S32-006

Diagnosis Of Damage in Forest Trees by Integrated Biochemical Parameters

C Kurz, V Schmitt, A Wild

Institute of General Botany, Johannes Gutenberg University, D-55099 Mainz, Germany
fax: +49 (0)6131 3923958, email: kurz@mail.uni-mainz.de

Keywords: antioxidants, pigments, stress, bioindication

Introduction

For the last twenty years novel forest decline has increasingly become more significant.

The results of over ten years of novel forest decline research suggest that the above mentioned phenomenon could be addressed as a so-called complex disease, caused by different biotic and abiotic factors such as extreme weather conditions, pathogens like fungi or insects and anthropogenic pollution (Wild et al., 1996; Tausz et al.; 1999). According to the model by Ulrich, bioindication parameters can be used to deduce information about processes that are performed in living systems, to enable responses about plant vitality (Ulrich, 1991). While the annual forest damage surveys in several European countries chiefly use visual parameters like foliage loss and yellowing, biochemical parameters like components of the photosynthetic apparatus, antioxidants, phytohormons, phenolic compounds and parameters that relate to water stress may serve as damage indicators. These indicators enable necessary supplementary information about plant vitality that may lead to an early risk assessement (Wild et al., 1996; Ribarik-Lasnik et al., 1999; Tausz et al., 1999).

Although literature focuses on alteration of biochemical parameters, especially phenolic compounds, polyamines and antioxidants, suitable for diagnosis of tree damage, these studies have up to now focused on information of only one of the observed parameters. Due to the high variability of the data and the non-uniform assignation to different metabolic processes, a prognosis by only one parameter has scarcely any evidential value (Tausz et al. 1998). Therefore, based on estimated threshold values deduced from comparative data from well known sites and experiments under controlled conditions we created the so called biochemical damage index (BDI) which may provide a tool for a more detailed and sensitive diagnosis of novel forest decline research.

Materials and Methods

The investigation presented here was carried out during three vegetation periods (1993-1995) at ten differently damaged natural habitats of Norway spruce trees (*Picea abies* L.) and one location that was used as a reference in South Western Germany. The selection of the sites and trees was carried out according to the degree of defoliation. Six trees of each site were chosen for the assessment as representative for the site of observation.

Plant material

Studies were carried out with needles from spruces harvested at different chosen sites. South to south-west exposed twigs bearing the second needle generation were taken from the

sixth whorl. After separation the two- years-old needles were immediately frozen in liquid nitrogen and stored at -80°C.

Analysis of phenolic compounds, putrescine, proline, phosphoenol pyruvate carboxylase (PEPC) and chlorophyll.

The method for extraction and analysis of the mentioned compounds is described exhaustly by Wild et al., 1996.

Statistical Analysis

Analysis of variance (ANOVA) was achieved to determine the level of significance $P \leq 0.01^{*}$, $< 0.005^{**}$, $\leq 0{,}001^{***}$.

BDI

The BDI was deduced from the parameters under investigation. For each parameter three threshold values were defined as undamaged, slightly damaged, and severely damaged represented as 0, 1, and 2 respectively. The threshold values were sumed up and divided through the number of parameters evaluated (BDI = [Σ valuation of parameter] n^{-1}). For more detailed information see Wild et al., 1996.

Results

BDI

There is a clearly visible maximum value of the BDI for all years observed at the heavily damaged site 61. We may explain this as a result of high pollution rates, because of the location nearby the industrial area Mannheim Ludwigshafen (BDI 93 = 1,73 ± 0,46; 94 = 1,67 ± 0,45; 95 = 1,53 ± 0,48). As we supposed, the lowest BDI levels were calculated at the reference stand, followed by site 13 that showed the lowest degree of defoliation besides the reference site. Furthermore we observed a strongly significant correlation (P 0,01) between the BDI and the rate of defoliation 1993-1998 for all years, see Tab. 2; Fig. 1

Discussion

The results clearly document the influence of air pollutants on the mentioned biochemical parameters that are responsible for stressors evaluated in this study. By far the highest BDI values were observed at site 61. Concerning to this, the highest rates of defoliation also reached maximum levels at these site. Furthermore the BDI at site 61 in 1993 was as high as in the other years though the defoliation rate increased between 1993 and 1995. The air pollution regime for these sites documents an increase in air pollutants, especially ozone, which has been blamed as a major contributor to oxidative stress in plants (Foyer et al., 1994; Tausz et al., 1999). However SO_2 emissions in South West Germany are of little importance because of emission preventing management. As a response to air pollution, scientific literature focuses on alterations in the parameters that were used in this study to create the BDI.

Alterations of putrescine values accompanied by air pollutants are well documented in literature (Wild et al., 1996). The simultaneous increase in putrescine content accompanied by an increase in other components that are well documented according to oxidative stress may probably confirm their function as radical scavengers (Foyer et al., 1994). Further we observed low levels of pigment values at the highly-damaged sites. According to these observations high ozone values cause reduction in pigment values, accompanied by alterations in chloroplast proteins (Foyer et al, 1994). We also observed high levels of catechine, PEPC activity and Proline at the highly-damaged sites. This can be explained as a

response to oxidative water shortage srtess. However both air pollutants and water shortage accompanied by reduction in photosynthesis may lead to the production of active oxygen species (Wellburn et al., 1996).

Tab 1 Average values and standard deviation; *, **, *** represent significant differences (P< 0,1, 0,05, 0,01 respectively) at the chosen sites (1993-1995) according to ANOVA.

	sites	putrescine [nmol g^{-1} DW]	catechine [µmol g^{-1} DW]	PEPC activity [µmol g^{-1} h^{-1} DW]	proline [µmol g^{-} DW]	chlorophyll [mg g^{-1} DW]
1993	61	1657,049*** ± 468,412	58,725*** ± 15,567	57,515*** ± 12,795	1,781*** ± 1,499	1,537*** ± 0,780
	reference	400,367*** ± 117,069	39,960*** ± 9,060	15,964*** ± 6,867	0,1934*** ± 0,018	3,450*** ± 0,221
	others	750,653*** ± 432,764	38,948*** ± 11,292	21,344*** ± 9,328	0,362*** ±0,161	2,463*** ± 0,414
1994	61	1363,644*** ± 229,479	63,608*** ± 18,037	67,039*** ± 21,285	1,273*** ± 1,253	2,020*** ± 0,856
	reference	404,336*** ± 137,076	37,925*** ± 6,652	15,555*** ± 6,207	0,152*** ± 0,007	3,687*** ± 0,388
	others	875,603*** ± 544,648	48,868*** ± 9,195	20,475*** ± 9,646	0,310*** ± 0,072	2,406*** ± 0,509
1995	61	2073,563*** ± 441,991	49,263*** ± 13,171	76,838*** ± 15,536	0,615*** ± 0,205	2,402*** ± 0,849
	reference	421,334*** ± 146,862	41,090*** ± 6,132	28,080*** ±12,224	0,270*** ± 0,023	3,704*** ± 0,401
	others	803,449*** ± 487,400	37,465*** ± 9,873	21,380*** ± 10,023	0,635*** ± 0,558	2,572*** ± 0,396

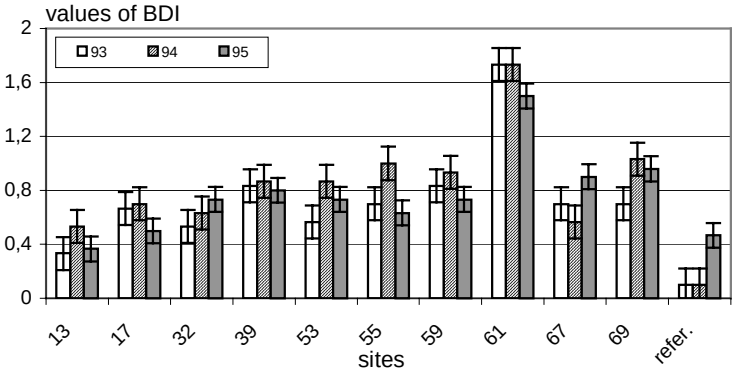

Fig. 1 Values of BDI; ref. = reference

Tab. 2 Levels of significance between the defoliaton rate and the BDI; *****, ****,** and ******* represent significant differences ($p \leq 0.05$; ≤ 0.01 and ≤ 0.001 respectively; DFP = rate of defoliation in %. according to Spearman

BDI		DFP	DFP	DFP	DFP	DFP	DFP
	average of	1993	1994	1995	1996	1997	1998
	defoliation	27,67	30,42	33,25	36,00	36,00	35,5
		± 11,33	± 9,54	± 9,33	± 13,17	± 10,80	± 12,24
1993	coeff of	0.331**	0.416***	0.435***	0.404***	0.502***	0.346**
	correlation	0,007	0,001	0,000	0.001	0.000	0.010
	niveau of signif.						
1994	coeff of		0.425***	0.434***	0.422***	0.518***	0.314*
	correlation		0.000	0.000	0.000	0.000	0.021
	level of signif.						
1995	coeff of		0.394***	0.406***	0.397***	0.493***	0.403**
	correlation		0.001	0.001	0.001	0.000	0.003
	level of signif.						

References

Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. *Physiologia Plantarum* **92**, 696-717.

Ribaric-Lasnik C, Tark B, Batic F, Grill D (1999) Antioxidants in Norway spruce needles at field plots in the vincinity of a thermal power plant in Slowenia. *Phyton-Annales Rei Botanicae* **39 (4),** 253-268.

Tausz M, Bytnerowicz A, Weidner, W, Arbaugh MJ, Padgett P, Gril D (1999) Changes in free radical scavengers describe the Susceptibility of Pinus Ponderosa to ozone in Southern Californian forests. *Water Air and Soil Pollution* **116 (1-2),** 249-254.

Tausz, M, Jimenez MS, Grill D (1998) Antioxidative defence and Photoprotection in Pine needles under field conditions. A multivariate approach to evaluate patterns of physiological responses of natural sites. *Physiologia Plantarum* **104 (4),** 760-764.

Ulrich B (1991) Folgerungen aus zehn Jahren Waldökosystem- und Waldschadensforschung. *Forst und Holz* **46**, 3-12.

Wellburn FAM, Lauk KK, Milling PMK et al. (1996) Drought and air pollution affect nitrogen cycling and free radical scavenging in Pinus Halepensis. *Journal of Experimental. Botany* **47**, 1361-1367.

Wild A, Schmitt V, Eis U, Strobel P, Wilksch W, Wohlfahrt S, (1996) Okulare und biochemische Schaddiagnose bei Fichten und Weißtannen *Forschungsbericht Forschungszentrum Karlsruhe Projekt "Europäisches Forschungszentrum für Maßnahmen zur Luftreinhaltung"* **149**.