



CHLOROPHYLLS AND CAROTENOIDS IN NEEDLES OF DAMAGED FIR (*ABIES ALBA* MILL.) FROM RISNJAK NATIONAL PARK IN CROATIA

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Chlorophyll and carotenoid content was studied in needles of damaged silver fir (*Abies alba* Mill.) trees growing in a polluted habitat, Risnjak National Park, and of almost undamaged ones growing at a much less polluted site, Donja Dobra, both locations in the Gorski Kotar region. Chlorophyll *a* and *b*, total chlorophylls and carotenoids were lower in needles from the polluted Risnjak site than in those from the unpolluted Donja Dobra site. The data indicate an increase of pigment content over the course of a year; and somewhat smaller content in the first in comparison with the second experimental year. The chlorophyll *a:b* ratios varied independently of pollution level and locality.

Key words: Pinaceae, *Abies alba* Mill., needles, chlorophylls, carotenoids, air pollution.

INTRODUCTION

It is well known that forests in Croatia, as in many other European countries, are declining (Huttunen, 1976a,b; Bernadzki, 1983; Schulze et al., 1989; Tikvić et al., 1995; etc). Forests of silver fir (*Abies alba* Mill.) are particularly affected. The process is most visible in the Gorski Kotar region, an area between the continental and Mediterranean climate zones, where the Risnjak National Park is situated. It is strongly impacted by different pollutants, particularly sulphur dioxide. Sulphur dioxide arrives from the Rijeka region and northern Italy, but also from Northern and Central Europe. According to Durbešić and Kerovec (1990), air pollution is the primary cause of silver fir damage in this area, although many factors such as insects and fungi are implicated in decline of forests (Opalički, 1972; Spaić, 1972; Glavaš, 1987; 1992a,b).

Previous investigations in Risnjak National Park, particularly those employing infrared color photography, found that none of more than 3000

monitored silver fir trees could be considered healthy (Bačić and Popović, 1998). It is estimated that the average damage of all of them is nearly 50% (Kušan et al., 1993). In view of data published by Prpić (1990) on the high level of sulphur dioxide in this area, especially that of transboundary origin (61%), and data of Komlenović and Rastovski (1992) on increased sulphur content, decreased levels of some metals, and numerous plastoglobules within chloroplast stroma in needles of damaged silver fir trees in this area, it is possible to attribute their decline to acid rain. In this work we study the condition of needles from damaged silver firs in the Risnjak National Park in greater detail.

MATERIALS AND METHODS

STUDY SITES

Two localities differing in air pollution levels were chosen: Risnjak and Donja Dobra. The first is a natural forest in the Risnjak National Park, and the



Fig. 1. Damaged fir trees (*Abies alba* Mill.) from the Risnjak site.



Fig. 2. Healthy fir trees (*Abies alba* Mill.) from the Donja Dobra site.

second is a planted site (control) about 30 km distant. Both are nearly the same age (about 150 years).

The Risnjak site is at 700 m altitude, on a dolomite base with a limestone surface of average pH 6.07, covered with a naturally growing silver fir forest of *Abieti-Fagetum* and *Calamagrosti-Abietum* populations. The Donja Dobra site is north of the Risnjak site, at 570 m altitude, also on a dolomite base but with a silicate surface of average pH 4.16, and its cultivated silver fir forest has overgrown a *Blechno-Abietum* population.

The silver fir trees in Risnjak are quite visibly damaged (Fig. 1). Their defoliation, chlorosis and crown architecture were assessed. Ten trees used for needle sampling had estimated damage ranging from 20% to 85% (35%, 55%, 30%, 35%, 85%, 75%, 45%, 45%, 20%, 45%). The silver fir trees at the Donja Dobra site looked almost completely healthy (Fig. 2). Here the estimated damage of ten selected trees used for needle sampling ranged from 5% to

10% (5%, 5%, 10%, 10%, 5%, 5%, 5%, 10%, 10% and 5%).

The visible damage to the silver firs at Risnjak is the result of pollution. Pollution, expressed as sulphur dioxide concentration and determined by the peroxide or British method (Amantaurić, 1981), was twice higher there than at the Donja Dobra site. For example, at Risnjak the concentration of sulphur dioxide was 47.5 $\mu\text{g}/\text{m}^3$ in December 2000, 41.3 $\mu\text{g}/\text{m}^3$ in July 2001, 32.2 $\mu\text{g}/\text{m}^3$ in October 2001 and 42.1 $\mu\text{g}/\text{m}^3$ in May 2002, whereas in Donja Dobra it amounted to 20.7 $\mu\text{g}/\text{m}^3$ in December 2000, 23.6 $\mu\text{g}/\text{m}^3$ in July 2001, 16.5 $\mu\text{g}/\text{m}^3$ in October 2001 and 23.9 $\mu\text{g}/\text{m}^3$ in May 2002.

There are also considerable differences in the soil of the two sites. Spectrophotometric analysis of the soil elements, determined by HClO_4 , HCl , HNO_3 and HF and detected with ICP-AES (Miko et al., 2000), showed that 10 of 22 elements (Al, Na, K, Fe, Ti, V, Cu, Co, Ba, Cr) are present at much greater

concentrations in the soil of the Donja Dobra site than at the Risnjak site, but at the Risnjak site higher concentrations are recorded for Zn, Mn, Mo, Mg and Ca, and particularly for S as well as for most heavy metals (Pb, Ni, Cd).

Hydrometeorological conditions differ little between the two sites. The hydrometeorological data for the two sites were obtained from Parg and Delnice. The Parg hydrometeorological station is near Risnjak and the Delnice station is near Donja Dobra. Measurements of temperature, relative humidity and rainfall during 1999 and 2000 indicate no essential differences between the two sites, except that the Parg site is slightly cooler, corresponding to the altitude.

SAMPLING

Current fir needles and of year-old needles were collected from each of ten silver fir trees of the two populations. Samples of ~1 g each were taken from the south side of the middle part of the tree canopy, three times per year, in the middle of May, July and September, in 1999 and 2000.

PIGMENT CONTENT MEASUREMENT

There are several methods for extraction and determination of chlorophylls and carotenoids (Barnes et al., 1992; Wellburn, 1994), but we used the traditional method for practical reasons (Holden, 1965; Urbach et al., 1976).

About 0.1 g of fresh current and year-old needles from each of the sites were taken for analysis. Pigments were isolated by extraction in 80% acetone and then quantitatively determined by the method of Holden (1965) and Urbach et al. (1976).

Content was calculated with the following equations:

- (1) $C_a = 12.7 \times E_{663} - 2.69 \times E_{645}$
- (2) $C_b = 22.9 \times E_{645} - 4.68 \times E_{663}$
- (3) $C_{a+b} = 20.2 \times E_{645} + 8.02 \times E_{663}$
- (4) $C_c = 4.75 \times E_{542.5} - 0.226 \times E_{a+b}$

where C_a , C_b , C_{a+b} and C_c is content (mg/g FW) of chlorophyll *a*, chlorophyll *b*, the sum of chlorophylls *a* and *b*, and carotenoids, respectively.

FW = fresh weight

STATISTICAL ANALYSIS

The results were statistically analyzed by analyses of variance and the t-test, using Microsoft Excel 7

and Origin 6.1. In the t-test the differences were significant at $p < 0.05$ (*).

RESULTS AND DISCUSSION

As shown in Tables 1–4, chlorophyll *a* content varied in the current-year needles from 0.26 mg/g FW to 0.94 mg/g FW in May and September 1999, respectively, and in year-old ones from 0.72 mg/g FW to 1.52 mg/g FW in July 1999 and 2000. Chlorophyll *b* content varied in current-year needles from 0.14 mg/g FW to 0.72 mg/g FW in May and September 1999, and in year-old needles from 0.31 mg/g FW to 1.07 mg/g FW in May 1999 and July 2000, respectively. Chlorophylls *a* + *b* ranged in the current-year needles from 0.40 mg/g FW to 1.67 mg/g FW in May and September 1999, and in year-old ones from 1.07 mg/g FW to 2.59 mg/g FW in May 1999 and July 2000. Carotenoids ranged in current-year needles from 0.07 mg/g FW to 0.15 mg/g FW in May 1999 and September 2000, and in year-old needles from 0.13 mg/g FW to 0.35 mg/g FW in July and May 1999.

The 1999 and 2000 values together, at the Risnjak site the values of the measured pigments in current-year needles varied from 0.26 mg/g FW to 0.54 mg/g FW (chlorophyll *a*), 0.29 mg/g FW to 0.47 mg/g FW (chlorophyll *b*), 0.40 mg/g FW to 1.21 mg/g FW (chlorophyll *a*+*b*) and 0.07 mg/g FW to 0.11 mg/g FW (carotenoids). In year-old needles the ranges were 0.72 mg/g FW to 1.08 mg/g FW (chlorophyll *a*), 0.31 mg/g FW to 0.79 mg/g FW (chlorophyll *b*), 1.07 mg/g FW to 1.88 mg/g FW (chlorophyll *a*+*b*) and 0.13 mg/g FW to 0.25 mg/g FW (carotenoids). At the Donja Dobra site the values in current-year needles were 0.36 mg/g FW to 0.94 mg/g FW (chlorophyll *a*), 0.15 mg/g FW to 0.72 mg/g FW (chlorophyll *b*), 0.55 mg/g FW to 1.67 mg/g FW (chlorophyll *a*+*b*) and 0.10 to 0.15 mg/g FW (carotenoids); in year-old needles they ranged from 0.91 mg/g FW to 1.52 mg/g FW (chlorophyll *a*), 0.47 mg/g FW to 1.07 mg/g FW (chlorophyll *b*), 1.58 mg/g FW to 2.59 mg/g FW (chlorophyll *a*+*b*) and 0.14 mg/g FW to 0.35 mg/g FW (carotenoids). The differences between sites generally were significant in both current and year-old needles (Tabs. 1–4).

Analysis of pigment content demonstrated an increase in levels of pigments in both current and year-old needles from May to September, and generally lower values at Risnjak than at Donja Dobra. Pigment content, both annual and in individual months, was higher in year-old needles than in current-year needles at both sites and in both experimental years.

TABLE 1. Chlorophyll *a* content (mg/g fresh weight; mean \pm SD, n = 10) in silver fir (*Abies alba* Mill.) needles collected from two localities, Risnjak and Donja Dobra

Month	Risnjak		Donja Dobra	
	Current-year needles	Year-old needles	Current-year needles	Year-old needles
May 1999	0.26 \pm 0.06	0.76 \pm 0.16	0.36 \pm 0.11*	1.12 \pm 0.16*
July 1999	0.45 \pm 0.09	0.72 \pm 0.14	0.69 \pm 0.13*	0.91 \pm 0.15*
September 1999	0.35 \pm 0.15	1.08 \pm 0.26	0.94 \pm 0.20*	1.26 \pm 0.24
May 2000	0.30 \pm 0.11	0.74 \pm 0.15	0.45 \pm 0.09*	1.03 \pm 0.17*
July 2000	0.54 \pm 0.15	0.86 \pm 0.14	0.78 \pm 0.21*	1.52 \pm 0.72*
September 2000	0.51 \pm 0.11	0.88 \pm 0.16	0.82 \pm 0.12*	1.08 \pm 0.16*

* Significant differences between Risnjak and Donja Dobra (p < 0.05).

TABLE 2. Chlorophyll *b* content (mg/g fresh weight; mean \pm SD, n = 10) in silver fir (*Abies alba* Mill.) needles collected from two localities, Risnjak and Donja Dobra

Month	Risnjak		Donja Dobra	
	Current-year needles	Year-old needles	Current-year needles	Year-old needles
May 1999	0.14 \pm 0.10	0.31 \pm 0.09	0.15 \pm 0.02	0.47 \pm 0.04*
July 1999	0.32 \pm 0.06	0.45 \pm 0.10	0.49 \pm 0.14*	0.66 \pm 0.12*
September 1999	0.47 \pm 0.17	0.79 \pm 0.16	0.72 \pm 0.20*	0.87 \pm 0.17
May 2000	0.29 \pm 0.14	0.58 \pm 0.09	0.37 \pm 0.07	0.95 \pm 0.18*
July 2000	0.40 \pm 0.12	0.67 \pm 0.16	0.54 \pm 0.16*	1.07 \pm 0.16*
September 2000	0.40 \pm 0.10	0.60 \pm 0.13	0.62 \pm 0.15*	0.82 \pm 0.15*

* Significant differences between Risnjak and Donja Dobra (p < 0.05).

TABLE 3. Chlorophyll *a* + *b* content (mg/g fresh weight; mean \pm SD, n = 10) in silver fir (*Abies alba* Mill.) needles collected from two localities, Risnjak and Donja Dobra

Month	Risnjak		Donja Dobra	
	Current-year needles	Year-old needles	Current-year needles	Year-old needles
May 1999	0.40 \pm 0.12	1.07 \pm 0.22	0.55 \pm 0.09*	1.60 \pm 0.21*
July 1999	0.78 \pm 0.16	1.17 \pm 0.21	1.18 \pm 0.26*	1.58 \pm 0.28*
September 1999	1.21 \pm 0.37	1.88 \pm 0.40	1.67 \pm 0.33*	2.14 \pm 0.41
May 2000	0.59 \pm 0.22	1.32 \pm 0.23	0.83 \pm 0.16*	1.99 \pm 0.29*
July 2000	0.96 \pm 0.28	1.54 \pm 0.30	1.29 \pm 0.37*	2.59 \pm 0.80*
September 2000	0.92 \pm 0.19	1.49 \pm 0.26	1.44 \pm 0.23*	1.90 \pm 0.25*

* Significant differences between Risnjak and Donja Dobra (p < 0.05).

Correlation coefficients (not shown) indicate that mean monthly temperature and relative humidity did not influence pigment content at the localities. There were no significant differences in those meteorological values between sites.

The concentration of Fe, a catalyst in the synthesis of chlorophylls (Taiz and Zeiger, 1987), was lower in the soil of the Risnjak site (1.75 %), and the concentration of Mg (6.25 %), a constituent of chlorophyll, was higher. There was no

deficiency of Mg or K in yellowish needles, unlike in Solberg et al.'s study (1998). At the Risnjak site, Vrbek and Gašparac (1992) found no Pb under the upper limit of 100 ppm, Zn was above the upper limit of 300 ppm only sporadically, and Cu was within the limits. Our investigation found the presence of heavy metals (Cd 0.57 mg/kg, Ni 30.6 mg/kg, Mo 6.0 mg/kg, Mn 520 mg/kg, Pb 52 mg/kg), and S, 0.11% at the Risnjak site, as well as a higher concentration of SO₂ in the air and 20–85% damaged trees, con-

TABLE 4. Carotenoid content (mg/g fresh weight; mean \pm SD, n = 10) in silver fir (*Abies alba* Mill.) needles collected from two localities, Risnjak and Donja Dobra

Month	Risnjak		Donja Dobra	
	Current-year needles	Year-old needles	Current-year needles	Year-old needles
May 1999	0.07 \pm 0.02	0.16 \pm 0.04	0.12 \pm 0.02*	0.35 \pm 0.10*
July 1999	0.09 \pm 0.01	0.13 \pm 0.03	0.14 \pm 0.04*	0.19 \pm 0.03*
September 1999	0.11 \pm 0.02	0.25 \pm 0.30	0.13 \pm 0.07	0.14 \pm 0.02
May 2000	0.11 \pm 0.05	0.14 \pm 0.05	0.10 \pm 0.02	0.26 \pm 0.05*
July 2000	0.11 \pm 0.03	0.18 \pm 0.04	0.12 \pm 0.03	0.25 \pm 0.12
September 2000	0.10 \pm 0.04	0.16 \pm 0.04	0.15 \pm 0.04*	0.18 \pm 0.04

* Significant differences between Risnjak and Donja Dobra ($p < 0.05$).

firming the Risnjak site as more polluted than Donja Dobra.

Earlier investigations pointed to the influence of air pollution on pigment content (Rao and Le Blanc, 1966; Skye, 1968; Syrrat and Wanstall, 1968; Kuziel, 1974; Swieboda, 1976; Siefermann-Harms, 1994). Our earlier results (Bačić and Ledić, 1993) and recent ones (Bačić and Zahirović, 2000) indicate decreasing pigment content in the needles from the polluted site, apparently corresponding with higher concentrations of SO₂ in the air but also suggesting a possible effect of tree age on pigment content.

As seen in Table 5, in 1999 the chlorophyll *a:b* ratio varied from 1.30 (September) to 2.36 (May) in current-year needles, and from 1.36 (July) to 2.43 (May) in year-old needles. In 2000 the values ranged from 1.03 (May) to 1.38 (July) in current-year needles and from 1.08 (in May) to 1.45 (in September) in year-old needles. In some months the ratios trended slightly higher in unpolluted Donja Dobra than in polluted Risnjak, but in other months the opposite was true and generally no regularity could be observed. Hence the chlorophyll *a:b* ratio fluctuated independently of the pollution level.

Researchers note different ranges of variation in the chlorophyll *a:b* ratio (Müller, 1957; Williams et al., 1971; Dässler, 1972). In pine tree needles it has been reported to vary from 2.07 to 2.66 (Langlet, 1942), 1.6 to 1.7 and 1.37 to 2.13 (Gerhold, 1959), 1.37 to 2.13 (Swieboda, 1976), 1.84 to 4.01 (Bačić and Ledić, 1993) 1.46 to 2.38, 1.31 to 2.49 and 1.52 to 2.18 (Bačić and Zahirović, 2000). The present study found no correlation between chlorophyll *a:b* ratios and pollution levels. These findings, along with Dässler's (1972) conclusion that chlorophyll *b* is not more resistant than chlorophyll *a*, support Swieboda's (1976) suggestion that the chlorophyll *a:b* ratio is an unreliable criterion of air pollution damage to plants.

TABLE 5. Chlorophyll *a : b* ratio in silver fir (*Abies alba* Mill.) needles collected from two localities, Risnjak and Donja Dobra (n = 10)

Month	Risnjak		Donja Dobra	
	Current-year needles	Year-old needles	Current-year needles	Year-old needles
May 1999	1.84 : 1	2.43 : 1	2.36 : 1	2.36 : 1
July 1999	1.42 : 1	1.61 : 1	1.41 : 1	1.36 : 1
September 1999	1.54 : 1	1.37 : 1	1.30 : 1	1.44 : 1
May 2000	1.03 : 1	1.28 : 1	1.21 : 1	1.08 : 1
July 2000	1.36 : 1	1.27 : 1	1.38 : 1	1.42 : 1
September 2000	1.26 : 1	1.45 : 1	1.30 : 1	1.31 : 1

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