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Antioxidants in Norway Spruce Needles at Field Plots in the Vicinity of a Thermal Power Plant in Slovenia

By

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Summary

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Results of antioxidant analyses such as vitamin C and E, water soluble thiols and β-carotene in Norway spruce needles at sampling sites influenced by the Thermal Power Plant in Šoštanj, Slovenia are presented. These antioxidants were analysed in current and one year old needles sampled in September 1997 at ten permanent sampling sites. The antioxidant response of Norway spruce needles is compared to the air pollution load, assessed by total sulphur content of needles, and general environmental stress factors connected with the sampling site. From the results obtained it is possible to draw logical conclusions about the physiological role and behaviour of the biochemical needle stress indicators analysed and to explain their status as a result of the air pollution load and environmental stresses.

Introduction

Among other effects, air pollution impact on tree physiology is manifested by free radical production, the process taking place during normal cellular metabolism. One free radical can begin the destructive process of removing an

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electron from stable compounds and forming many reactive radical species, transforming the stable compounds into a variety of free radicals such as superoxide, superoxide conjugated acid, a hydroxyl radical, an organic free radical, or a peroxy free radical (SUN 1990). Other triggers of free radicals include exposure to certain chemicals, radiation, ultraviolet light and drought, (WINSTON 1990, TAUSZ & al. 1996b, BYTNEROWICZ 1996). Free radicals can be detrimental by reacting with, and sometimes destroying, critical cellular components including the polyunsaturated fatty acid that is a constituent of lipoprotein particles and plasma membranes. Reactive oxygen species attack the unsaturated bonds of fatty acids in lipid membranes, altering membrane structure and functions (SUN 1990). Among different cell defense systems some small molecular compounds act directly to interfere with the propagation of free radical generation. Such molecules are vitamin E, vitamin C, β-carotene, gluthathione and carotenoides BERMADINGER & al. 1990, TAUSZ & al. 1996a).

Lipid-soluble vitamin E has been identified as an endogenous free radical scavenger, slowing down the damage process in plants. The antioxidative action of vitamin E seems to be determined by a synergism with the water-soluble antioxidant vitamin C (KUNERT & EDERER 1985), and vitamin E has been proposed to act as the primary antioxidant, while vitamin C reductively regenerates oxidized vitamin E.

There is no single bioindication method to determine stress in plant tissues exposed to air pollutants or natural stresses, and a combination of several physiological stress analyses is necessary (BERMADINGER & al. 1990, TAUSZ & al. 1996a, b). In previous investigations combinations of total needle sulphur content and photosynthetic pigments, ascorbic acid and water soluble thiol content were used as needle stress indicators (BATIČ & al. 1995, RIBARIČ-LASNIK & al. 1996)

The aim of this study was to determine the oxidative stress in Norway spruce needles exposed to air pollutants from the Thermal Power Plant in Šoštanj (yearly (1997) emissions 53.093 tons of SO_2 and 11.750 tons of NO_x) by analyses of vitamin C and E, watersoluble thiols and β-carotene, and to connect them to total sulphur content and important site parameters.

Material and Methods

Choice of sampling sites, sampling procedure and sample processing was carried out as described in previous papers (BATIČ & al. 1995, RIBARIČ-LASNIK & al. 1996, KRAIGHER & al. 1996, SIMONČIČ 1996). The most polluted sites are either those which are very close to the thermal power plant (Veliki vrh, Šoštanj, Lajše, Topolšica) or those which are highly polluted due to climatic conditions and topography (Zavodnje, Graška gora) but more distant from the thermal power plant. Sites with relatively clean air are remote sites at the higher altitudes (Smrekovec, Kope, Brneško sedlo, Kramarica) or sites in the sheltered position in relation to the wind direction from the thermal power plant (Laze, Razbor).

Air pollution data are available for some of the sampling sites for 1997 and are presented in Table 1.

Sampling was carried out using the ICP-Forest recommendation (ANONYMOUS 1987). Samples were taken from eleven sites at the end of September, 1997. Five trees per site were chosen for sampling.

β-Carotene, ascorbic acid, tocopherol and water soluble thiols were analysed by the HPLC methods described by PFEIFHOFER 1989, BUI-NGUYEN 1980, WIMANLASIRI & WILLS 1983 and GRILL & ESTERBAUER 1973.

Total sulphur content was determined with a SULMPHOMAT 12-ADG at the Slovenian Forestry Institute (SIMONČIČ & KALAN 1996).

Results and Discussion

Analyses of water soluble thiols and total sulphur content are presented in Fig. 1. (a. Total sulphur content; b. Thiols). The highest contents of sulphur were measured in needles from very polluted sites close to the thermal power plant (Veliki vrh, Zavodnje, Graška gora) and were accompanied by high levels of thiols. The only difference is at the high elevation site of Smrekovec, with a low sulphur content and high thiols due to greater oxidation stress at higher altitudes. The content of sulphur is also much smaller at low altitude site Laze, which is protected from the pollutants from thermal power plant. Total sulphur content decreases with altitude of the sampling site and increases with the age of needles. Thiols content does not increase with altitude in current year needles what can explained as air pollution effect, but one year old needles show already more natural pattern of thiols content, e.g. slight increase with altitude.

Table 1. Annual sulphur (a) (% DWG) and water soluble thiols (b) (μmol/g) contents in needles sampled in September 1997.

Sampling site	Altitude (m)	O_3 (μg/m^3)	SO_2 (μg/m^3)
ŠOŠTANJ	400	-	29
TOPOLŠICA	400	-	18
ZAVODNJE	760	72	42
GRAŠKA GORA	730	-	36
VELENJE	360	35	11
VELIKI VRH	570	-	53

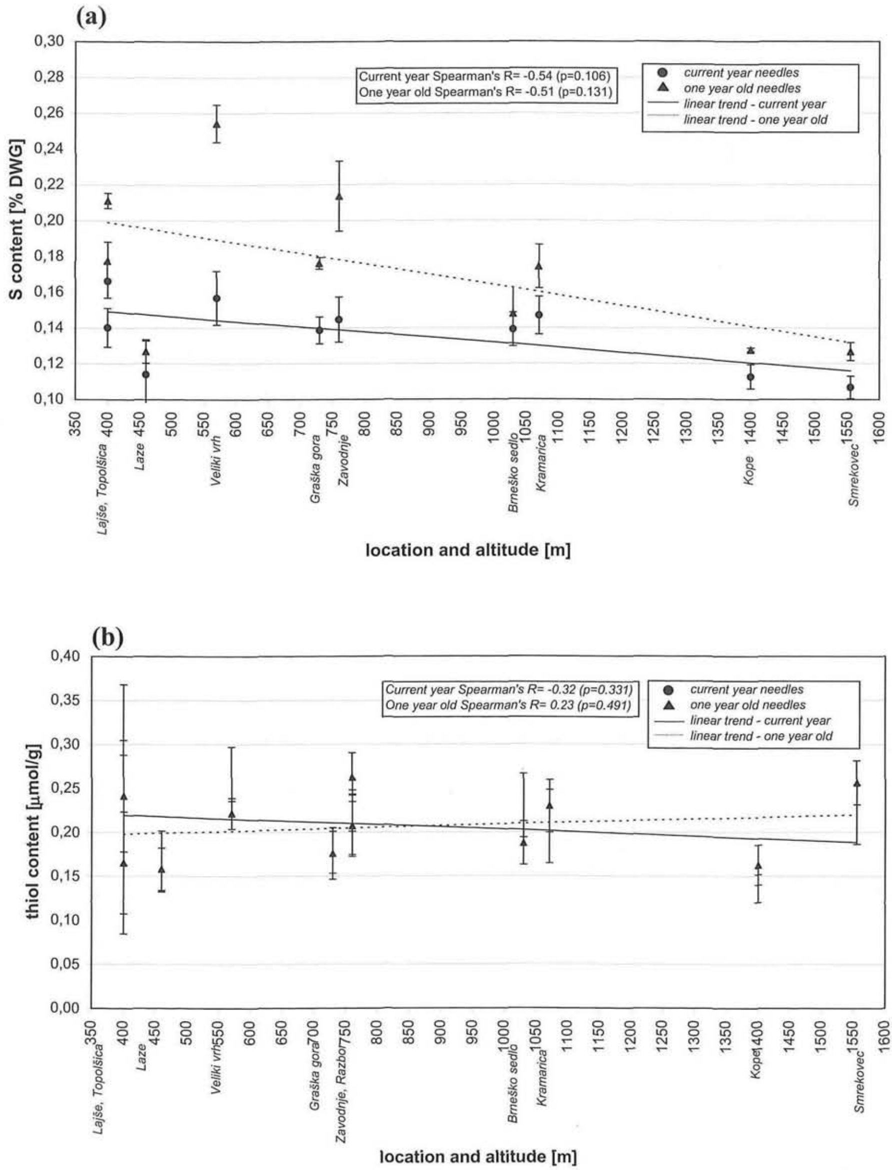

Fig. 1. Total sulphur (a) (% DWG) and water soluble thiols (b) (µmol/g) contents in needles sampled in September 1997.

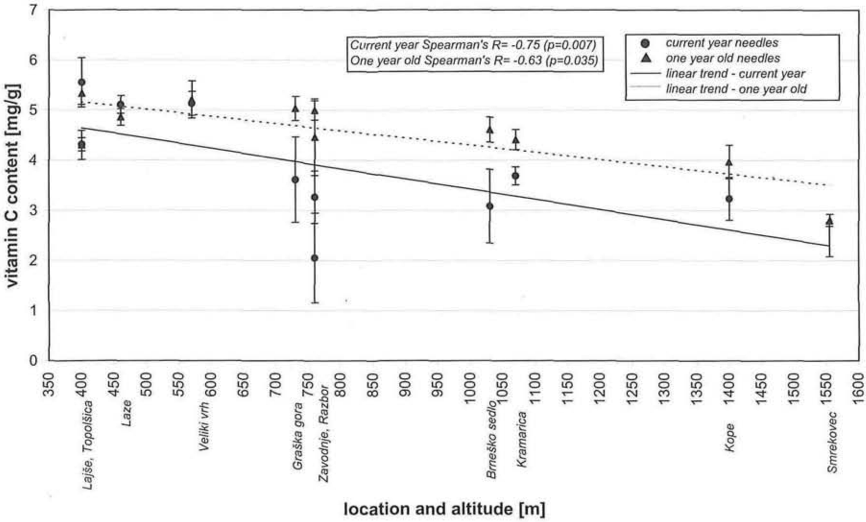

Fig. 2. Vitamin C content in needles (mg/g) sampled in September 1997.

The vitamin C and E content of Norway spruce needles decreases with altitude (Fig. 2, 3) which is the opposite to the normal situation. Higher levels of these two antioxidants at low elevation sites polluted by emissions from the thermal power plant indicate high stress conditions. The defense mechanism of Norway spruce needles is exceeded at the Zavodnje site, as indicated by the severe drop of ascorbic acid content. The altitudinal pattern of vitamin E becomes more normal in one year old needles, indicating that the water phase of cells is more affected than the lipid phase where tocopherol is situated. According to literature data the ratio vitamin C/E should be greater than ten. In our results this ratio is higher in needles from all sites, except at the very polluted Zavodnje site (Fig. 4), again showing the impact of sulphur pollution exceeds defense mechanism. The concentration of β-carotene in needles did not show any significant pattern in regard to the air pollution of the site or altitude. The content of vitamin C and E increases with needle age (Fig. 2, 3), very probably as a stress accumulation effect.

The antioxidants vitamin E and C have been identified as non-enzymatic protectors against peroxidation of lipids (KUNERT & EDERER 1985). It was reported that chlorophyll damage, a severe phytotoxic consequence of lipid peroxidation, is prevented by vitamin E. In the present studies, we found an increase of the vitamin E and C content in Norway spruce needles and we assume that enhanced age-related peroxidative events are limited by the higher amount of potent lipid-soluble vitamin E produced during aging. Both the amount and the concentration ratio of the anti-oxidants vitamin C and E and β-carotene are important factors that can determine the sensitivity of plants to peroxidation. A concentration ratio of vitamin C to vitamin E of about 10:1 to 15:1 was highly protective against oxidative damage (KUNERT & EDERER 1985). An excess of vitamin C is necessary to provide efficient regeneration of vitamin E. In our study this is the case at all sites. The ratio is close to 10 at highly polluted site Zavodnje, and below 10 at high elevation site Smrekovec, which is out of the inner emission area but more exposed to stresses typical for higher altitudes.

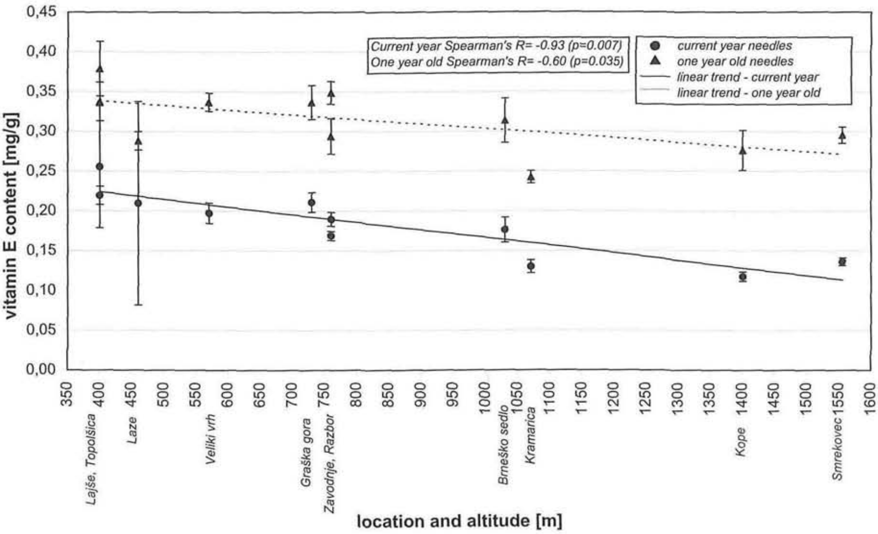

Fig. 3. Vitamin E content (mg/g) in needles sampled in September 1997.

Conclusions

All the biochemical stress physiological indicators in needles can be efficiently used for air pollution and other stress evaluation when compared to site characteristics and air quality. On the most polluted site the production of antioxidants is severely impaired. This can lead to damage, expressed first as chlorosis and necrosis of needles (defoliation!), and later to forest decline. Normally the concentration of antioxidants increases with altitude and when the opposite occurs as at sites close to the thermal power plant in Šoštanj, this is the consequence of air pollution. The response of Norway spruce to air pollution is not dependent solely on the concentration and duration of air pollution but also on all site dependent climate and soil characteristics which change with time and to biotic stress factors. Therefore we need to use as many stress indicators as possible to determine the action of stress and its effect on the vitality of trees.

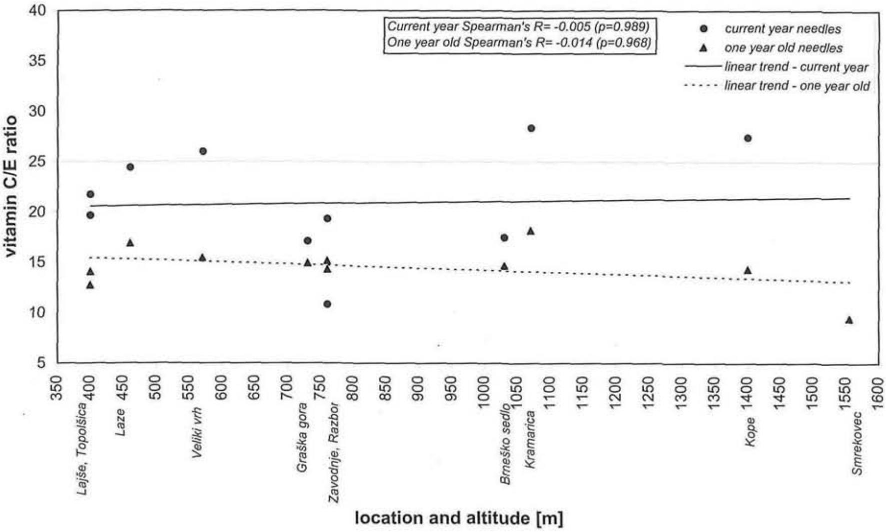

Fig. 4. Ratio between vitamins C / E in needles sampled in September 1997.

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