE-30 and 3% PEG columns described earlier [1, 3, 5]. Since 1:8-cineole cannot be separated satisfactorily from limonene and β-phellandrene on these columns, additional isothermal runs were carried out on an ethylene glycol-*bis*-propionitrile column (2 m + 0·3 mm OD, 5% on Chromosorb W, 60–80 mesh). The relative percentages of the individual components were determined by integration and summation of the peak areas with electronic digital integrators. The dry wt of plant material was determined by desiccation to constant weight in a vacuum oven at 85°.

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REFERENCES

1. von Rudloff, E. (1972) *Can. J. Botany* **50**, 1595.
2. Hrutfiord, B. F., Hopley, S. M. and Gara, R. I. (1974) *Phytochemistry* **13**, 2167.
3. von Rudloff, E. (1975) *Biochem. Systematics Ecology*, **2**, 131.
4. von Rudloff, E. (1967) *Can. J. Botany* **45**, 1703.
5. von Rudloff, E. (1967) *Can. J. Botany* **45**, 891.
6. Murray, M. J. (1960) *Genetics* **45**, 931.
7. Hanover, J. W. (1966) *Heredity* **21**, 73; *Forest Science* **12**, 447.
8. Forde, M. B. (1964) *New Zealand J. Botany* **2**, 53.
9. Juvonen, S. (1966) *Acta Bot. Fennica* **71**, 5.
10. Zavarin, E. (1968) *Phytochemistry* **7**, 92.