# Phenolic Compounds as a Tool of Bioindication for Novel Forest Decline at Numerous Spruce Tree Sites in Germany

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Within a project that applied biochemical criteria to the diagnosis of damage to Norway spruce, 43 sites in western and eastern Germany showing only moderate tree damage were screened for the amounts of methanol soluble phenolic compounds in spruce needles. The concentrations of most of the main compounds – especially catechin – positively correlated with needle loss and the altitude of the site. It was also found that it is necessary to differentiate between trees younger and older than 60 years of age. The correlations between the increase of the phenolic compounds studied and the needle loss or the damage class are stronger in the younger trees, possibly implicating differences in metabolic state or disturbances in protectective mechanisms in the older trees.

## Introduction

Bioindication studies can be started from different approaches, e.g. cell, organism, and ecosystem. The annual forest damage survey in Germany evaluates visible symptoms like loss of foliage and yellowing and in this way considers the whole organisms. Biochemical indication refers to events on a cellular level thus providing necessary additional information. As physiological changes in response to stress can be discovered before symptoms become visible on the whole organism, this opens the possibility to diagnose an early impairment of the vitality of a tree.

Over the last years much research was aimed at physiological parameters suitable for diagnosis of tree damage (e.g. Wild *et al.*, 1990; MURL, 1993; Wild *et al.*, 1994; KfK-PEF, 1994; Wild *et al.*, in press). Among various physiologically reactive parameters meeting the necessary requirements for biomarkers (Tenter and Wild, 1991; Tietz and Wild, 1991; Wild and Schmitt, 1994), the amounts of various methanol soluble phenolic compounds – especially catechin – in spruce needles of the second age class proved a useful indicator because they increased with damage, were constant during the course of the day and the year, and their absolute amounts were quantitatively comparable between different sites (Richter and Wild, 1992, 1994). These observations agree with earlier findings of increased biosynthesis of phenolic compounds in response to various kinds of stress (Howell, 1974; Tingey *et al.*, 1976; Rubin *et al.*, 1983; Kicinski *et al.*, 1988).

Within an extensive research project on various appropriate cellular parameters like phosphoenolpyruvate carboxylase, mineral nutrients, components of the thylakoid membrane, antioxidants, and polyamines and the evaluation of their bioindicative potential, a great number of forest stands mainly in the subalpine mountain ranges in western and eastern Germany was screened for the mentioned phenolic compounds. This paper presents correlations found between various phenolic compounds and visible symptoms of damage, thus providing a tool towards a more sophisticated damage diagnosis.

## Materials and Methods

### *Description of the sites*

Needles were taken from spruce trees of 43 sites in various regions of Germany. Twelve sites are located in eastern Germany, in the Harz Mountains, the Thuringian Forest, and in the Ore Mountains at altitudes between 500 and 750 m above sea level. The trees were moderately damaged. Their needle losses amounted to 20–40%, and for each

*Abbreviations:* DW, dry weight; PTG, piceatannol glucoside.

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site average damage classes from 1.3 to 1.9 were calculated (for damage classes see Waldschadenserhebung, 1991, 1992). Only the trees of two sites were assigned to average damage classes below 1. The age of the chosen trees ranged between 55 and 90 years. The soils of most sites are poor in nutrients, and the main air pollutant at these sites has been sulfur dioxide (Lauchert, 1994).

The twelve sites studied in Rhineland-Palatinate (Rheinland-Pfalz) are found in the Palatinate Forest, the Hunsrück Mountains, the Westerwald Mountains, and in the Eifel Mountains at altitudes between 450 and 700 m above sea level. The age of the trees ranged between 70 and 120 years. Needle losses from 9 to 23% were determined resulting in average damage classes from 0.2 to 1.5 for the sites. Apart from a reference site showing almost no damage symptoms, most soils are poor in nutrients (Lauchert, 1994).

Nineteen sites were studied in Northrhine-Westphalia in the Eifel, Rothaar, Ebbe, and Egge Mountains and in the Sauerland at altitudes from 200 to 610 m above sea-level. According to an average damage class assignment of 0–0.8, the 40–90-year-old trees were only slightly damaged (needle loss 0–37%). At these sites nutrient deficiencies were also detected (Wild *et al.*, 1994). The sites in western Germany are characterized by elevated ozone concentrations in the ambient air (ZIMEN, 1991; TEMES, 1991).

Sampling was carried out from the end of August to the beginning of September 1991. Needles of the second age class (i.e. sprouted in the previous year) were taken from twelve trees at each site (six trees in Northrhine-Westphalia) and were combined to bulk samples for each site. Needles were frozen in liquid nitrogen immediately at the site and stored at -75 °C.

### *Analysis of phenolic compounds*

Aqueous methanolic extracts (1 g/ml) containing 0.9 mM gallic acid as internal standard were prepared from deep-frozen needles according to Richter and Wild (1992, 1994). The extracts were purified by filtration and solid phase extraction. The analysis of phenolic compounds was carried out by means of reversed-phase HPLC (LKB, column RP-18 Macherey-Nagel, Düren) using a four-step elution gradient composed of 0.5% natrium acetate buffer (pH 3.3) and methanol, running from 17% to 80% methanol. The elutes were detected and quantitatively determined by measuring UV-absorption at $\lambda = 275$ nm (Shimadzu). For details see Richter and Wild (1992). From each needle sample three extracts were prepared each of which was chromatographed twice.

Fig. 1 shows two representative chromatograms of the extracted phenolic compounds derived from sites in the Rothaar Mountains (Northrhine-Westphalia) and in the Thuringian Forest, respectively.

## Results

The chromatograms obtained in this study show similar patterns of methanol-soluble phenolic compounds in spruce needles for all sites studied in the various regions of Germany. In Fig. 1 examples from two distant regions with even different air pollution profiles are presented. The main compounds detected in significant quantities, which were used in calculations, are picein, catechin, epicatechin, piceatannol glucoside, and two other compounds (retention times 5.5 and 9.1 min).

In relation to needle loss and damage class, the latter of which is based on the former, the amounts of catechin, epicatechin, and the two still unidentified compounds (see Richter and Wild, 1992) increase with increasing damage (Tables I and II, Fig. 2 a). This was not found for picein (Tables I and II). The sum of all studied phenolic compounds, however, also shows a positive correlation

Table I. Coefficients for correlations between the content of phenolic compounds and various parameters based on results obtained from 43 sites (significance levels: * < 0.01; ** < 0.001). Unidentified compounds are characterized by their retention times.

| Compound/parameter | Needle loss | Damage class | Altitude |
|---|---|---|---|
| Picein | 0.3999 | 0.3486 | 0.4402* |
| Peak 5.5' | 0.5649** | 0.5454** | 0.5950** |
| Catechin | 0.6011** | 0.6156** | 0.7425** |
| Peak 9.1' | 0.5293** | 0.5339** | 0.6838** |
| Epicatechin | 0.5364** | 0.5531** | 0.6424** |
| PTG | 0.2541 | 0.2779 | 0.5677** |
| Sum | 0.5638** | 0.5767** | 0.7264** |
| Sum minus picein | 0.5922** | 0.6047** | 0.7616** |

Table II. Coefficients for correlations between the content of phenolic compounds and needle loss in relation to the age of the trees (for further explanations see Table I).

| Compound/age | All sites | < 60 Years $n$=15 sites | > 60 Years $n$=28 sites |
|---|---|---|---|
| Picein | 0.3999 | 0.3080 | 0.4087 |
| Peak 5.5' | 0.5649** | 0.7784** | 0.4122 |
| Catechin | 0.6011** | 0.7846** | 0.4645* |
| Peak 9.1' | 0.5293** | 0.6182** | 0.4742* |
| Epicatechin | 0.5364** | 0.8017** | 0.3523 |
| PTG | 0.2541 | 0.2984 | 0.1936 |
| Sum | 0.5638** | 0.6713** | 0.5059* |
| Sum minus picein | 0.5922** | 0.7576** | 0.4640* |

with the damage symptoms (Tables I and II, Fig. 3a). With regard to parameters other than damage symptoms, all studied phenolic compounds – apart from picein – increase with the altitude of the site (Table I, Figs. 2 b and 3 b). The presented correlation analysis is based on the results of all 43 studied sites. When regarding each region independently, less and weaker correlations of single phenolic compounds with the considered parameters could be found (data not shown).

Closer examination revealed that the correlation between the studied phenolic compounds and needle loss is linked to the age of the trees, while age itself is not. Table II demonstrates that below the threshold of an age of 60 years there is a stronger correlation between the amounts of most of the phenolic compounds studied and needle loss than if integrating all trees. Older trees show almost no correlations.

## Discussion

The pattern of methanol soluble phenolic compounds in spruce needles at all sites described here agree well with earlier published results for sites in the Black Forest, the Hunsrück and the Westerwald Mountains (Richter and Wild, 1992, 1994).

The screening of 43 spruce tree sites showing slight to moderate tree damage resulted in positive linear correlations between most studied phenolic compounds and needle loss or damage class as well as the altitude of the site.

In relation to visible damage, which is expressed as needle loss and damage class, the amounts of catechin, epicatechin, and two still unidentified compounds increase with increasing damage. This

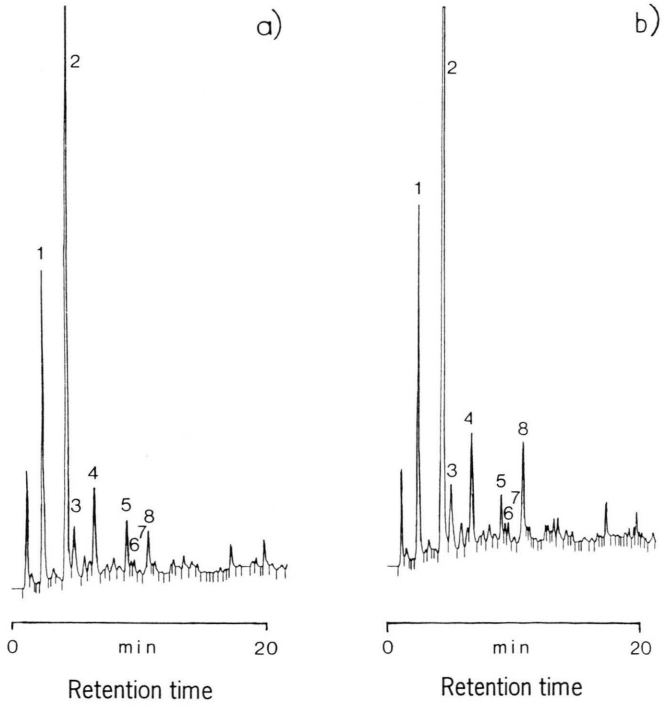


Fig. 1. Chromatograms of phenolic compounds from spruce needles sampled at a) Hilchenbach (Rothaar Mountains; average needle loss 3%), and b) Großbreitenbach/Altenfeld (Thuringian Forest; average needle loss 38%). 1 gallic acid (internal standard), 2 picein, 3 "peak 5.5'", 4 catechin, 5 "peak 9.1'", 6 epicatechin, 7 pHAP, 8 piceatannol glucoside.

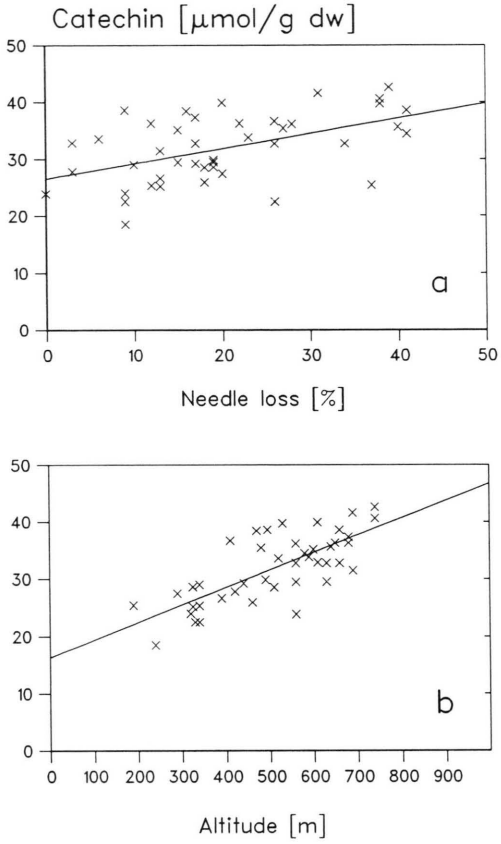


Fig. 2. Relations of **catechin** content to needle loss (a), altitude of the site (b). Mean values for each of the 43 sites are given.

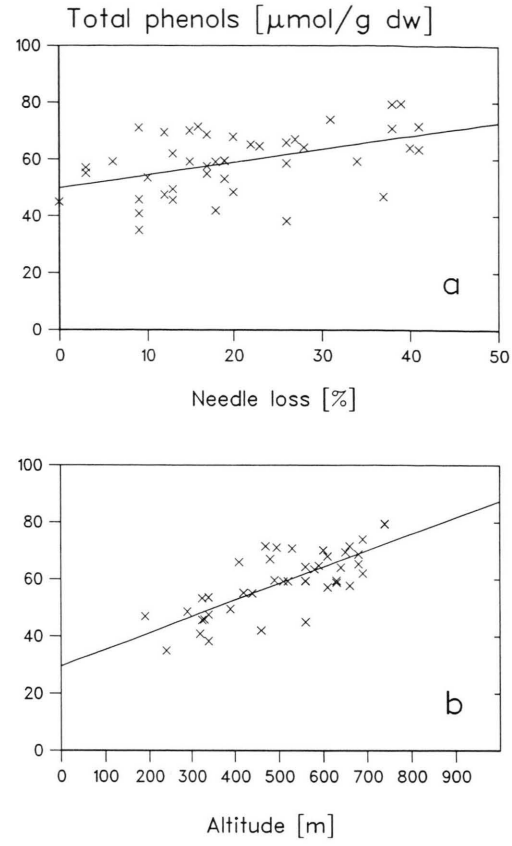


Fig. 3. Relations of the content of all studied phenolic compounds (sum of the values) to needle loss (a) and altitude of the site (b). Mean values for each of the 43 sites are given.

confirms our results obtained in studies at three other sites (Richter and Wild, 1992 and 1994). As before, this correlation could not be determined for picein because quantities were again inconsistent between different sites. Earlier findings also demonstrate that amounts of picein vary greatly among individual trees (Osswald and Benz, 1989, Heller *et al.*, 1990; Richter and Wild, 1992, 1994). Piceatannol glucoside, an important stilbene in spruce, could not be correlated with damage in these studies, either, which can be explained by individual variations between single trees (Solhaug, 1990; Richter and Wild, 1994). In our previous studies, where we did observe a clear increase in the content of PTG, we dealt with severely damaged trees (Richter and Wild, 1992), while in this project we included only trees with slight to moderate damage. However, the sum of all phenolic compounds studied, which also includes the components showing no distinct linkage to damage, shows a positive correlation with damage symptoms. This again confirms our previous observations and those of other authors (Yee-Meiler, 1974; Kicinski *et al.*, 1988; Richter and Wild, 1992, 1994) and adds to the concept of enhanced protection mechanisms in stressed and damaged trees (Swain, 1977; Rhodes, 1985).

For the first time we studied the amounts of phenolic compounds in trees older than 60 years. The results show that the damage-related increase of phenolic compounds in needles is not as obvious in trees older than 60 years as it is in the younger trees. Reasonable explanation may be found in damage independent needle loss due to hormonal changes (Elstner and Osswald, 1984), or in loss of vitality of the older trees affecting the biosynthesis

of protectants, or in an increased need of phenolic compounds as precursors for following reactions like lignin biosynthesis (Rhodes, 1985; Freytag and Hahlbrock, 1992). In any case, the described observations must be taken into account when attempting to assess the physiological damage status of spruce trees.

Apart from picein, all studied phenolic compounds additionally increase with the altitude of the site. Heller *et al.* (1991) and Ziegler and Kraus (1989) also observed an increase of various phenolic compounds in spruce needles in relation to the altitude of the site. This may be related to light conditions, like greater intensities of UV-radiation in higher altitudes. Several authors (Caldwell *et al.*, 1983; Flint *et al.*, 1985; Weissenböck *et al.*, 1986) reported on stimulations of biosynthetic enzymes and about accumulations of phenolic compounds, especially flavonoids, in various plant species in relation to increased doses of UV-light. Mainly accumulated in the epidermis, these UV-absorbing substances act as internal filters against the damaging radiation. A connection between these phenomena and damaged-related accumulation of most studied phenolics may be found in the fact that high light intensities do represent additional stress for trees weakened by anthropogeneous and/or other stress factors (Wild, 1987).

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