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Seasonal Variation of Abscisic Acid in Needles of *Pinus cembra* L. at the Alpine Timberline and Possible Relations to Frost Resistance and Water Status

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K e y w o r d s : *Pinus cembra* L., abscisic acid, frost resistance, water relations, alpine timberline.

S u m m a r y

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The seasonal variation of abscisic acid (ABA) and frost resistance was studied in needles of five *Pinus cembra* trees at the alpine timberline at Mt. Patscherkofel near Innsbruck. The mean ABA level was distinctly higher in the winter than during the growing season. While frost resistance remained constantly high in winter, concentration of ABA varied considerably for trees and sampling dates. It is suggested that the high ABA level during the cold season is responsible for a continuous closure of stomata.

Two hypotheses are put forward to explain the high ABA concentration in needles during winter: (a) Stress during diurnal freeze-thaw cycles triggers ABA synthesis or (b) photodestruction of xanthophylls leads to an increased level of ABA in *Pinus cembra* needles, possibly in combination with diminished ABA catabolism during winter. In both cases, variation in needle exposure would explain the large variation of ABA over time and among trees .

Introduction

For conifers at the margin of the distribution of a species, such as the timberline, the development of adequate frost resistance is especially important,

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and indeed such trees achieve extreme frost hardiness with the beginning of the cold season (cf. PISEK & SCHIESSL 1947). Both short days and low temperatures in autumn are environmental stimuli that trigger the biochemical and physiological changes associated with acclimation to cold in temperate zone trees (GUY 1990). Application of the plant hormone abscisic acid (ABA), which is involved in plant responses to stress of different kinds (ZEEVAART & CREELMAN 1988), can induce frost-hardening in plants in the absence of low temperature (MOHAPATRA & al. 1988, LÅNG & al. 1989, CHEN & al. 1983), and common polypeptides are induced in *Arabidopsis* by both ABA and low temperature (LÅNG & al. 1989). Therefore ABA is probably part of the transduction chain between environmental signals and changes in gene expression which lead to the development of frost resistance in plants. However, plants acclimated by low temperature treatment survive still lower temperatures than plants treated only with exogenously applied ABA (MOHAPATRA & al. 1988, LALK & DÖRFFLING 1985) which indicates that low temperatures also activate signal cascades in which ABA is not involved.

During cold acclimation, transient increases of ABA concentration have been found in above-ground parts of potato (CHEN & al. 1983), in spinach (GUY & HASKELL 1988), in wild type *Arabidopsis* plants (LÅNG & al. 1994), in a frost-resistant barley cultivar (MURELLI & al. 1995) and in spruce trees (QAMARUDDIN & al. 1995), suggesting that a temporal increase of ABA is sufficient to trigger biochemical and physiological changes associated with acclimation to cold (cf. CHEN & al. 1983).

Nothing is known so far about the interrelation of water status, ABA and frost hardiness of leaves under the climatic conditions at timberline. We therefore studied these parameters in needles of *Pinus cembra* L. trees at the alpine timberline at Mt. Patscherkofel near Innsbruck over the course of a whole year.

Material and Methods

Needle samples were taken from five *Pinus cembra* trees (49 to 82 years old) at the alpine timberline at Mt. Patscherkofel (1950 m a.s.l.) near Innsbruck every two weeks from November 1994 until October 1995. During periods which seemed important with respect to frost hardening, sampling was done weekly. Twigs were cut in the morning from south-exposed branches and brought into the lab within 30 minutes and, if necessary, were allowed to thaw in plastic bags at 5°C. Needles formed in 1993, 1994 and 1995 were sampled separately from each twig. For ABA analyses subsamples were frozen immediately in liquid nitrogen, freeze-dried, ground to fine powder and stored at -20°C over silica gel until they were analysed. All analyses were performed with needles formed in 1994; needles formed in 1993 and 1995 were included for selected dates only. ABA was extracted from the needles and extracts were purified as described by CHRISTMANN & al. 1995. ABA and its inactive isomer trans-ABA were quantified by means of capillary gas chromatography using an electron capture detector. Corrections were made for losses of ABA during the purification procedure (20-30%) as calculated from the recovery of [^{14}C]-ABA added as an internal standard to the samples at the beginning of the extraction.

Water potential of the needles was measured according to SCHOLANDER & al. 1965. Needle water content and saturation deficit were calculated after water saturation and drying of subsamples. Frost resistance was determined on the same twigs which were used for water potential and ABA measurements. Subsamples were enclosed in plastic bags and divided into 5 groups which were frozen simultaneously to different target temperatures within the expected range of frost

resistance, with intervalls of about 2° C, in a deep freezer. Cooling and thawing rates were about 10K/h and twigs were exposed to target temperatures for 12 hrs. For the development of frost damage, twigs were placed with recut ends into beakers with water and kept in indirect daylight at 15-20°C. After at least four weeks of post-culture (with weekly changes of water and recutting of the twig axes) percentage of needle damage was determined visually on each twig and compared to unfrozen control twigs. Air temperature and relative humidity were recorded continuously at the site. CO_2 gas-exchange was measured from October 1994 until May 1995, and needle and stem temperatures were measured until October 1995 on a *P. cembra* neighbouring the 5 experimental trees (cf. WIESER 1997 and unpublished results).

Correlations between ABA, frost resistance and needle water status were calculated using the Spearman rank correlation coefficient. Differences between mean ABA concentrations during winter and summer were evaluated by the Wilcoxon test (cf. SACHS 1984).

Results

Seasonal variation of ABA concentration

Apart from short-term variations, a general trend was observed in the ABA content of needles (figs. 1-3): On average, ABA concentration was high from November through April and then started to decline during May until a minimum was reached in September. Tree-to-tree variation was considerable with a mean coefficient of variation of 59% at the sampling dates. However, the ABA concentration was significantly ($p<0.05$) higher during "winter" than during "summer".

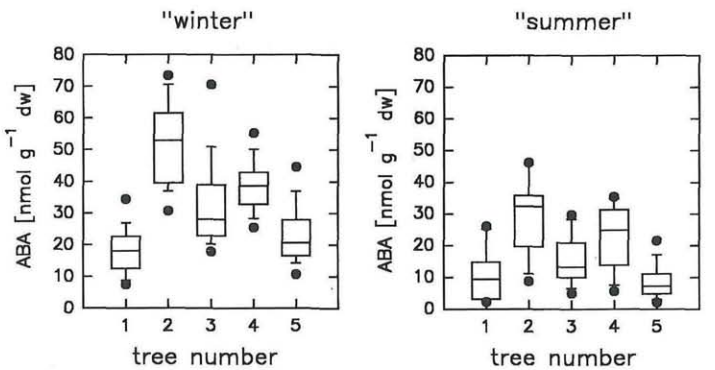

Fig. 1. Box plot of ABA concentrations during "winter" (23.11.94 - 21.04.95) and "summer" (18.05.95 - 11.10.95) of five *Pinus cembra* trees. Box displays the 25th, 50th and 75th percentile, marks represent the 10th and 90th percentile.

ABA concentrations of needles formed in 1994 were clearly higher in early winter 1994 than in early winter 1995. This seems to be mainly a needle age-related phenomenon since ABA concentrations measured at the same dates were

always lower in the older needles as shown in Fig. 3 for 1993 vs. 1994 needles (tree 1), and 1994 vs. 1995 needles (trees 3 & 5).

The seasonal variation of needle trans-ABA was similar to that of ABA and consequently, the percentage of ca. 5% of t-ABA in the sum of ABA and t-ABA varied only little, i.e. no accumulation of t-ABA was found in response to low temperatures as was observed in herbaceous plants (CAPELL & DÖRFFLING 1989).

ABA, frost resistance and needle water status

Frost resistance of the needles was high between November and April, i.e. during the period with a high ABA-level (Fig. 2). Frost resistance rather seemed to precede the decrease of ABA in spring and the bulk increase in September. A weak but statistically significant positive correlation (trees 1-5: $0.309 < r_S < 0.771$, $0.0001 < p < 0.070$) was found between frost resistance and ABA content of the samples.

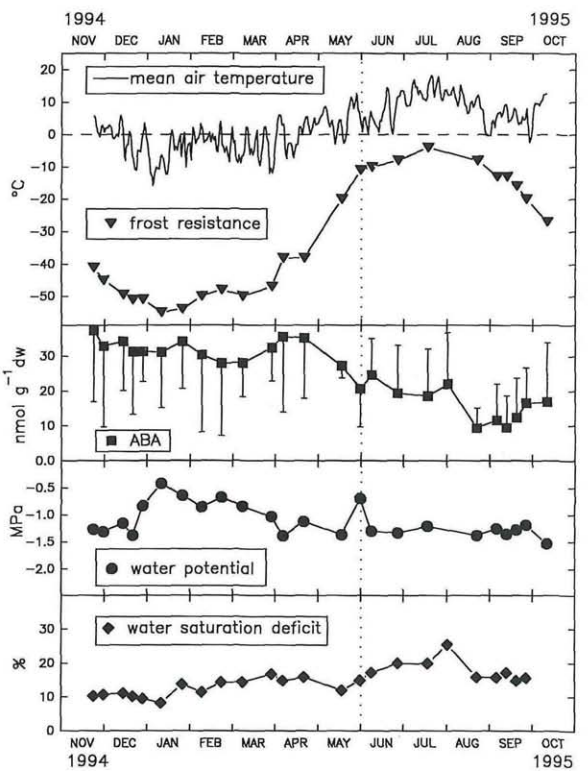

Fig. 2. Mean seasonal variation of the ABA concentration of five *Pinus cembra* trees, and of frost resistance, water potential and water saturation deficit in needles formed in 1994 as compared with the seasonal course of the air temperature. Bars indicate SD (n=5), broken line indicates budbreak.

Water potential of the needles ranged between -0.4 and -1.4 MPa (Fig. 2). High values were usually measured during dormancy when water saturation deficits of the needles were low. In contrast, water potential was low during the growing season and correspondingly, the water saturation deficit of the needles was higher during that period. No significant correlation was found between water potential and ABA content of the needles during "winter" or during "summer" (cf. Fig. 2). Moreover, the water saturation deficits varied independently of levels of ABA during both winter and summer. During September, when ABA levels reached their minimum, transient increases of needle ABA levels were observed in the trees (Fig. 3).

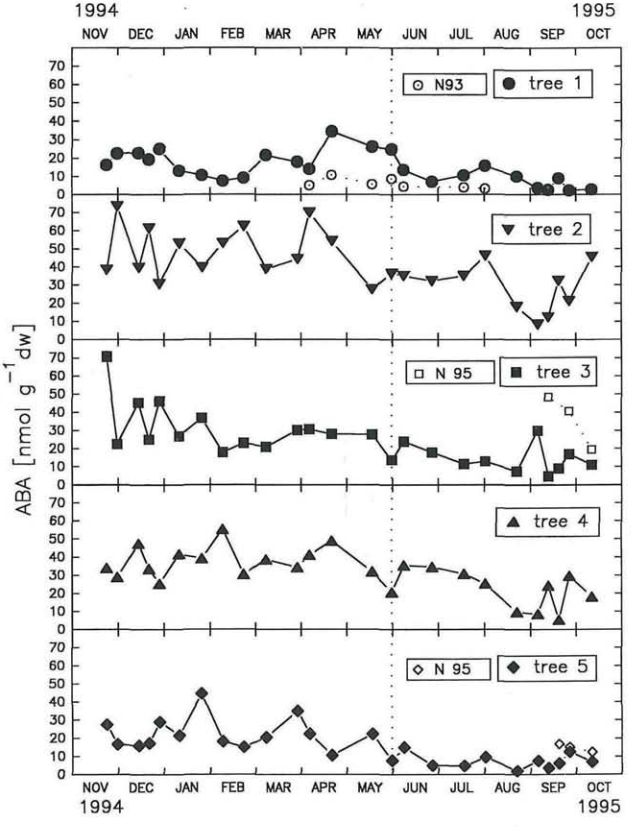

Fig. 3. Seasonal variation of ABA level in needles formed in 1994 for five *Pinus cembra* trees. ABA concentration in needles formed in 1993 and 1995 is added for selected sampling dates for trees 1, 3 & 5. Broken line indicates budbreak.

Discussion

The mean ABA level paralleled the seasonal trend of frost resistance but while frost resistance remained continuously high in all sample trees, ABA concentration showed considerable variation during winter. These findings are in agreement with the view that ABA plays an important role in triggering the frost hardening process but is not necessary to keep a plant organ in the frost-hardened state once this state has been reached (cf. LÅNG & al. 1994). However, the high concentration of ABA in *Pinus cembra* might be involved in the closure of stomata during that period (cf. HAVRANEK & TRANQUILLINI 1995).

During winter, the amount of ABA which affected the stomata probably did not only rise due to higher bulk needle ABA, but also because of a winter increase in apoplastic pH (TAULAVUORI & al. 1997) which changes the ABA distribution within the needles in favour of the apoplast (cf. WILKINSON & DAVIES 1997). Closed stomata during winter were likely to be the reason for the low water saturation deficits and high water potentials of the needles. Continuous gas-exchange measurements on a *Pinus cembra* tree at the same site confirmed that net photosynthesis was suppressed from the beginning of December 1994 until mid-April 1995 (WIESER 1997) and that transpiration during this period did not exceed cuticular transpiration rates (unpublished results). This means that needles did not suffer from water stress during this period and therefore the high level of ABA has to be attributed to other factors. It must be the result of enhanced ABA biosynthesis and/or of a restricted conversion to phaseic acid in the needles. An import of ABA from the root system seems unlikely since transport with the xylem water was severely restricted as stems were frozen for most of the time. Conjugated forms of ABA are also ruled out as a source of free ABA during periods with elevated level of ABA (ZEEVAART & CREELMAN 1988).

We propose two possible explanations for an enhanced ABA biosynthesis in needles in winter which we intend to test in future experiments: one explanation builds on temperature-induced stress and the other on photooxidative stress.

Needles of *Pinus cembra* trees were frozen for extended periods and, additionally, went through rapid freeze-thaw cycles on many days with low air temperatures but strong radiation (cf. GROSS 1989). During such cycles cells are subjected to a multitude of stresses including osmotic, thermal, mechanical, chemical and possibly electrical perturbations (STEPONKUS 1984). We suppose that the alterations in the plasma membrane associated with these perturbations (cf. COSTER & al. 1976) are transformed into signals which enhance ABA biosynthesis. It is also possible that changes in the hydration state of the cells within the frozen needles are registered and transformed into appropriate signals (cf. GUY & al. 1992). If the stress experienced by the cells triggers ABA biosynthesis the subsequent rise in ABA level will probably take place while needles are thawed and reach temperatures well above zero degrees since ABA synthesis has been reported to be repressed at low temperatures (ZEEVAART & CREELMAN 1988). There will be some variability in the intensity of freeze-thaw cycles experienced by needles due to temporal snow cover and/or partial shading (less overheating of

needles) which could explain variation in ABA level even among samples from similar, south-exposed parts of the crown as used in this study.

Photooxidative stress is well-known to affect needles in winter at the alpine timberline. Although a high level of xanthophyll cycle pigments and an appropriate state of this cycle are involved in photoprotection of conifer needles (ADAMS III & DEMMIG-ADAMS 1994) some photodestruction of the xanthophyll cycle carotenoid violaxanthin might occur during such periods and increase the immediate precursors of ABA (cf. WALTON & LI 1995), thus contributing to the elevated level of ABA. In this case, variation in ABA level among samples during winter would be due to different needle exposure to sunlight. It is in agreement with both hypotheses that ABA level was lower in older needles with a more sheltered position within the crown than in younger needles. Both mechanisms, however, imply that the variation over time of the ABA content of one tree may not always have reflected real ABA changes typical of the whole tree but also differences within the tree. Nevertheless, the higher ABA concentrations during winter were statistically significant compared to summer values. However, there were surprisingly great differences in the average ABA level of individual trees.

The variations in ABA level during the growing season cannot be explained by the water status of the needles either. This is in contrast to studies of silver fir and spruce needles where ABA level was found to be closely related to needle water status (CHRISTMANN & al. 1995). We suppose that soil water stress at this particular site was very low due to frequent precipitation and only occasionally ABA variation may have been due to decreases in soil water potential.

As mentioned before, transient increases of needle ABA content were observed in September, but it is not possible to decide whether these increases are related to photoperiod or whether they are a response to freezing stress which occurred already in September in 1995. It is still a matter of debate, if such ABA-peaks, which have been suggested to trigger the hardening process (cf. QAMARUDDIN & al. 1995) do appear under natural conditions in conifer trees. However, if such peaks are present for only few days, they might be difficult to detect under natural conditions if sampling is done once a week as in our study.

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