WITHIN-TREE VARIATION OF CORTICAL MONOTERPENES DUE TO DEFOLIATION

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ABSTRACT.--Defoliation of Scotch pine (*Pinus sylvestris* L.) by sawflies resulted in significant changes in year-old branch cortical monoterpene composition. The concentration of a-pinene and total monoterpenes were significantly increased in defoliated branches compared to branches with foliage of the same tree. In some trees defoliation also resulted in marked changes in the concentration of B-pinene and 3-carene. Trees with moderate to heavy defoliation exhibited the largest monoterpene changes. In chemotaxonomic and inheritance studies, which utilize branch cortical oleoresin, only trees of good health and vigor should be selected. Tree defoliated by insects or damaged by winterburn injury should be avoided.

Genetic studies have shown that monoterpene composition is strongly inherited in a variety of coniferous species (Squillace, 1976). This is an important feature in chemotaxonomic studies and other investigations using terpenes as genetic markers. However, some modification of terpene composition has been observed as a result of mechanical wounding (Roberts, 1968), site factors (Hodges and Lorio, 1975), or diseases (Krupa et al., 1973).

While investigating the effects of planting site on monoterpene composition of Scotch pine (*Pinus sylvestris* L.), I found that the average level of a-pinene concentration was significantly higher at one of two Michigan plantings (Tobolski, 1968). It was suspected that this increase as well as other changes may have been due to defoliation by the European pine sawfly (*Neodiprion sertifer* (Geoff.)).

The objective of this study was to determine the within-tree changes of monoterpene composition that results from defoliation by sawflies.

MATERIALS AND METHODS

Oleoresin samples were obtained from a Michigan State University test plantation established at the Rose Lake Wildlife Experiment Station, located 10 miles northeast of East Lansing, Michigan. This plantation was established in 1961 with 2-0 stock and was 9 years old at the time of sampling. The plantation contains 75 origins of trees planted in an 8-by-8 foot spacing in four-tree plots and eight replications (Wright and Bull, 1963).

To determine the effects of defoliation, samples were obtained from defoliated and non-defoliated branches of each of 31 trees. Of the trees sampled, 9 were classified as lightly defoliated (5 to 25 percent of the branches defoliated) while 12 and 10 trees were classified in the medium to heavy defoliation classes, 35-65 and 75-95 percent of branches defoliated, respectively. Normally only year-old needles are eaten by the sawfly and current-year needles are not damaged. Because the plantation was infested by sawflies for several years, repeated defoliation had removed all but the current year needles on some trees. The samples were collected in mid-July from 1 year-old cortex tissue in the upper two-thirds of the crown. Twenty microliters of oleoresin were obtained from cortical incisions on a defoliated and non-defoliated branch of each tree. The samples were placed in small vials or centrifuge tubes stored at 35°C and analyzed by gas-liquid chromatography within 10 days.

At the time of analysis each sample was diluted with acetone and a three microliter aliquot was immediately injected into an F and M model 700 gas chromatograph. It was equipped with a thermal conductivity detector, a Hewlett Packard automatic attenuator model 50B, and a Honeywell disc-chart integrator Model 227. The chromatograph column was stainless steel, 1/4-inch in diameter and 8 feet long, and packed with 10% polypropylene glycol on 60/80 mesh chromasorb G-AW. Column temperatures were 95°C-105°C, injection port and detector temperatures were 190°C-200°C, and the helium flow rate was 100-110 ml/min.

The monoterpenes were tentatively identified by comparing relative retention times of the unknowns with those of known compounds. Monoterpene concentrations were derived from area integrator values on a column in which standard curves were prepared from a series of known monoterpene concentrations. The same conditions were used when analyzing the unknown oleoresin samples.

RESULTS AND DISCUSSION

Differences in monoterpene concentrations were small and not significant between defoliated and normal (non-defoliated)

Table 1.--The effect of individual trees and defoliation by the European pine sawfly on the monoterpene composition of Scotch pine.

Monoterpenes	Monoterpene Concentration: Range of Tree Means	Monoterpene Concentration: Mean Increase (+) or Decrease (-) Due to Defoliation	Portion of Total Variance Due to:[1] Tree	Portion of Total Variance Due to:[1] Defoliation	Portion of Total Variance Due to:[1] Error
	Percent of oleoresin		Percent		
a -Pinene	2.1 - 24.6	+1.26	93**	3**	4
0 -Pinene	1.0 - 20.6	+ .23	96**	0	4
Myrcene	1.2 - 13.9	+ .06	98**	0	2
3-Carene	.0 - 21.3	+ .43	96**	0	4
Limonene	.3 - 11.1	+ .19	98**	0	2
1 -Phellandrene[2]	.4 - 4.4	- .06			
Y-Terpinene	.0 - .4	.00			
Cymene	.0 - .5	+ .01			
Terpinolene	.0 - 2.2	+ .04			
Camphene	.3 - 1.1	+ .03			
-Terpinene	.0 - .1	- .02			
Total Monoterpenes	25.6 - 40.0	+2.17	60**	8**	32

**Significant at the 1 percent level.

[1] Only the medium to heavy defoliation classes (35-95 percent of year-old needles) are included in the analysis.

[2] Concentration determined from the calibration curve for limonene.

branches of lightly defoliated trees. However, differences did occur within the medium and heavy defoliated trees. Since the effects were similar in both classes of defoliation, their data was combined and analyzed together (Table 1).

There was a significant increase in the concentration of a -pinene (1.26 percent) and in the total monoterpene concentration (2.17 percent) of defoliated branches. Increases of individual trees ranged from 0 to 185 percent in a-pinene and from 0 to 40 percent in the concentration of total monoterpenes. To explain these changes, it is helpful to compare differences between defoliated and normal branches in each of the three defoliation classes.

The monoterpene concentration in defoliated branches increased (+) or decreased (-) as indicated in Table 2.

Table 2.--Changes in monoterpene concentration associated with different degrees of defoliation.

Degree of Defoliation	Nonoterpene				
	a-Pinene	B-Pinene	3-Carene	Y-Terpinene	Total
	Percent of Oleoresin				
Light	+ .18	+ .12	+ .51	+.02	+ .49
Medium	+1.36***	+1.06*	+1.23*	-.06	+3.86*
Heavy	+1.16**	- .59	- .40	+.02	+ .45

*, **, ***Significant at the 10, 5, and 1 percent levels, respectively.

Note that trees which were moderately defoliated exhibited the largest changes in monoterpene composition. In lightly defoliated trees no significant change occurred, and the small differences shown are primarily due to experimental error. With heavy defoliation, differences between defoliated and non-defoliated branches decreased and only the concentration of a-pinene was significantly different. Apparently the almost total lack of foliage also influenced the monoterpenes in the few remaining foliated branches decreasing the difference between the normal and defoliated branches. This effect seems to be reversed in lightly defoliated trees where the presence of foliage overrides the effect of defoliation.

Thus, changes in monoterpene composition reflect the degree of defoliation. As defoliation increases the mononterpene composition is subsequently altered, but the exact degree of this change in unknown in heavy defoliated trees since the monoterpene composition in the remaining foliated branched is probably also affected. Hodges and Lorio (1975) also found similar increases in the concentration of a-pinene and total monoterpenes in xylem oleoresin of loblolly pine (*Pinus taeda* L.) which were under severe moisture stress.

The mechanisms involved in these monoterpene changes are unknown. Foliage or its absence may be altering substrates, enzyme activities and/or enzyme levels involved in monoterpene metabolism.

In chemotaxonomic and inheritance studies care should be exercised to avoid trees that are under stress as a result of mechanical injury or lack of water or exhibit foliar damage because of insects, winter-burn injury or pollution.

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