Carotenoid Composition of Needles of *Picea abies* L. Showing Signs of Photodamage

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Z. Naturforsch. **45c**, 1111–1116 (1990); received August 3/September 24, 1990

Picea abies L., Norway Spruce, Carotenoids, Chlorosis, High Performance Liquid Chromatography

The pigment composition of needles of *Picea abies* L. taken from sites in the Bavarian forest has been determined. Needles were removed from (a) healthy, control trees, (b) trees which showed visible signs of damage for either 6 months or 3.5 years, and (c) trees which had received supplemental fertilizer application. In general, needles taken from these trees could be placed into three categories; (i) green, (ii) partially chlorotic, and (iii) partially necrotic (brown). Whilst the pigment composition of needles taken from healthy trees was typical of any higher plant, substantial changes in the chlorophyll and carotenoid content of the damaged needles were recorded. Particularly noticeable was an increase in the ratio of total carotenoid/chlorophyll and losses of neoxanthin and violaxanthin. Levels of total carotene (α- and β-carotene) and, especially, of lutein remained high in damaged needles. Levels of zeaxanthin were highest in the brown, partially necrotic, needles.

Introduction

Atmospheric pollutants, mainly SO_2 and oxidants such as NO_x, O_3 and peroxyacetonitriles (PAN), are often held responsible for the injury to the forest ecosystem of Central Europe. One of the visible symptoms of "Waldsterben" is the yellow chlorosis (partial bleaching) of plant material followed by necrosis of the needle or leaf [1]. The general action of these pollutants in plant physiology has been described [2], but unfortunately there are few reports on the effect of mixtures of air on plants [3], a situation which is perhaps more realistic.

Chronic injury symptoms such as chlorosis in leaves and needles have been reported [2] whilst more acute symptoms include orange-red pigmentation in injured tissue of conifers and white bleaching in other species. At high SO_2 concentrations decomposition of chlorophyll to phaeophytin has often been reported [4–6]. It is particularly interesting to note that, in contrast to SO_2, degradation of carotene specifically has been reported in NO_x treated tissues [7].

In addition to the direct effects of atmospheric pollutants Elstner and co-workers [8] have identified both acid precipitation and infection by parasites (viruses, bacteria etc.) as possible sources of damage. The direct effects of acid rainfall on plant material are not well reported although Cowling and Linthurst [9] report the formation of necrotic lesions and loss of nutrients, especially Mg^{2+} and Ca^{2+}, through leaching. This may, in turn, give rise to nutrient-induced chlorosis as described by Kinzel [10].

Pigment bleaching is one of the major visible symptoms of damage to needles. Although carotenoids are well known for their protective role against photooxidation in the chloroplast, few studies have investigated the fate of carotenoids in damaged needles. The aim of this study is to determine the carotenoid composition of undamaged spruce needles and to identify patterns of pigment destruction in chlorotic and necrotic needles. The pigment composition of a range of needle ages has been determined.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/1100–1111 $ 01.30/0

Materials and Methods

Needles of spruce (*Picea abies* L.) were obtained from the Bavarian Forest in April. All samples were taken from branches from the 11th whorl of 28 year-old trees in the "Ludwigsreuth" area which is located 845 m above sea level. The levels of pollutants at the nearby permanent monitoring station of Brotjacklriegel are shown in Fig. 1. Material was collected from 5 trees: (I) control, (II) a tree showing visible signs of damage for 6 months or, (III) for 3.5 years, (IV) a former damaged tree which has received fertilizer application (NPKMg) and, (V) as (IV) but infected with an endophytic needle fungus. All needles from trees (I) and (IV) were green, those of (IV) being much darker in colour. Needles from trees (II), (III), and (V) were either light-green (current year), partially chlorotic (previous year and older) and partially or totally necrotic (previous year and older). Three sets of needles of different ages (current year, previous year and year 4) were taken for analysis by high performance liquid chromatography.

Pigment analysis

Analyses of chlorophylls and carotenoids were made on ethanolic (redistilled) extracts of approximately 100 mg (wet weight) of needles. Reversed-phase high performance liquid chromatography (HPLC) was performed with a Zorbax-ODS column (5 μm, 4.6 mm × 25 cm) and a solvent gradient of 0–60% ethyl acetate acetonitrile/H_2O) (9:1) over 16 min followed by 14 min isocratic at 60% ethyl acetate. Carotenoids were identified by their retention times and electronic absorption spectra (λ_{max}), or in the case of chlorophylls by their "Soret" peaks. Spectra were determined on-line by means of a HP 1040 A Diode Array Detector (Hewlett-Packard). Mass spectrometry was used to confirm identifications.

Results and Discussion

All branches of the damaged trees taken for pigment analysis exhibit the characteristic yellowing symptom. The current year's needles remain green until new growth commences in late May or June (for Ludwigsreuth). Yellowing starts at the tip of the previous year's needles and proceeds, preferentially on the upper (sun exposed) side, towards the needle base. Such yellowed needles do not necessarily fall immediately but may remain on the tree for many years.

The HPLC separation of carotenoids and chlorophylls found in *P. abies* is shown in Fig. 1. The pigment composition is typical of higher plants with α- and β-carotene, lutein, violaxanthin and neoxanthin present as the major carotenoids. In addition, small amounts of antheraxanthin, lutein-5,6-epoxide, zeaxanthin and, occasionally, β-carotene-5,6-epoxide were detected in the needles. Table I shows the pigment composition of the control needles. Current year, light-green and 4 year-old dark-green control needles collected from a single branch. Although there was little overall difference in the carotenoid composition of these needles, to-

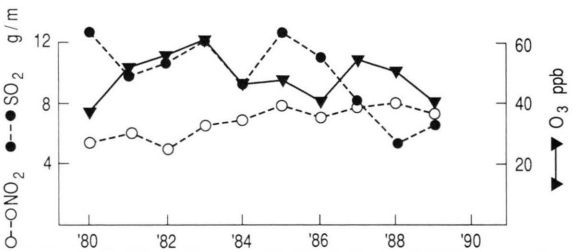

Fig. 1. Course of NO_2 and SO_2 concentrations at the permanent monitoring station at Brotjacklriegel (Bavarian Forest) of the Bundesumweltamt. The O_3 concentrations are mean values for the months May–August.

Table I. Pigment composition of spruce needles from control trees (I): (a) current-year light-green, needles; (b) 4 year-old, dark-green, needles. Values are given as a percentage of total carotenoids or total chlorophyll. Three sets of needles have been analyzed in each case. The data are presented as mean ± standard error.

	(a) Current year needles	(b) 4 Year-old needles
Neoxanthin	7.8 ± 1.29	8.2 ± 0.3
Violaxanthin	4.9 ± 0.7	10.2 ± 0.5
Lutein-5,6-epoxide	1.2 ± 0.1	2.0 ± 0.1
Antheraxanthin	1.4 ± 0.2	0.8 ± 0.2
Lutein	46.5 ± 3.3	50.4 ± 0.8
Zeaxanthin	1.0 ± 0.2	0.8 ± 0.3
α-Carotene	3.6 ± 1.6	1.2 ± 0.9
β-Carotene	34.6 ± 4.3	26.5 ± 0.8
Carotenoid/chlorophyll ratio	0.49:1	0.45:1
Lutein: total carotene ratio	1.21:1	1.80:1
Chlorophyll *a*/ chlorophyll *b* ratio	2.64:1	2.84:1

tal carotene (α- and β-carotene) levels were higher in the current year needles. Chlorophyll *a*/*b* ratios, which are suggested to be indicative of sun/shade responses [1] showed little variation. Interestingly, violaxanthin levels were higher in the older dark-green needles, possibly due to adaptation to different light regimes or even prolonged exposure to low levels of pollutants (see below). It is not known why this should be so; the violaxanthin cycle (*i.e.* the de-epoxidation of violaxanthin to zeaxanthin *via* antheraxanthin) may be involved although the reduction in violaxanthin content is not reflected in any subsequent rise in levels of zeaxanthin.

Needles from trees that had shown signs of damage for a total of either 6 months (II) or 3.5 years (III) prior to analysis could be placed into three categories as shown in Table II: (i) fully green though lower in pigmentation than the controls, (ii) partially chlorotic with yellow tips, and (iii) partially or wholly necrotic brown needles. Generally type (i) consisted of current year needles, whereas types (ii) and (iii) came from previous and older (years 3 and 4) needles.

In the type (i), apparently healthy green needles, from the damaged trees, the pigment composition was similar to that of the control, current year, needles. However, in the partially chlorotic needles of the previous year (type ii) overall xanthophyll levels were much reduced. Again a reduction in violaxanthin levels was observed, although there was no corresponding increase in antheraxanthin or zeaxanthin levels. This would suggest that the violaxanthin cycle is not involved and that pigments have been destroyed. Relative levels of α- and β-carotene, although variable, were higher in the previous year (type ii) than in the green, current year, needles (type i) from the same trees, again suggesting preferential loss of xanthophylls over the usually more susceptible carotenes.

In the case of the brown, type (iii), necrotic needles (year 4) the recorded pigment loss was particularly large, especially the reduction in neoxanthin and violaxanthin levels (Fig. 2). The minor xanthophylls, lutein-5,6-epoxide and antheraxanthin, could not be detected in these needles. Zeaxanthin levels, however, were elevated in comparison to those in either the healthy green or partially chlorotic needles. In the necrotic needles therefore the reduction in levels of violaxanthin could apparently be compensated, at least in part, by de-epoxidation to zeaxanthin. It is also possible that at least some of the zeaxanthin seen in necrotic tissues may have been formed *de novo*. Such a response is seen in many tissues as a consequence of long-term adaptation to "stress" conditions (*e.g.* transfer of plants to high light). In a number of photooxidative treatments involving photosynthetic tissues,

Table II. Pigment composition of needles taken from trees showing a typical yellowing of their previous year and older needles for 6 months (tree II) and 3.5 years (tree III). Values are given as a percentage of total carotenoid or total chlorophyll. Three sets of needles have been analyzed in each case. The data are presented as mean ± standard error (−, not detected).

	(I) Current year needles Light-green		(II) Previous year needles Partially chlorotic		(III) 3 and 4 year-old needles Partially chlorotic	
	3.5 years	6 months	3.5 years	6 months	3.5 years	6 months
Neoxanthin	12.1 ± 0.3	11.5 ± 0.5	6.2 ± 0.9	6.7 ± 0.8	6.4 ± 0.3	4.2 ± 0.1
Violaxanthin	11.8 ± 2.2	11.7 ± 1.4	7.1 ± 0.5	8.8 ± 1.1	0.9 ± 0.4	0.9 ± 0.7
Lutein-5,6-epoxide	1.5 ± 0.2	1.5 ± 0.1	2.1 ± 0.1	1.4 ± 0.2	0.2 ± 0.1	−
Antheraxanthin	0.6 ± 0.0	1.4 ± 0.6	0.50 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	−
Lutein	46.4 ± 1.0	43.8 ± 0.2	39.0 ± 1.6	47.0 ± 2.0	53.8 ± 1.5	55.5 ± 2.0
Zeaxanthin	−	0.4 ± 0.3	0.5 ± 0.2	0.7 ± 0.3	3.9 ± 0.3	4.7 ± 0.0
α-Carotene	3.7 ± 0.3	6.0 ± 0.5	6.0 ± 0.4	6.1 ± 0.6	2.9 ± 1.2	5.3 ± 0.1
β-Carotene	23.6 ± 1.3	23.5 ± 2.5	38.1 ± 3.2	28.2 ± 3.3	31.7 ± 0.5	29.2 ± 1.3
Carotenoid/chlorophyll ratio	0.39:1	0.38:1	0.44:1	0.40:1	1.03:1	1.18:1
Lutein/total carotene ratio	1.69:1	1.48:1	0.88:1	1.37:1	1.55:1	1.61:1
Chlorophyll *a*/chlorophyll *b* ratio	2.69:1	3.07:1	3.72:1	3.37:1	0.93:1	1.29:1

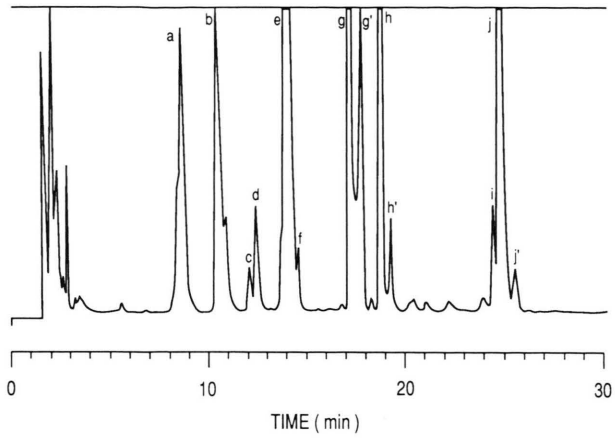

Fig. 2. Reversed-phase HPLC chromatogram of pigments from control, fully green, needles monitored at 447 nm; a, neoxanthin; b, violaxanthin; c, lutein-5,6-epoxide; d, antheraxanthin; e, lutein; f, zeaxanthin; g, g', chlorophyll *b;* h, h', chlorophyll *a;* i, α-carotene; j, j', β-carotene.

both *in vivo* and *in vitro,* neoxanthin has been found to be as sensitive as β-carotene to photodestruction [11].

Wolfenden and colleagues [12] analyzed the carotenoid content of plant material from field sites where O_3 was thought to be the major pollutant and suggested that exposure to O_3 may promote the epoxidation of zeaxanthin to antheraxanthin and violaxanthin. In a series of experiments carried out under controlled conditions in open-top chambers Senser and co-workers [13] observed that exposure of young spruce trees to a combined O_3/acid mist stimulated an increase in the violaxanthin content of needles. Such changes could from part of a regulatory process leading to protection from light-induced stress, *via* the "violaxanthin cycle" and production of zeaxanthin [14]. However, exposure of barley seedlings to high levels O_3 did not greatly affect these "violaxanthin-cycle" pigments [15] and did not promote either the de-epoxidation or epoxidation parts of the cycle preferentially. The possibility of a photoprotective role for the "violaxanthin-cycle" pigments in plants exposed to atmospheric pollutants should be assessed.

Lichtenthaler and Buschmann [16] found an increase in xanthophyll/carotene ratios in brown, and particularly, in yellow needles. They also noted a general increase in carotenoid/chlorophyll ratio in all damaged needles, similar to the findings of the present study. Such data is also consistent with the pattern seen during the senescence of photosynthetic tissues in a range of plants (unpublished data).

Addition of fertilizer to the soil around previously damaged trees (IV) appears to restore the needles to a healthy condition, as reflected by their carotenoid composition (Table III). As with many of the other samples there was a large degree of variation between needles of different ages on a single branch. Particularly noticeable was a large variation in the total carotene levels as reflected in the lutein/total carotene ratio. This ranged from 2.48:1 for current year needles branch to 1.39:1 for 4 year-old needles. Such variation does, of course, make interpretation of the data more difficult. The carotenoid content of needles taken from the tree (V) infected with an endophytic fungus (Table III) was very similar to those obtained for other damaged needles, in which there was no evidence of fungal infection (Table II). This may suggest that the destructive processes involved in the degradation of the pigment-protein complexes and their pigments in both these cases are similar.

Both chlorotic and necrotic needles have a high carotenoid/chlorophyll ratio in comparison to the control, suggesting a preferential loss of chlorophylls. In general, chlorophyll *a* levels were reduced to a greater extent than chlorophyll *b,* especially in necrotic needles. This may indicate that the reaction centres of PS I and PS II which are rich in chlorophyll *a* are damaged to a greater extent than the light-harvesting complexes which are rich in chlorophyll *b*. However, a corresponding loss of β-carotene, the single major carotenoid in the reaction centres, was not seen and the lutein/total-carotene ratio remained relatively constant in all types of damaged needles. Lichtenthaler and Buschmann [16] noted that in spruce needles exposed to SO_2 and NO_2, chlorophyll *a* was destroyed at a quicker rate than chlorophyll *b,* and β-carotene more rapidly than the xanthophylls. Such data is consistant with oxidative processes leading to the direct degradation of carotenoids and chlorophylls in the pigment-protein complexes.

In the needles examined in this study the relative levels of both α- and β-carotene remained high in both chlorotic and necrotic tissues (Tables II and III) whilst neoxanthin and, especially, violaxan-

Table III. Pigment composition of needles collected from previously damaged spruce trees that have received additional fertilizer. Dark-green needles from a tree showing successful recovery following fertilizer application (IV) and needles from a tree infected with an endophytic fungus (V) have been analyzed. Data are shown for A) current year, B) previous year and C) 4 year-old needles. Values are given as a percentage of total carotenoid or total chlorophyll (n = 3, mean ± standard error) (−, not detected).

	Tree IV			Tree V		
	(A) Current year (i) Dark-green	(B) Previous year (ii) Dark-green	(C) 4 Year-old (iii) Dark-green	(A) Current year (i) Light-green	(B) Previous year (ii) Partially chlorotic	(B) 4 Year-old (iii) Partially necrotic
Neoxanthin	12.3 ± 1.4	12.1 ± 1.0	9.8 ± 0.4	10.5 ± 1.2	9.9 ± 0.7	8.3 ± 1.9
Violaxanthin	21.1 ± 0.0	3.6 ± 1.2	8.9 ± 2.0	9.2 ± 1.9	14.5 ± 0.7	1.5 ± 0.9
Lutein-5,6-epoxide	1.0 ± 0.1	0.6 ± 0.0	1.2 ± 0.4	0.9 ± 0.0	2.4 ± 0.0	–
Antheraxanthin	2.5 ± 0.8	0.6 ± 0.0	1.0 ± 0.3	1.3 ± 0.3	1.9 ± 0.3	–
Lutein	50.1 ± 0.4	53.2 ± 1.6	45.5 ± 1.6	48.1 ± 2.8	47.4 ± 1.1	62.3 ± 1.0
Zeaxanthin	1.5 ± 0.4	0.9 ± 0.4	0.4 ± 0.3	0.8 ± 0.1	1.0 ± 0.5	5.5 ± 1.5
β-Carotene-5,6-epoxide	–	–	0.2 ± 0.1	–	1.0 ± 0.4	–
α-Carotene	1.8 ± 0.8	4.5 ± 0.3	3.1 ± 1.8	1.7 ± 0.8	2.2 ± 0.3	1.7 ± 0.7
β-Carotene	18.4 ± 1.2	24.2 ± 1.2	29.6 ± 5.0	27.5 ± 6.2	20.0 ± 1.9	20.8 ± 0.7
Carotenoid/chlorophyll ratio	0.48:1	0.46:1	0.44:1	0.44:1	0.55:1	0.85:1
Lutein/total carotene ratio	2.48:1	1.79:1	1.39:1	1.64:1	2.13:1	2.75:1
Chlorophyll *a*/chlorophyll *b* ratio	2.60:1	1.83:1	1.74:1	2.49:1	3.02:1	1.67:1

thin levels may be much reduced. The recorded stability of β-carotene in these needles could be due to the formation of plastoglobuli into which β-carotene, released from pigment-protein complexes, could be incorporated. Such plastoglobuli are commonly formed during the senescence of photosynthetic tissues and are rich in lutein and β-carotene (unpublished data). These needles however did not contain any xanthophyll-acyl esters which are commonly found in some other plastoglobuli-containing plant tissues and may indicate their presence.

The results suggest that the destruction of chlorophylls and carotenoids in these spruce needles may be the result of a senescent-type process possibly promoted by the poor nutrient status of the soils. The effect of some gaseous pollutants (*e.g.* O_3) can be, however, to induce pigment bleaching which is indistinguishable from the normal pattern of senescence seen in the same plant tissues [11]. There is no evidence from the analysis of the pigment content of these spruce needles that direct photooxidative destruction of carotenoids has taken place in the manner suggested by Elstner and colleagues [8]. In addition, pollutant levels in this part of Southern Germany have remained relatively constant over the past decade (Fig. 1).

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