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Antioxidants and Manganese Deficiency in Needles of Norway Spruce (*Picea abies* L.) Trees[1]

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ABSTRACT

Chlorotic and green needles from Norway spruce (*Picea abies* L.) trees were sampled in the Calcareous Bavarian Alps in winter. The needles were used for analysis of the mineral and pigment contents, the levels of antioxidants (ascorbate, glutathione), and the activities of protective enzymes (superoxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate radical reductase, dehydroascorbate reductase, glutathione reductase). In addition, the activities of two respiratory enzymes (glucose-6-phosphate dehydrogenase, NAD-malate dehydrogenase), which might provide the NADPH necessary for functioning of the antioxidative system, were determined. We found that chlorotic needles were severely manganese deficient (3 to 6 micrograms Mn per gram dry weight as compared with up to 190 micrograms Mn per gram dry weight in green needles) but had a similar dry weight to fresh weight ratio, had a similar protein content, and showed no evidence for enhanced lipid peroxidation as compared with green needles. In chlorotic needles, the level of total ascorbate and the activities of superoxide dismutase, monodehydroascorbate radical reductase, NAD-malate dehydrogenase, and glucose-6-phosphate dehydrogenase were significantly increased, whereas the levels of ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase, and glutathione were not affected. The ratio of ascorbate to dehydroascorbate was similar in both green and chlorotic needles. These results suggest that in spruce needles monodehydroascorbate radical reductase is the key enzyme involved in maintaining ascorbate in its reduced state. The reductant necessary for this process may have been supplied at the expense of photosynthate.

In an oxygen-containing atmosphere, the formation of toxic oxygen species such as O_2^- and H_2O_2 is a potential threat to cellular constituents (6). To prevent oxidative damage, plant cells are equipped with a scavenging system consisting of low molecular weight antioxidants and protective enzymes that operate in the following pathway: radicals are removed by superoxide dismutases (EC 1.15.1.1) (15). The product of this reaction, H_2O_2, can be detoxified with a specific peroxidase oxidizing ascorbate to the free radical (EC 1.11.1.11) (5). Ascorbate-free radicals can be reduced enzymically by a monodehydroascorbate radical reductase (EC 1.1.5.4) utilizing NADPH as reductant (1, 10) or can dismutate spontaneously to yield ascorbate and dehydroascorbate (2). The reduction of dehydroascorbate to ascorbate is achieved by glutathione in a nonenzymic or enzymic reaction catalyzed by dehydroascorbate reductase (EC 1.8.5.1). Glutathione disulfide formed in this reaction is reduced by glutathione reductase (EC 1.6.4.2) at the expense of NADPH (9). The functioning of this pathway depends on reducing power that can be supplied directly via light-driven electron transport reactions in the chloroplast or supplied via secondary enzymic activities, such as glucose-6-phosphate dehydrogenase (EC 1.1.6.49) and NAD-malate dehydrogenase (EC 1.1.1.37).

Under an increased oxidative stress that is encountered, for instance, at low temperatures in combination with high light intensities, adjustments of enzymic activities and antioxidant levels have been observed in herbaceous plants such as cold-acclimated spinach (22) and pea leaves (32), as well as in conifer needles in winter (7). Additional stress factors such as nutrient deficiencies can modulate these responses. In leaves of Mg-deficient beans the levels of glutathione, ascorbate, superoxide dismutase, ascorbate peroxidase, and glutathione reductase were enhanced under high light intensities as compared with Mg-sufficient plants (4). In addition, high light intensities enhanced chlorosis in leaves of Mg-deficient plants (14).

In spruce needles, chlorotic symptoms were also often correlated with nutrient deficiencies, *e.g.* with Mg deficiency when older needle age classes were chlorotic (30) and with Mn and/or K deficiency when younger needles were affected (3). It was reported that chlorotic spruce needles contained higher ascorbate and glutathione levels than green needles (18). In a spruce forest in the Calcareous Alps (Bavaria, Federal Republic of Germany), we observed chlorotic symptoms in the youngest needles that were most severe at sun-exposed sides during winter. Because this observation suggested a link between oxidative stress, needle chlorosis, and nutrient deficiencies, we investigated chlorotic needles during two winter periods with the following objectives: (a) whether or not needle chlorosis was correlated with nutrient disorders and (b) how nutrient status correlated with the antioxidant system.

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MATERIALS AND METHODS

Plant Material and Site Characteristics

Chlorotic and green needles were obtained from about 100-year-old spruce (*Picea abies* L., Karst.) trees grown at two field sites, Kramer and Katzenstein mountains, respectively. Most spruce trees grown at the edge of a forest at the foot of Kramer mountain showed chlorotic symptoms. The chlorosis was more prevalent during winter than summer. Chlorotic symptoms were not observed in spruce trees at the foot of Katzenstein mountain. The two sampling sites were located in the Calcareous Alps at a distance of about 2000 m at valley level (approximately 800 m above sea level) near Garmisch-Partenkirchen (Bavaria, Federal Republic of Germany). Climatic data and concentrations of air pollutants were obtained from a meteorological station (Fraunhofer Institut, Garmisch-Partenkirchen) located at a distance of about 500 m from the Katzenstein site and about 2000 m from the Kramer site. During the period of needle emergence and growth, the average pollutant concentrations were about 30 nL L^{-1} O_3, 2 nL L^{-1} NO_x, and 5 nL L^{-1} SO_2 (R. Sladkovic, personal communication). In January 1990 and in February 1991, when samples were taken, the respective average air pollutant concentrations (O_3, 14.4 and 20.1 nL L^{-1}; SO_2, 4.1 and 5.4 nL L^{-1}; NO_x, 17.7 and 11.7 nL L^{-1}) and climatic data (average monthly temperatures, −3.5 and −3.7°C; sum of monthly precipitation, 31 and 12 mm) were similar, except for global radiation (1229 and 2165 W m^{-2} d^{-1}) (R. Sladkovic, personal communication).

Sampling Conditions

During the winters of 1990 and 1991, needles formed in 1989 and 1990 were obtained from eight and seven spruce trees grown at Kramer and Katzenstein, as indicated in Table I. Small twigs were collected between 8:00 and 8:30 AM from southerly branches approximately 2 m above ground. The material was transported to the laboratory within 15 min and separated into different needle age classes.

Analytical Procedures

Extracts for the determination of enzymic activities were prepared from fresh needles as described previously (21). The extracts were desalted on Sephadex G-25 (PD-10 column, Pharmacia, Federal Republic of Germany) and then assayed at 25°C using methods tested for spruce needle extracts (20, 21, 31). With the exception of superoxide dismutase and catalase for which extracts were kept frozen until analysis, all other enzymic activities were measured immediately in fresh extracts. Because ascorbate peroxidase was labile in the absence of ascorbate (20), 5 mM ascorbate was included for the extraction of this enzyme.

For the analysis of antioxidants the needles were frozen in liquid nitrogen and stored at −80°C until use. For the determination of reduced ascorbate, needles were powdered in liquid nitrogen and processed by the following protocol: 0.2 g of frozen needle powder was transferred to a centrifuge tube containing 5 mL of 0.1 N HCL, 1 μM EDTA, and 0.4 g of washed, insoluble PVP. The mixture was stirred slowly for 5 min (avoiding air bubbles), centrifuged at 12000*g* for 15 min, diluted with 0.1 N HCl as appropriate (usually by a factor of 5), and subjected to HPLC analysis according to the method of Lee *et al.* (11). Ascorbate was detected at 268 nm by comparison with standards that had been treated in the same manner as the samples. To investigate the specificity of the peak in spruce extracts, samples and standards were oxidized by ascorbate oxidase (1 mg/mL) for 20 min at 25°C after adjusting the pH value of the HCl-extracts to pH 5 by addition of 2 M sodium acetate buffer (pH 6.2). The oxidized samples were diluted with 0.1 N HCl to the same extent as the reduced samples and analyzed by HPLC. An unspecific signal with the same retention time as ascorbate was present in the samples and had to be subtracted from each sample. To determine losses of ascorbate during the extraction of the needles, aliquots of the samples were internally standardized with ascorbate. The recovery amounted to 86.4 ± 4.0% (n = 28) for both green and chlorotic needles.

Total ascorbate was determined in the HCl-extracts after oxidation and derivatization of the samples with *o*-phenyldiamine using an HPLC technique adapted for spruce extracts (20). The samples were internally standardized with ascorbate. The recovery amounted to 87.3 ± 2.5% (n = 28) for both green and chlorotic needles.

Total glutathione was determined in needle extracts after reduction with DTE and derivatization with monobromobimanes using a HPLC technique adapted for spruce samples (24). The samples were internally standardized with glutathione. The recovery amounted to 86.2 ± 10.1% (n = 12) for both chlorotic and green needles.

The protein content was determined in extracts purified over Sephadex G-25 (PD-10 column) with the bicinchoninic acid reagent (Pierce, Amsterdam, The Netherlands). The pigment content was determined spectrophotometrically in 80% acetone and calculated with the extinction coefficients given by Lichtenthaler and Wellburn (13). The malondialdehyde content was determined according to the procedure reported in ref. 19 using aliquots of 0.2 g of freshly prepared needle powder (see above). The dry weight of the needles was determined after drying for 72 h at 80°C. Dry needles were powdered, and aliquots of 0.1 g of needle powder were digested in 10 mL of HNO_3 for determination of foliar element contents (23, 26). Statistical analysis was performed with the software STATGRAPHICS comparing samples by t

Table I. *Sampling Scheme for Chlorotic and Green Needles*

T, Acutal air temperature when samples were taken; *N*, number of different spruce trees used to collect needles.

Date	T (°C)	Site	Needle Color	*N*	Needle Age Class
Winter 1990					
Jan 23	−5.9	Kramer	Chlorotic	4	1989
Jan 25	+0.4	Katzenstein	Green	4	1989
Jan 30	−1.3	Kramer	Chlorotic	4	1989
Feb 01	+1.1	Katzenstein	Green	4	1989
Winter 1991					
Feb 15	−5.4	Kramer	Chlorotic	4	1989, 1990
Feb 19	−6.9	Katzenstein	Green	4	1989, 1990
Feb 21	−6.0	Kramer	Chlorotic	3	1989, 1990
	−6.1	Katzenstein	Green	3	1989, 1990

Table II. *General Characteristics of Chlorotic and Green Spruce Needles*

Results are means ± SD ($n = 7$) for needles formed in 1989 and 1990 and harvested in February 1991 at Kramer (chlorotic) and Katzenstein mountain (green). P, Significance level for age-dependent differences (P_{age}) and site-dependent differences (P_{site}). Further details are given in "Materials and Methods."

Parameter	Chlorotic Needles (Kramer)			Green Needles (Katzenstein)			P_{site} for Age Class	
	1990	1989	P_{age}	1990	1989	P_{age}	1989	1990
Chl ($\mu g\ g^{-1}$ dry wt)	356 ± 93	906 ± 286	***	1710 ± 224	2017 ± 358	*	***	***
Carotenoids ($\mu g\ g^{-1}$ dry wt)	263 ± 48	318 ± 63	*	445 ± 55	481 ± 74	NS	***	***
Protein (mg g^{-1} dry wt)	52.4 ± 11.7	53.7 ± 11.1	NS	63.1 ± 9.3	60.8 ± 10.5	NS	NS	NS
Dry wt (mg $needle^{-1}$)	2.92 ± 0.60	3.55 ± 0.56	NS	5.69 ± 1.92	6.35 ± 2.40	NS	*	**
Dry wt/fresh wt	0.418 ± 0.022	0.443 ± 0.018	*	0.415 ± 0.057	0.455 ± 0.013	NS	NS	NS

test analysis. Significance levels are indicated as follows: ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, and NS.

RESULTS

Foliar Element Concentration and General Characteristics of Chlorotic and Green Spruce Needles

To characterize the chlorotic and green needles used in the present investigation, specific needle mass, dry weight to fresh weight ratio, and protein and pigment contents were determined. Both Chl and carotenoid contents were significantly lower in chlorotic than in green needles (Table II). The decrease in Chl amounted to about a factor of 5 in the youngest needle age class but improved with advancing age (Table II). This was also macroscopically detected as an improvement in needle color. The protein content and the dry weight to fresh weight ratio were not affected in chlorotic needles. However, growth was apparently decreased, because the weight of the chlorotic needles was lower than for green needles by a factor of about 2 (Table II).

Foliar analysis for pollutants revealed only noncritical levels of Al (<40 $\mu g\ g^{-1}$ dry weight), Pb (<0.3 $\mu g\ g^{-1}$ dry weight), and Cd (<0.01 $\mu g\ g^{-1}$ dry weight) in needles from both sites ($n = 16$). To investigate whether chlorosis was correlated with nutrient imbalances, the foliar content of micro- and macronutrients was determined. In the needle age classes 1989 and 1990, the elements C, N, P, Fe, Zn, and B showed neither significant age-dependent nor site-specific differences. Means ($n = 28$) ± SD for both needle age classes sampled at both sites in February 1991 were: C, 485 ± 6 mg g^{-1} dry weight; N, 10.9 ± 1.2 mg g^{-1} dry weight; P, 1229 ± 218 $\mu g\ g^{-1}$ dry weight; Fe, 47.2 ± 15.2 $\mu g\ g^{-1}$ dry weight; Zn, 43.5 ± 3.8 $\mu g\ g^{-1}$ dry weight; and B, 14.2 ± 3.6 $\mu g\ g^{-1}$ dry weight. These data indicate slight limitations of N, P, and B at both sites but exclude Fe or Zn deficiency as a cause of the chlorosis (ranges for optimum element concentrations for spruce trees grown in Bavaria, Federal Republic of Germany, were taken from ref. 8).

Analysis of the content of the nutrients S, K, Ca, Mg, Mn, and Cu revealed significant differences either between different needle ages or between chlorotic and green needles (Table III). Although some of the variations with age or needle color were considerable, *e.g.* Mg and Ca, none of the observed concentrations indicated starvation, except for Mn. Mn was severely deficient in chlorotic needles and sufficiently present in green needles. The decrease in Mn in chlorotic needles amounted to about a factor of 3 to 7, as compared with the threshold range of 10 to 20 μg Mn g^{-1} dry weight (8), and to a factor of 50, as compared to the average Mn content found in green needles.

However, Mn cannot be considered the only factor affecting Chl content because the Mn content did not change in older needles, yet the Chl content increased threefold in the previous year's needles from Kramer mountain (Table II). Because Mg can substitute for Mn to a certain extent, the ratio of both elements might be more important than their absolute amounts. In this respect, it is noteworthy that the Mg concentration in chlorotic needles was approximately

Table III. *Element Concentrations in Chlorotic and Green Spruce Needles*

Conditions are as described for Table II. Results are means ± SD ($n = 7$).

Element	Chlorotic Needles (Kramer)			Green Needles (Katzenstein)			P_{site} for Age Class	
	1990	1989	P_{age}	1990	1989	P_{age}	1989	1990
	$\mu g\ g^{-1}$ dry wt			$\mu g\ g^{-1}$ dry wt				
S	804 ± 34	789 ± 54	NS	870 ± 58	876 ± 61	NS	*	*
K	7627 ± 2671	4872 ± 1703	*	5494 ± 98	4717 ± 1349	NS	NS	NS
Ca	3883 ± 622	7068 ± 1607	***	6800 ± 1558	12244 ± 2162	***	***	***
Mg	1446 ± 179	1897 ± 438	*	966 ± 117	883 ± 108	NS	***	***
Mn	3.69 ± 2.81	3.56 ± 1.83	NS	147.9 ± 126	192.6 ± 161.3	NS	**	**
Cu	2.51 ± 0.42	2.04 ± 0.28	*	3.24 ± 0.46	2.90 ± 0.27	NS	***	*

twofold higher than in green needles and increased with advancing age, whereas the Mg content in green needles did not change.

The same set of parameters was also investigated in January 1990 in the needle age class 1989, which was the youngest age class at that time. Significant differences between these data and those reported for the investigation performed in 1991 (Tables II and III, needle age class 1990) were not observed.

Antioxidants in Chlorotic and Green Spruce Needles

Enzyme activities (superoxide dismutase, ascorbate peroxidase, monodehydroascorbate radical reductase, dehydroascorbate reductase, glutathione reductase, catalase, glucose-6-P dehydrogenase, and NAD-malate dehydrogenase) and antioxidants (ascorbate, glutathione) necessary for the scavenging of O_2^- and H_2O_2 were investigated for 2 years during the winter season. The absolute glutathione and ascorbate contents and enzymatic activities found in green needles are indicated numerically under the bars in Figure 1. In general, the antioxidant status of the green tissue was slightly higher in February 1991 than in January 1990 (Fig. 1, A and B). It is possible that this enhancement was caused by lower actual temperatures (Table I) or by higher radiation stress in February 1991 than in January 1990 (see "Materials and Methods").

The antioxidant levels and enzymatic activities found in green needles were set to 100% and used to calculate relative changes found in chlorotic needles. In chlorotic needles sampled in 1990, the level of total ascorbate and the activities of monodehydroascorbate radical reductase and glucose 6-P dehydrogenase were enhanced by 80 to 90% as compared to green needles (Fig. 1A). The increase in superoxide dismutase activity was also significant but much smaller (30%). The levels of glutathione, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase were not significantly higher in chlorotic than in green needles. Activities of catalase were also investigated but not detected during that time of year.

To assess lipid peroxidation in chlorotic and green needles sampled in 1990, the amount of malondialdehyde was determined. In both sets of needles, low malondialdehyde contents were found, amounting to 55.7 ± 9.4 nmol g^{-1} fresh weight in green (n = 8) and 57.2 ± 10.3 nmol g^{-1} fresh weight in chlorotic needles (n = 8). Thus, no evidence for enhanced lipid peroxidation was detected in chlorotic needles.

The sites were reinvestigated in 1991, analyzing both needles formed in 1989 and 1990. The same trends emerged as in the previous year (Fig. 1B). Significantly higher levels of total ascorbate, monodehydroascorbate radical reductase, superoxide dismutase, and glucose 6-P dehydrogenase were observed in chlorotic needles. The same was true for NAD-malate dehydrogenase, which was also investigated. However, the enhancements were less pronounced than in the previous year (Fig. 1A). This was, perhaps, due to the fact that the control levels in green needles were generally higher in February 1991 than in January 1990 (Fig. 1). The activity of dehydroascorbate reductase in February 1991, which was very low in January 1990, was not detectable above the background rates of nonenzymic reduction of dehydroascorbate by glutathione of 5.4 μmol/min.

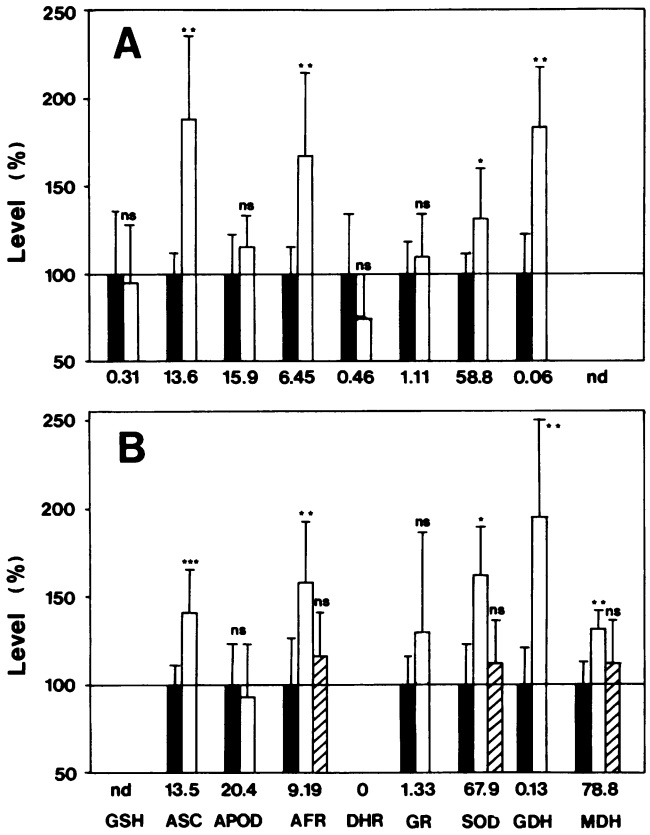

Figure 1. Comparison of antioxidant levels and enzymic activities in chlorotic and green needles sampled during two winters, 1990 (A) and 1991 (B). Bars, Relative means ± SD of antioxidant and enzyme activity levels (black, green; white, chlorotic needles); numerical values under bars, absolute amounts of antioxidants (μmol/g fresh weight) or enzymatic activities (nkat/mg protein) found in green needles (=100%). A, Young needles (1989) (n = 8 at each site); B, young (1990) and previous year's needles (1989). No age-dependent differences occurred among the green needles (black bars, n = 14). When age-related differences were apparent ($P < 0.05$) in the chlorotic needles, they were reported separately (1990, white bar; 1989, hatched bar, n = 7). Significance levels refer to the comparison with green needles. Glutathione, GSH; total ascorbate, ASC; ascorbate peroxidase, APOD; monodehydroascorbate radical reductase, AFR; dehydroascorbate reductase, DHR; glutathione reductase, GR; superoxide dismutase (units mg^{-1} protein as defined previously [21]), SOD; glucose 6-P dehydrogenase, GDH; NAD-malate dehydrogenase, MDH; nd, not determined.

Significant age-related differences in enzymic activities or antioxidant levels between the youngest and the second age class (1989, 1990) of green needles were not observed. In chlorotic needles, monodehydroascorbate radical reductase, superoxide dismutase, and NAD-malate dehydrogenase showed a significant age-dependent decrease in activity, thereby approximating levels found in green needles (hatched bars in Fig. 1B). These decreases in enzymic activity in the previous year's needles from the Kramer site correlated with the much improved Chl content (Table II). These results

indicate that chlorotic needles recover with advancing age and, thus, might have less need for detoxification. However, enhanced levels of total ascorbate were maintained in the previous year's needles from Kramer mountain. To investigate the question as to whether the reductive capacity was sufficient to keep ascorbate in its functional state in both age classes of needles from Kramer mountain, the amount of reduced ascorbate was determined. However, significant age-related differences were not observed. The relative portion of reduced ascorbate amounted to 86 and 84% in the needle age classes 1989 and 1990, respectively. The same proportion of reduced ascorbate was found in green needles.

DISCUSSION

In the present paper we have shown that chlorotic needles from Kramer mountain were Mn deficient. Because lime soils with a low availability for Mn are frequently found in the Calcareous Alps, it seems likely that the Mn deficiency at Kramer mountain was caused by soil-borne factors. However, soil analyses, which could clarify this question, are lacking at the Kramer site.

Mn deficiency impairs various metabolic functions because this element is an essential cofactor for arginases, phosphotransferases, and the water-splitting complex in photosynthesis (12). In spruce needles, Mn deficiency causes symptoms of chlorosis (3). A range of other nutrient deficiencies, such as lack of K, P, Mg, and Zn, was also reported to induce symptoms of chlorosis in spruce (16, 25, 30) as well as in herbaceous plants such as beans (4, 14). Chlorosis increased at high light intensities in nutrient-deficient bean leaves (14) and was less prevalent on northerly branches of spruce trees at the Kramer mountain. These observations suggest that radiation played a role in the development of chlorotic symptoms and might imply that the decrease in Chl was not a specific response to the lack of a specific nutrient but a more general secondary effect.

In fact, it was possible to observe chlorotic symptoms in the absence of specific stress factors in transgenic tobacco plants in which sugar export was inhibited (28). Stitt *et al.* (28) showed that in these transgenic plants the Calvin cycle was inhibited and that respiration and the level of hexose phosphates were increased. Similar metabolic disorders, such as increased sugar content and increased respiration rates, were found in chlorotic spruce needles (17) and might also be inferred from the increased activities of respiratory enzymes observed in the present investigation. These "over-reducing" conditions would, paradoxically, generate oxidative stress because photosynthetically produced reductant that cannot be directed into the Calvin cycle will be transferred to molecular oxygen and yield $O_2^{\cdot-}$. To prevent damage, the function of the antioxidative system would be to divert electrons into harmless detoxification pathways. The present results suggest that oxidative damage was successfully prevented by the antioxidative system because evidence for enhanced lipid peroxidation was not observed in chlorotic needles. Further investigations are needed to determine whether the low Chl content indicates injury or an adaptation to a decreased demand for photosynthetic electron transport.

To cope with an increased production of toxic oxygen species in chlorotic needles, the levels of superoxide dismutase and monodehydroascorbate radical reductase activity and ascorbate were increased (Fig. 1). Increased levels of ascorbate free radicals have been detected in needles from a declining spruce forest by means of EPR spectroscopy (27). We have shown that neither dehydroascorbate reductase activity nor the redox state of ascorbate/dehydroascorbate was affected in chlorotic needles. These observations support the proposal of Arrigoni and coworkers (1) that the monodehydroascorbate radical reductase is the key enzyme involved in maintaining ascorbate in its reduced state.

Significant increases in glutathione and glutathione reductase were not found in chlorotic needles (Fig. 1), indicating that these components were not limiting factors in an ascorbate-glutathione-related detoxification pathway. At variance with our results, enhanced levels of glutathione were observed previously in chlorotic spruce needles (18), and enhanced activities of glutathione reductase were found in chlorotic bean leaves (4). However, neither investigation was performed under low-temperature conditions in which background levels of glutathione and glutathione reductase are generally high. For instance, in spruce needles, glutathione content and glutathione reductase activity were approximately 3 times higher during winter than summer (7). High background levels of glutathione and glutathione reductase might have prevented a further increase of these components in chlorotic spruce needles. Because both glutathione and glutathione reductase activity showed a high variability from tree to tree and marked fluctuations between the sampling dates (*cf.* SD values in Fig. 1), small increases might not have been recognized on a statistical basis from the eight to 14 samples investigated.

In plants, H_2O_2 is enzymically reduced by catalases and peroxidases. Catalase activity played no apparent role in H_2O_2 scavenging in winter, because it was detected neither in chlorotic nor green needles. Because catalase was found at other times of the year (20), the activity appeared to be cold sensitive as in other plant species (22, 29). Ascorbate-specific peroxidase activity was not affected in chlorotic needles, whereas the activities of "nonspecific" peroxidases determined with guaiacol as substrate were twofold higher than in green needles (K. Chakrabarti and A. Polle, unpublished results). These differences support our previous results that suggested that the ascorbate-specific peroxidase and the nonspecific peroxidases in spruce needles were two classes of enzymes with different characteristics (20).

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