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Manganese-induced physiological iron deficiency in Douglas fir.

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Introduction

Since the middle of the sixties, an apparently new disease affecting Douglas fir (*Pseudotsuga menziesii* var. *viridis*) is observed in SW-Germany (Schöne 1987). Symptoms of this disease, which is obviously not primarily caused by fungal or other pathogens, are needle chlorosis, crown thinning, internal bark necrosis, stem resin efflux, and growth anomalies. Based on soil and needle analyses, an excessive uptake of manganese is discussed as the main cause of the disease. Because the patterns of needle chlorosis in affected trees are quite similar to an iron deficiency chlorosis, a close examination of the iron balance seemed to be of great interest. For comparison, trees from healthy and diseased natural Douglas fir were studied. The investigation also included a Douglas fir stand on calcareous soil, showing symptoms of a 'true' lime-induced iron deficiency. The research in the presented poster focuses on manganese and iron content, iron-dependent biochemical parameters as well as chloroplast ultrastructure.

Materials and methods

The three different forest sites under study are located in the Eifel mountain area (380-520 m a.s.l.) in SW Germany. Each 8-10 about 50-year-old Douglas fir trees were selected and grouped into the following respective variants:

Table 1: An overall view of the Douglas fir tree variants under study.

R	'reference trees'; undamaged trees from an apparently undamaged site
C	'negative reference trees'; trees growing on calcareous soil, showing 'true' iron deficiency chlorosis
G	symptome-free trees from a site with trees showing 'new' damage symptoms
Fe	damaged trees from a site with trees showing 'new' damage symptoms
FeP	damaged trees from a site with trees showing 'new' damage symptoms, supplied with 'Promi-Ferro' iron capsules as stem implantates for iron fertilization

From every Douglas fir, needles of the current, second and third needle generation were separately taken from twigs of the 7th whorl in October 1995 and October 1996. The R- and C-variant were harvested in 1996 only. For needle element analysis, oven-dried needles were ground to powder and decomposed with nitric acid. Element analyses including manganese and iron were carried out in an ICP-AES analyzer. Soluble iron was extracted from the needles with citrate buffer according to Booß et al. (1983). Ferrous and ferric iron were determined spectrophotometrically using bathophenanthrolin as chelating agent (Pollock and Miguel 1967). Chlorophyll was extracted from the needles with dimethylsulfoxide (DMSO) and measured spectrophotometrically according to Hiscox and Israelstam (1979). Catalase activity was assayed on basis of oxygen evolution after addition of hydrogen peroxide to needle extracts using a Clark type oxygen electrode. For the study of the ultrastructure of Douglas fir needle chloroplasts, needles sections were prepared for transmission electron microscopy according to Forschner et al. (1989).

Results

Douglas fir trees showed needle age-dependent increasing amounts of manganese (cf. fig. 1). The manganese content was extraordinary high in trees growing on the site showing symptoms of ‘new’ Douglas fir damage, particularly in visually damaged trees (variants Fe and FeP). According to Mengel (1991), needle manganese contents exceeding 3.000 ppm are considered as toxic. In contrast, the trees of the C variant growing on calcareous soil showed very low levels of manganese.

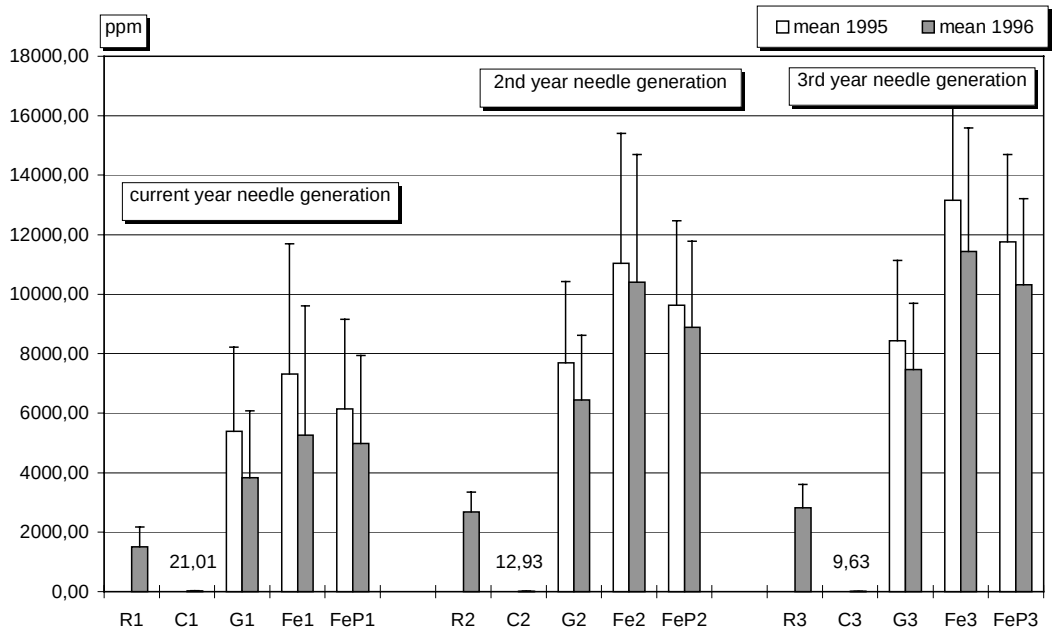

Fig. 1: Manganese contents in [ppm] in needles of five Douglas fir sample variants (3 needle generations). Arithmetic means and standard deviations of 8-10 trees per variant.

The findings regarding the iron content are summarized in table 2. The total iron contents showed, like manganese, an needle age-dependent increase. The undamaged reference trees (var. R) had higher iron contents as compared to the trees growing on the site showing ‘new’

damage symptoms (vars. G, Fe and FeP). However even the trees of the var. C had iron contents above the value of 20-30 ppm which is regarded as iron deficiency level (Mengel 1991). Therefore, total iron levels solely did not point to iron deficiency. In order to detect a potential 'physiological' iron deficiency, the different oxidation stages of soluble iron (ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+})) were studied. While the ferrous iron contents did not vary much between the variants, the amounts of ferric iron showed a considerable difference, with high amounts in undamaged (var. R) and symptom-free (var. G) trees, respectively.

Because of the dependency of chlorophyll biosynthesis and haemin formation on iron both, chlorophyll content and catalase activity were considered as presumable indicators of a 'physiological' iron deficieny.

Table 2: Contents of total iron, soluble iron, Fe^{2+} and Fe^{3+} contents in [ppm] in three subsequent needle generations of five Douglas fir sampling variants, arithmetic mean ± standard deviation. The results of the year 1996 are shown.

Needle age	**Variant**				
Current year	**R**	**C**	**G**	**Fe**	**FeP**
total Fe	134,4 ± 30,1	84,5 ± 21,2	96,9 ± 35,6	74,6 ± 35,2	70,9 ± 35,0
soluble Fe	38,1 ± 7,2	25,5 ± 9,1	39,8 ± 6,8	25,3 ± 4,5	23,0 ± 4,4
ferrous	13,6 ± 5,4	10,8 ± 6,2	9,9 ± 4,1	13,5 ± 5,6	6,6 ± 3,6
ferric	24,5 ± 6,3	14,7 ± 8,9	29,9 ± 8,0	11,9 ± 5,9	16,4 ± 4,3
2nd year	**R**	**C**	**G**	**Fe**	**FeP**
total Fe	143,5 ± 29,7	105,2 ± 20,4	125,5 ± 35,7	116,9 ± 33,9	109,6 ± 34,2
soluble Fe	50,0 ± 11,5	29,5 ± 10,0	44,7 ± 10,6	30,8 ± 6,7	26,2 ± 3,4
ferrous	15,1 ± 6,4	19,2 ± 5,9	11,1 ± 5,4	13,3 ± 5,9	7,5 ± 6,7
ferric	32,9 ± 12,7	10,3 ± 6,3	33,6 ± 8,9	17,5 ± 5,1	18,7 ± 2,6
3rd year	**R**	**C**	**G**	**Fe**	**FeP**
total Fe	180,3 ± 31,6	106,6 ± 20,2	152,3 ± 36,1	135,0 ± 33,6	129,9 ± 35,1
soluble Fe	36,3 ± 9,7	30,6 ± 7,8	47,7 ± 17,9	31,3 ± 3,2	30,1 ± 5,6
ferrous	13,2 ± 7,8	12,6 ± 8,2	10,4 ± 4,7	13,7 ± 6,5	11,6 ± 5,3
ferric	23,1 ± 13,3	18,0 ± 3,8	37,3 ± 18,0	17,6 ± 5,6	18,5 ± 3,1

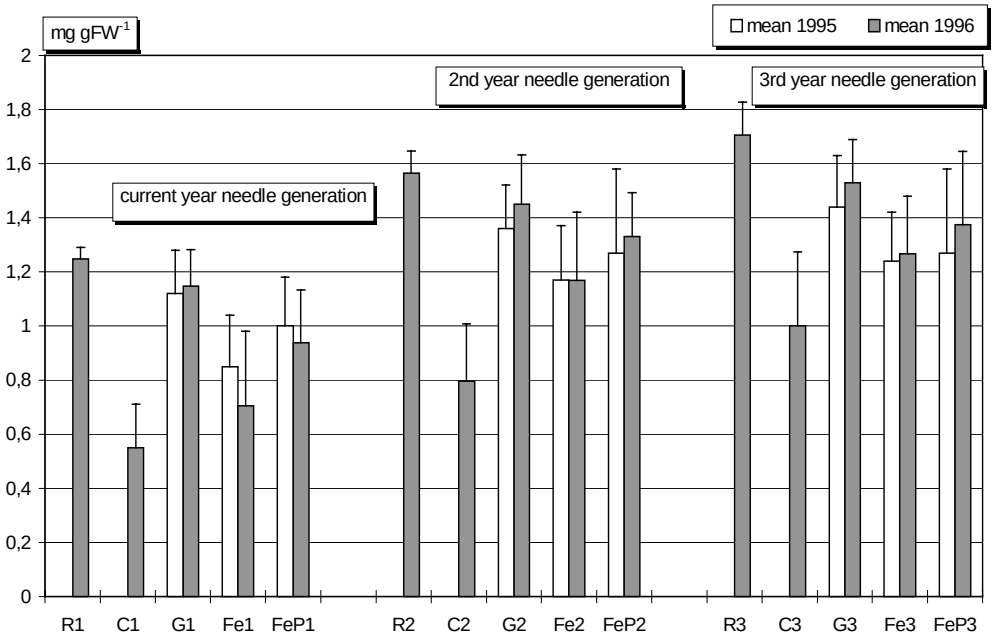

Fig. 2: Chlorophyll content in [mg g FW^{-1}] of five Douglas fir sample variants (3 needle generations). Arithmetic means and standard deviations of 8-10 trees per variant.

The mean chlorophyll contents of the trees showing 'new' damage damage symptoms (vars. Fe and FeP) ranked clearly below both, the symptom-free trees from the same site (var. G) and the undamaged reference trees (cf. fig. 2). By far the lowest chlorophyll levels were noticed in trees with presumable 'true' iron deficiency chlorosis (var. C).

The activity of the haemin-containing enzyme catalase was measured in the 1996 investigation period (data not shown). In all variants, catalase activity based on needle fresh weight increased with needle age. A marked difference between the variants was noted especially in the current-year needles, with the highest mean activity in the needles of the R variant and lowest mean activities in the Fe and FeP variants.

Because of ca. 75% of the total iron content beeing located in the chloroplasts, their ultrastructure was studied in comparison. Needles of the reference trees showed chloroplasts with a well-developed thylakoid system, few plastoglobuli and only little starch grains. Damage to chloroplasts manifested itself in rounding of the chloroplast shape, swelling as well as reduction in number of the thylakoid membranes and an increase of the plastoglobuli in size and number. Damage symptoms in the current-year needle generation were pronounced already in needles of the variants C, Fe and FeP, while variant G occupied an intermediate position. Additionally, huge starch grains were noted in needles of the variant C. Heavy deposition of electron-dense material was noted in the cell walls of 2^{nd} and 3^{rd} year needle generation of the Fe and FeP variants.

Discussion

The presented data support the hypothesis, that the 'new' damage to Douglas fir is chiefly caused by manganese surplus and a coincident 'physiological' iron deficiency. While the total

iron content seems not suitable for the indication of iron deficiency, the soluble ferric iron content may serve as marker. Damage to the trees was accompanied by low contents in ferric iron. This result was quite surprising, because several authors postulated on the contrary a manganese-induced decrease in ferrous iron, which is considered the physiologically active form (e.g. Olsen et al., 1982). Furthermore, decreased chlorophyll contents as well as decreased catalase activities reflect disturbances in the iron economy (Iturbe-Ormaetxe et al. 1995).

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