BIOINDICATION OF DIFFERENT STRESSES IN FOREST DECLINE STUDIES IN SLOVENIA

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Abstract. Determination of the stresses operating on a forest ecosystem demands the use of several bioindication methods. Air quality indicators were assessed from an inventory of forest decline based on the assessment of tree crowns and epiphytic lichens. Photosynthetic pigments, ascorbic acid and major macronutrients were studied in Norway spruce needles as indicators of physiological and biochemical stress. Analyses were carried out on selected forest plots in polluted areas (Zasavje district,vicinity of thermal power plants) and predominantly unpolluted areas (Triglav National Park, Julian Alps, Slovenia). For some bioindication methods, there was good agreement with measured air quality and climatological parameters. The best agreement was found between total foliar sulphur in needles and epiphytic lichens, especially in more polluted areas. Agreement with forest decline inventories and analyses of some needle stress physiological/biochemical parameters was less convincing. The strength of agreement was further decreased by soil characteristics and climatic parameters, influenced also by biotic parameters and forest stand history and management. It was concluded that there is no simple bioindication method available to evaluate the vitality of a forest.

Keywords: forest decline, stress, air pollution, bioindication, Norway spruce, photosynthetic pigments, ascorbic acid, macro nutrients, epiphytic lichens, Slovenia

1. Introduction

The concept of using bioindication and stress physiology to study forest decline has been promoted in Slovenia, and culminated in an international cooperative Tempus project dedicated specifically to bioindicators of forest site pollution (Kraigher *et al.*, 1996). In this paper two investigation areas are briefly presented, describing forest decline inventories which were later extended to include stress factors. Triglav National Park, representing a rather unpolluted area, was contrasted with the Zasavje district, historically one of the most air polluted regions in Slovenia due to mining and thermal power plant activity.

Photosynthetic pigments, ascorbic acid, thiols and plant hormone are often used as stress indicators for early diagnosis of disturbances in forest trees (Grill and Esterbauer, 1973; Darrall and Jäger, 1984; Wolfenden *et al.*, 1988; Pfeifhofer and Grill, 1989; Bermadinger *et al.*, 1989; Tausz *et al.*, 1996a). Combination of analyses of different stress physiological parameters in forest trees with air pollution measurements, analysis of mineral nutrition of forest trees and use of some other sensitive bioindicators like epiphytic lichens provide a more complete explanation of the process of forest decline (Tausz *et al.*, 1996b; Batič and Mayrhofer, 1996). The pattern of photosynthetic pigments in Norway spruce needles is widely used as an unspecific vitality indicator (Wolfenden *et al.*, 1988; Pfeifhofer and Grill, 1989). Analyses of thiols and macronutrients in combination with ascorbic acid analysis in needles can distinguish between stresses caused by air pollutants and those caused by natural phenomena like

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frost, drought, UV radiation etc. (Bermadinger *et al,*. 1989; Tausz *et al.*, 1996 b).

2. Material and methods

The Triglav National Park (TNP) is situated in the Julian Alps, in the western part of Slovenia. All forests are natural, or close to natural, due to forest management practice and inaccessible terrain. Norway spruce (*Picea abies* (L.) Karst.) is the commonest tree species, followed by the common beech (*Fagus sylvatica* L.), European larch (*Larix decidua* Mill.) and mountain pine (*Pinus mugo* Turra), which represents a shrub belt of "Krumholz" above the tree and forest limit.

The forest decline inventories were carried out in 1987, 1993 and 1995 (Šolar, 1989; Šolar, 1991; Gomišček, 1997) using the methodology developed by the ICP-Forest (Anonymous 1994), slightly modified by Slovenian foresters (Šolar 1991). Air quality was assessed at all forest decline inventory plots, using a very simple mapping methodology based on assessment of cover and frequency of major lichen thalli types, e.g. crustose, foliose and fructicose lichens (Batič and Kralj, 1989; Batič and Mayrhofe,r 1996). Subsequently, epiphytic lichen species were mapped along defined transects (Primožič, 1998; Surina, 1998).

Three transects, Trenta, Pokljuka and Kranjska gora were chosen, along which air pollutant concentrations were measured, and Norway spruce needles were sampled for physiological stress analyses. The transects were chosen in the south-western, eastern and northern parts of the park, representing major park forest types. Only results from the transects Kranjska gora (TNP Kranjska gora, 1A) and Trenta (TNP Trenta, 1B) are presented in this paper. Concentrations of SO_2 and NO_x were measured for two week periods using passive samplers during the growing season, from March to November. On the high plateau, Pokljuka, O_3, SO_2, NO_x, VOC and climatological parameters were analysed during campaigns lasting for one week in spring, summer and autumn. Foliar macro nutrients were measured (Simončič and Kalan, 1996), and photosynthetic pigments and ascorbic acid were analysed by HPLC (Pfeifhofer, 1989; Bermadinger *et al.*, 1990).

The contrasting, polluted area, the Zasavje district is situated in the middle of Slovenia. The main air pollutor is the thermal power plant in Trbovlje-TET (125 MW), which began operation in 1968. Due to its location in a deep and narrow valley, it caused serious air pollution in the 1960s when half hour concentrations of SO_2 reached as high as 20 mg m^{-3} in the most exposed localities. At that time forest damage was 10 fold higher than in other parts of Slovenia, and more than 84% of all damage was ascribed to air pollution (Šolar, 1989). Although Norway spruce is not an indigenous species in this district of Slovenia, it is very common due to historic forest practices, and after silver fir was most affected. Typical symptoms of damage were defoliation, chlorotic and necrotic needles, and changes in the branching system, all of which were included in the methodology of the assessment of forest decline (Šolar, 1989; Vidergar-Gorjup, 1998). Within this area 10 forest stands were selected according to forest damage level, topography and climatic circumstances. The forest decline inventory was carried out at the same time as in the TNP and the same measurements were made.

3. Results and discussion

Data obtained by measurements of climatological parameters are not presented and only selected air pollution values are presented. Further selected results include Norway spruce needle anlyses and epiphytic lichen mapping from both areas. Defoliation at the clean site (TNP) was lower than the national average 13 cf 22% respectively, indicating the healthy state of the park forests (Gomišček, 1997).

TABLE I

Concentrations of N, P and S (mg g^{-1} DW), N/P ratio and N/S ratio and common S content class in current year Norway spruce (*Picea abies* (L.) Karst.) needles sampled on the profiles TNP Kranjska gora (**1A**) and Trenta (**1B**) in autumn 1994 and Zasavje (**2**) district in autumn 1995. Data are the average of two sampled trees (K. Gora, Trenta) and three trees (Zasavje).

(1A) TNP Kranjska gora	altitude	N mg g^{-1}	P mg g^{-1}	N/P	S_{94} mg g^{-1}	N/S_{94}	S_{93} mg g^{-1}	S conent class S_{94+93}	No. of lichen species
Za žago	920	11.9	0.90	13.0	1.10	10.8	0.96	2	19
Greben 1	1020	9.5	0.77	12.0	0.89	10.7	1.12	1	28
Greben 2	1130	9.9	0.80	12.0	0.91	10.9	0.94	1	31
Plato	1250	10.5	0.79	13.0	0.85	12.4	0.83	1	42
Skalna glava	1360	9.9	0.84	12.0	0.86	11.5	0.94	1	37
Gozdna jasa	1450	11.6	1.38	8.4			0.98	1	37
Pod kurjim vrhom	1540	12.0	1.50	8.0	0.85	14.1	0.82	1	37

(1B) TNP Trenta	altitude	N mg g^{-1}	P mg g^{-1}	N/P	S_{94} mg g^{-1}	N/S_{94}	S_{93} mg g^{-1}	S content class S_{94+93}	No. of lichen species
Kamp korita	520	11.4	1.80	6.3	1.10	10.8	0.96	2	29
Pod vršičem	610	10.6	1.24	8.5	0.89	10.7	1.12	1	29
Za vršičem	700	11.7	1.36	8.6	0.91	10.9	0.94	1	75
Nad vršičem	800	11.6	1.00	11.6	0.85	12.4	0.83	1	40
Skokar	910	10.5	0.90	11.7	0.86	11.5	0.94	1	62
Ovinek	1000	13.1	1.61	11.3			0.98	1	16
Ob cesti	1100	9.5	0.99	9.6	0.85	14.1	0.82	1	16
Polog	1215	13.3	1.39	9.6					16
Glava	1215	11.6	0.92	12.6					16
Za glavo	1400	12.7	1.86	6.8					29
V Plazeh	1620	12.2	1.71	7.1					40

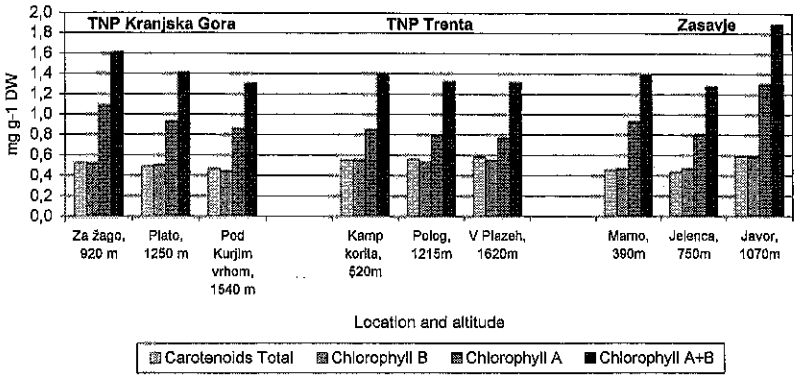
(2) Zasavje	altitude	N mg g^{-1}	P mg g^{-1}	N/P	S_{95} mg g^{-1}	N/S_{95}	S_{94} mg g^{-1}	S content class S_{95+94}	No. of lichen species
Ostenk	390	14.8	1.21	12.3	1.43	10.3	1.52	3	6
Marno	390	12.5	1.13	11.1	1.72	6.8	1.50	4	7
Ravenska	480	11.7	1.16	10.1	1.53	8.2	1.87	3	7
Retje	490	13.3	1.47	9.0	1.30	10.2	1.44	3	7
Kovk	590	14.3	1.55	9.2	1.64	8.7	1.90	4	5
Jelenca	750	13.2	0.85	15.5	1.07	12.3	1.10	2	23
Dobovec	790	12.4	1.06	11.7	1.65	7.5	1.61	4	5
Gore	880	13.5	1.03	13.1	1.31	10.3	1.24	3	21
Kljucevca	1070	14.4	1.57	9.2	1.13	12.7	1.19	2	19
Javor	1070	14.0	1.26	11.1	1.24	11.3	1.23	3	21

Concentrations of SO_2 and NO_x were low, especially in summer. At Trenta SO_2 reduces from 3.34 μg m^{-3} in the valley bottom (520m) to 2.2. μg m^{-3} at 1620m. Measured values for NO_2 for the same transect and period were from 2.7 μg m^{-3} in the valley and 0.80 μg m^{-3} at the highest sampling site. At Kranjska gora values for SO_2 decreased from 6.93 μg m^{-3} in the valley (920 m) to 1.10 μg m^{-3} at the highest sampling site (1540 m). Values for NO_2 were between 3.32 μg m^{-3} and 0.72 μg m^{-3} (Gomišček, 1997). Bulk N deposition (NH_4-N, NO_3-N) and S deposition (SO_4-S) based on measurements on Pokljuka plateau between 1996 and 1998 were between 10 and 12 kg N ha^{-1} yr^{-1} and 12 and 13 kg S ha^{-1} yr^{-1} (unpublished data, Simončič, Slovenian Forestry Institute), too small to damage the forest. However, measurements in open areas at low altitudes underestimate inputs to forests via occult deposition as can be seen from S analyses and the epiphytic lichens (Table 1 (1A)). O_3 concentrations (campaigns, Pokljuka) were relatively high (maxima 80-100 ppb), exceeding the threshold causing damage to sensitive crops and natural vegetation (40 ppb) (Gomišček, 1997). With the exception of some sites at lower altitudes at Trenta and Kranjska gora, due to pollution from the valley and transboundary sources, foliar S was low. N concentrations were just sufficient, and slightly increased in the needles from Trenta (Table 1 (IB)). The higher SO_2 concentration prevailing on the lower slopes could be seen in reduced number of epiphytic lichens particulary in Kranjska gora.

The age pattern of chlorophylls, the carotenoid content and their ratio reflect the SO_2 pollution and/or photooxidants and sampling altitude (Batič *et al.*, 1995; Bermadinger *et al.*, 1990; Grill *et al.*, 1979). Ascorbic acid content is a good indicator of oxidative stress in needles and also their reductive capability (Bermadinger *et al,* 1990). Its content in non polluted areas increases with altitude (UV light, O_3, drought, etc.) and is decreased by very high SO_2 pollution (Bermadinger *et al,*. 1989; Tausz *et al.* ,1996). The results from Triglav area are consistent with a non polluted situation. There was a slight decrease in chlorophyll, especially chlorophyll a, with sampling altitude (Figure 1), and an increase in ascorbic acid content, especially at Trenta (Figure 2), and in the Kranjska gora (Figure 2) in the TNP. Epihytic lichen populations reflect air pollutants earlier than spruce needles. The number of epiphytic lichen species in the south-west part of Julian Alps (transect Trenta) between 1000 and 1500 m, were drastically reduced and although no air pollution data is available (Primožič, 1998), occult deposition may be responsible (Gomišček, 1997).

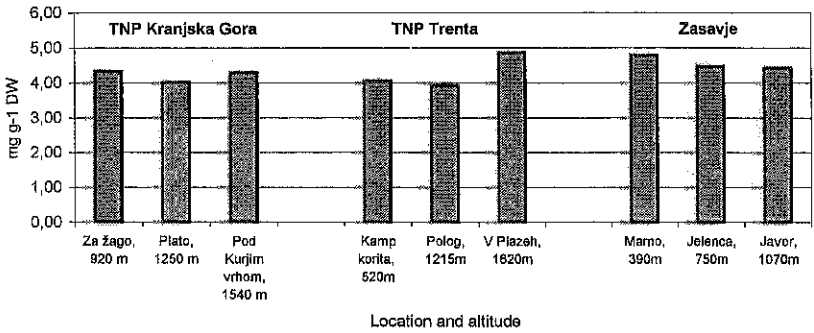
In the Zasavje district the concentrations of SO_2 were very high; NO_x and O_3 measured at Kovk exceeded the threshold values for sensitive vegetation. Bulk deposition in the open was 13 kg N ha^{-1} yr^{-1} and 25 kg S ha^{-1} yr^{-1} (Anon., 1998). At other sites in this area S deposition can exceed 30 kg S ha^{-1} yr^{-1} (Anon., 1998) and 83-100% of the assessed Norway spruce in damage classes 2-4 (Šolar, 1989). This damage can be largely ascribed to pollution from the thermal power plant and to the impact of SO_2 because almost all trees are grouped within classes 4 and 3 according to total foliar S concentrations (Table 1; II). Analyses of photosynthetic pigments and ascorbic acid gave quite different results in comparison with TNP (Figs. 1 and 2). Due to very high SO_2 concentrations in the valley the content of photosynthetic pigment increases with altitude while the ascorbic acid content decreases.

Fig.1 Concentration of Pigments in Needles (Average for 1992-95 Needle Age Classes)



The number of epiphytic lichen species found on all sites in Zasavje clearly decreased with decreasing altitude (Tables I and II). This trend was less obvious in TNP, although expressed in the same way.

Fig. 2 Concentration of Ascorbic Acid in Needles (Average for 1992-95 Needle Age Classes)



The forest decline inventories, epiphytic lichen mapping, air pollutant measurements and analyses of Norway spruce needles confirm that forests in the Triglav National Park are not very polluted by comparison with forests in the Zasavje region. The impact of air pollution in the TNP is small and of two kinds: the first, observed especially in the east-northly part (Kranjska gora) has come from the steel plant in Jesenice, manifest as elevated foliar S concentration and in lower photosysnthetic pigment and ascorbic acid content, especially in the valley bottom. The second is the impact of eutrophication, expressed as higher foliar N concentration, especially at Trenta, in the south-western part of the park. Between 1000 and 1500m die-back of sensitive macro lichens is common and increasing cover of algae and the pollution resistant lichen species *Scoliciosporum chlorococcum* is observed. Eutrophication most probably originates from long range transport of pollutants from the south-west, very probably from industrial north Italy, which is the prevailing wind direction.

At the Zasavje the small number of epiphytic lichens present are primarily

represented by species higly resistant to air pollutants (*Scoliciosporum chlorococcum, Lecanora conizaeoides, Lepraria* species) (Vidergar-Gorjup, 1998). Both, the epiphytic lichens and spruce needles have a high S content consistent with a low content of photosynthetic pigments and ascorbic acid. By comparison with the less polluted park the effects of altitude were negligible.

Comparison of the different bioindicator methods used in these two case studies (forest tree-defoliation, epiphytic lichens, S concentration, macronutrients, photosynthetic pigments, ascorbic acid) indicates that some of the responses could be explained and ascribed to the impact of SO_2 and to a lesser extent N deposition. However natural stresses (drought, light, biotic stress) may also affect forest health and their contribution could not be assessed by these methods.

References

Anonymous: 1994, Manual on methods and criteria for harmonize sampling, assessment, monitoring and analysis of the effects of air pollution on forests, 1-177, PCC WEST, BFH Hamburg, PCC East, Jiloviste-Strnady.

Anonymous: 1998, Air Pollution in Slovenia. Year 1996. Hydrometeorologica Institute of Slovenia, p 146, Ljubljana.

Batič, F. and Kralj, T: 1989, *Zbornik gozdarstva in lesarstva* **34**: 51-70.

Batič, F., Grill, D., Kalan, P. and Ribarič-Lasnik, C.: 1995, *Acta Pharm.* 45, 191-197.

Batič, F. and Mayrhofer, H.: 1996, *Phyton* **36**: (85)-(96).

Bermadinger, E., Grill, D. and Guttenberger, H.: 1989, *Phyton* **29**: (163)-(185).

Bermadinger, E., Guttenberger, H. and Grill, D.: 1990, *Environ. Pollut.* **68**, 319-330.

Darrall, N. M. and Jager, H. J.: 1984, *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M.J.Koziol and F.R. Whatley), 333-351. Butterworths, London.

Gomišček, S.: 1997, Onesnaženje zraka v Triglavskem narodnem parku in njen vpliv na gozd in vode.- Atmosfera in nacionalni park. Final Report, Contract No.: S2-6241, pp., 1-133. Ministrstvo za znanost in tehnologijo Republike Slovenije. Ljubljana.

Grill, D. and Esterbauer, H.: 1973, *Eur. J. For. Path.* **3**: 65-71.

Grill, D., Esterbauer, H. and Klosch, U.: 1979, *Environmental Pollution (Series A)* **17**, 187-194.

Kraigher, H., Batič, F., Guttenberger, H., Grill, D., Agerer, R. and Hanke, D. E.: 1996, *Phyton* **36**: 1-188,

Pfeifhofer, H., and Grill, D.: 1988, In: Fuhrer E., Neuhuber, F., *FIW- Symposium 1988*, Wien, 290-291.

Pfeifhofer, H.: 1989, *Eur.J. For. Path.* **19**: 363-369.

Primožič, K.: 1998, *Graduation Thesis*, 81 University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana.

Simončič, P. and Kalan, P.: 1996. *Phyton* 36(3): 81-84.

Surina, B.: 1998, *Graduation thesis*, 1-52. University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana.

Šolar, M.: 1989, *Zbornik gozdarstva in lesarstva (Ljubljana)* **34**, 71-97.

Šolar, M.: 1991, *Graduation thesis*, 1-150, Sveučilište u Zagrebu, Hrvatska.

Tausz, M., Batič, F., and Grill, D.: 1996a, *Phyton* **36**: 7-14.

Tausz, M., Grill, D., Guttenberger, H.: 1996b, *Phyton* **36**: 31-34.

Vidergar-Gorjup, N.: 1998, *Master of Science Thesis*, 170. University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana

Wolfenden, J., Robinson, D., Cape, J. N., Paterson, I. S., Francis, B. J., Mehlhorn, H., Wellburn A. R.: 1988, *New Phytol.* **109**: 85-95.