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Effects of decline and/or air pollution on the terpene metabolism of *Picea abies* needles

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Introduction

The terpene metabolism (mevalonic acid pathway) is a secondary metabolism present in all plants. Terpenes are elaborated by successive condensation of isoprene units (C_5). C_{10} and C_{15} terpene molecules are the main constituents of the volatile oils. These volatile terpenes are formed in secretory systems. Leucoplasts, non-green plastids (Carde, 1984), are involved in their synthesis (Gleizes *et al.*, 1983). In conifer needles, these plastids are localized in the epithelial cells of the subepidermal resin ducts. In *Picea abies* needles, the secretory ducts are longitudinal and discontinuous.

Forest decline is an important problem which appears in several countries in Europe and North America (McLaughlin, 1985). Most of the damaged forests are coniferous forests containing mainly spruce (*Picea*). Among the potential causes of forest decline, air pollution has received particular attention (McLaughlin, 1985). Previous studies have shown that the resin content of pine tissues greatly increases after mechanical or chemical injuries: wounding (Vassiliev and Carde, 1976), infestation by insects and infection by fungi (Cheniclet, 1987) or treatment with herbicides (Brown and Nix, 1975).

The intention of this study was to elucidate a possible relationship between the stress factors and a variation in terpene metabolism.

Materials and Methods

Different samples of needles were collected in the spruce *(P. abies)* stands which were located in the Donon forest where 3 decline classes were defined with respect to needle loss: class 2 (0–10% needle loss), class 3a (10–20%) and class 3b (35–50% with yellowish chlorosis). Needles of 3 consecutive yr from about 12 trees of each decline class were collected. The other samples of 5 yr old clones ("lac de Constance" (LC), "Gérardmer" (GER), "Istebna" (IST) were collected in the open top chambers of Montardon and Donon. These plants were placed under controlled conditions of air pollution which were equivalent to the pollution recorded in the Donon forest. The 3 clones were placed in air-filtered open top chambers or fumigated with ozone (O_3) or sulfur dioxide (SO_2) alone or a mixture. In Montardon, a mobile roof protects the trees from the rain.

Cytological observations were made with an electron microscope. RuBPCase was localized on ultrathin sections using immunogold labeling

techniques (Shaw and Henwood, 1985). The leucoplastidial volume density *(LVD)* (% of the cell volume occupied by leucoplasts) was estimated using a morphometric technique (Weibel, 1969).

For analytical studies, oxygenated and hydrocarbon terpene fractions were separated on a silica column after pentane extraction and analyzed with a gas chromatograph, using an apolar capillary column (Belingheri *et al.*, 1988). A 'desorption concentration injection' system (DCI, Delsi, France) was also used. About 5 needles were inserted into a heating block. The volatile compounds were swept by a carrier gas and trapped in a tenax cartridge attached directly to the injector of the gas chromatographic apparatus (the injection consists of a thermal desorption of the trapped compounds).

The statistical evaluations of our data included an analysis of variance and a technique for testing all differences between pairs of means (multiple comparisons among pair of means: *T'*-method) (Spjotvoll and Stoline, 1973; Sokal and Rohlf, 1981).

Cytological studies

Study of the leucoplastidome

Leucoplastidome and decline

In the Donon forest, the mean volume densities of leucoplasts were 10% for the 'healthy' trees and 15, 18 and 19% for the classes 2, 3a and 3b, respectively. Results of the *T'*-method are presented in Table I. The pairs of damaged classes (2–3a, 2–3b and 3a–3b) did not show any significant differences between each other. But a significant difference did exist between the *LVD* of healthy trees and the *LVD* of all the other classes.

Leucoplastidome and air pollution

For spruces fumigated with air pollutants in open top chambers, the estimation of the *LVD* was different between the trees from Montardon and these from Donon experiments.

In the first case, the *LVD* was about 20% of the cell volume and no significant difference between trees fumigated with O_3, SO_2, $O_3 + SO_2$ or charcoal-filtered air could be shown.

Samples from Donon showed a higher *LVD* for fumigated trees (24%) than for non-fumigated ones (13%).

Table I. Pairs comparison of the decline classes as regards the leucoplastidial volume density: '*T'*-method'.

Compared classes	T'	*Significant difference if* T'>*or*=Q' $_{0.05\ (4.120)}$[a]
healthy 2	3.700	+
healthy 3a	5.791	++
healthy 3b	6.763	+++
2–3a	2.359	–
2-3b	3.149	–
3a–3b	1.106	–

For each pair of classes the sample statistic *T'* was calculated.
[a]Critical value of the studentized augmented range table: $Q'_{0.05\ (4.120)} = 3.685$

Study of the chloroplasts (RuBPCase labeling)

About 20 plastids were investigated on ultrathin sections for each decline state. Variance analysis of these results verified that RuBPCase labeling was not equivalent for the different classes. The average densities of the gold labeling (number of gold particles per μm^2 of chloroplast section) was 96 for 'healthy' trees and 126, 121 and 59 for classes 2, 3a and 3b, respectively. Only the 3b state showed significant differences with the 3 other classes (Table II).

Table II. Pairs comparison of the decline classes as regards the RuBPCase density: '*T*'-method'.

Compared classes	T'	*Significant difference* $T' > or = Q'_{0.05\,(4.60)}$[a]
healthy 2	4.208	+
healthy 3a	3.308	–
healthy 3b	5.066	+
2–3a	0.572	– –
2–3b	9.504	+++
3a–3b	7.979	++

For each pair of classes, the sample statistic *T'* was calculated.
[a] Critical value of the studentized augmented range table: $Q'_{0.05(4.60)} = 3.737$.

Terpene composition

Donon forest: declined trees

No significant variation of terpene composition was observed in correlation with the decline.

Open top chambers

The investigations of needle samples of IST from the Donon forest showed a constant terpene hydrocarbon composition. In a polluted atmosphere, the proportion of bornyl acetate increased, while the proportion of camphor decreased. The 2 other clones (GER and LC) presented larger proportions of pinene and camphene and smaller proportions of limonene in needles of fumigated trees.

In the Montardon forest, the terpene composition of IST and LC was independent of air pollution conditions during the growth of the needles. For GER the concentrations of limonene and bornyl acetate seemed to be different between fumigated and non-fumigated trees. However, on fully grown needles, differences in the terpene composition were no longer observed. The varying terpene patterns found in these needles were dependent upon the origin of the plants and not upon on the conditions of pollution.

Discussion

There are significant differences between the leucoplastidial volume density of 'healthy' and damaged trees of the Donon forest. But the increase of the *LVD* and the decline of the trees did not seem to correlate to any variation of terpene composition in the needles of *P. abies.* Therefore, the *LVD* differences could be due to a shifting of cell differentiation in relation to the different localizations of the healthy and damaged tree classes. The lower labeling density of RuBPCase for the trees with bleached needles (state 3b) would be due to an irreversible disturbance of the metabolism. But another experiment with a larger plastid sampling must be done in order to confirm this first result.

In the open top chambers of the Montardon forest, there was no change of the leucoplastidial density and of the composition of the terpene hydrocarbons. In needle samples from the Donon open top chambers, the decreased *LVD* was correlated to changes in the composition of the oxygenated compounds for IST. The terpene composition of GER and LC was also modified. These results suggest that fumigation *and* natural rain are necessary to produce a modification of the terpene metabolism under controlled conditions.

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