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# Needle Contents of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) as Biomarkers for Assessment of Vitality in Comparison with the Crown Condition

By

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Key words: Biomarker, bioindication, forest condition, *Picea abies*, *Pinus sylvestris*.


Summary

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Monitoring crown and canopy characteristics is therefore a crucial issue for intensive and continuous monitoring programs of forest ecosystem status. The defoliation class is exclusively concerned with damage registration and doesn't provide information between causes (e.g. weather, air pollution) and effects (adaptation or [latent] damages). This study was carried out to investigate the suitability and efficiency of biomarkers in comparison with defoliation class evaluations. The correlations between crown condition and biomarker are limited to a few parameters. Therefore, defoliation class rating and biomarker-based evaluations cannot be replaced against each other but they complete each other in a meaningful way. Biomarkers make a supplementary contribution to the exclusive assessment of crown thinning particularly regarding tree populations with moderate damages.

The most relevant and „economical" biomarkers for the tree species spruce and pine were presented in this paper and they are therefore suggested for the integration into the forest environmental monitoring.



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## Introduction

Since the mid-1980s the state of health of European forests is assessed annually on the basis of the outside crown condition of forest trees in comparatively close monitoring plots (at least 16 x 16 km grid) established within the EC-UN/ECE program "Intensive Monitoring (Level II) of Forest Ecosystems" (e.g. EC-UN/ECE 2004).

In addition several studies have been initiated in the last two decades to explain the vitality condition of trees on the basis of biochemical needle contents, in particular concerning the main tree species Scots pine and Norway spruce (TAUSZ & al. 1994, WILD & al. 1996, SCHULZ & al. 1999, WIENHAUS & al. 2002, GRILL & TAUSZ 2003, KÄTZEL 2003). In these studies the physiological and/or biochemical parameters, which react to environmental influences, are called biomarkers. Biomarkers are suitable for the measurement of environmental effects on organizational levels below the organism (molecule, cell, tissue, organ) (mod. according to ZIMMERMANN 1996). If biomarkers are used in field conditions and under consideration of other general conditions (KÄTZEL & al. 2004), they make an early determination of potential risks possible.

Although the suitability of the crown condition as an indicator of tree vitality was questioned several times, the relation between the crown condition and the biochemical composition of the needles has almost not been examined yet.

Table 1. Selected biomarkers for determination of the biochemical index of vitality.

<table>
<tr><th>Norway spruce, Picea abies L. KARST.</th><th>Scots pine, Pinus sylvestris L.</th></tr>
<tr><td colspan="2">100 needle dry mass [g]</td></tr>
<tr><td colspan="2">needle water content [%]</td></tr>
<tr><td colspan="2">chlorophyll [mg g$^{-1}$ dm]</td></tr>
<tr><td colspan="2">ascorbate [mg g$^{-1}$ dm]</td></tr>
<tr><td colspan="2">soluble carbohydrate [µmol glycosyl units g$^{-1}$ dm]</td></tr>
<tr><td>→ phenolic compounds [µmol g$^{-1}$ dm]</td><td>→ soluble starch [µmol glycosyl units g$^{-1}$ dm]</td></tr>
<tr><td>→ CAPE index - only applicable for the 3rd needle age class</td><td>→ soluble amino acids [µmol g$^{-1}$ dm]</td></tr>
<tr><td></td><td>→ proline [µmol g$^{-1}$ dm]</td></tr>
</table>

Based on this background information more than 30 biomarkers of the tree species Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) were examined regarding their suitability for the assessment of tree vitality conditions in the context of a research project. The objective of this research project was to limit the number of biomarkers by means of multivariate statistics to select parameters particularly suitable for the forest monitoring program. As a result specific biomarkers for the different tree species were determined as indicated in Table 1 (WIENHAUS & al. 2002). Cluster analyses based on the constellation of "biomarker-factors" provided specific biomarker patterns, which could be assigned to potential risks. These results were used for the examination of the relation between biomarker patterns and slightly recognizable crown thinning (defoliation class [DC] 1).

## Material and Methods

Trial areas and sample trees

In order to ensure a high information density concerning the ecosystem influence factors in the examination period, the studies were carried out in Saxony and Brandenburg using available permanent plots or other intensive monitoring plots (Level II) of the forest monitoring environmental program.

The spruce trial areas (Table 2a) are near Oberbärenburg (OB) in the Osterzgebirge and in Tharandt Forest (TW) in immediate proximity of long term intensive monitoring stations of-the Technical University of Dresden, which have been operated for many years. The age of the spruce trees at the beginning of the examination was approx. 40 years (TW) and 45 years (OB), respectively. 20 trees out of each plot were selected as sample trees. The assignment of the 20 sample trees into two vitality groups (VG 1 and VG 2) was carried out on the basis of their relative crown thinning (Table 4) according BMVEL 2001. The two vitality groups marked as VG 1 represented spruces, which were assigned to defoliation class 0 on an average (below 10 % needle loss). The spruces of VG 2 were characterized by a light crown thinning (defoliation class 1, 10 - 25 % needle loss). Trees damaged visibly were not examined. The trees of the two vitality groups were identical regarding their tree height and their diameter at breast high (dbh 1.3 m) (Table 3.).

Table 2a. Characterization of the Saxon spruce permanent monitoring plots.

| site | Tharandter Wald (TW) | Oberbärenburg (OB) |
|---|---|---|
| Stand establishment | 1958 | 1953 |
| Elevation [m] | 380 | 735 |
| Mean annual precipitation [mm] | 720 - 840 | 940 - 1000 |
| Mean annual air temperature [°C] | 7.0 - 8.2 | 4.5 - 5.5 |
| Air pollution pattern | $O_3$ (low concentration) | $O_3$ (higher concentration) |

Table 2b. Characterization of the Brandenburg pine permanent monitoring plots.

| site | Groß Schöne-beck (GR) | Kahlenberg (KA) | Lichterfelde (LI) |
|---|---|---|---|
| Stand establishment | 1904 | 1916 | 1912 |
| Trees/ha | 371 | 740 | 770 |
| Elevation [m] | 40 | 50 | 40 |
| Mean annual precipitation [mm] | 585 | 561 | 561 |
| Mean annual air temperature [°C] | 17.5 | 17.5 | 17.5 |
| Forest locality class | Z2m | M2 | M2 |

The examinations of the pine trees were carried out in a middle-age pure pine stand on two intensive monitoring areas of the research program "Ecosystem Research Eberswalde" conducted by the Institute for Forest Ecology and Forest Inventory Eberswalde of the Federal Research Centre for Forestry and Forest Products Hamburg (Revier [forest range] Britz [Lichterfelde, LI] and Revier [forest range] Kahlenberg [KA]) and an EU-intensive monitoring plot (Level II) of the federal state Brandenburg (Revier [forest range] Kienhorst [Groß Schönebeck, GR]), which represent the predominant-site conditions/ecosystem-types of pines in North-East German Lowland (Table 2b). Prior to the examinations two tree collectives (n = 10 pines) were selected out of each stand, which were slightly different regarding their degree of crown thinning. Therefore, the first vitality group of the pines represented the average trees of the stand (AT) while the second vitality group represented the most vigorous and above average trees of the stand (elite trees ET). Trees damaged visibly were not examined. Some of the selected tree vitality groups were significantly different regarding their growth performance (Table 3).

Table 3. Comparison of the tree high and the diameter at breast high (dbh 1.3 m) depending on the vitality groups (VG) on the permanent monitoring plots for spruce and pines. Asterisk denotes statistical significance: (*) p = 0.05, (***) p = 0.001).

| SITE NORWAY SPRUCE | high [m] | | diameter at breast high [cm] | |
|---|---|---|---|---|
| | VG 1 | VG 2 | VG 1 | VG 2 |
| Tharandter Wald | 14.0 ± 0.7 | 14.1 ± 0.8 | 19.8 ± 2.7 | 19.3 ± 4.0 |
| Oberbärenburg | 16.1 ± 0.8 | 16.7 ± 0.7 | 22.3 ± 2.1 | 22.7 ± 1.7 |
| SITE SCOTS PINE | high [m] | | diameter at breast high [cm] | |
| | VG ET | VG AT | VG ET | VG AT |
| Groß Schönebeck | 23.6 ± 1.8 | 22.1 ± 1.7 | 34.2 ± 4.1 | 30.5* ± 3.6 |
| Kahlenberg | 26.6 ± 1.3 | 24.6* ± 2.6 | 31.0 ± 4.6 | 28.2 ± 3.4 |
| Lichterfelde | 19.7 ± 2.0 | 17.7* ± 1.5 | 27.4 ± 3.3 | 21.7*** ± 0.8 |

Sampling and biochemical examination

The sampling of needle material from pines and spruces was carried out from 1998 to 2001 according to the guidelines (BMELF 1994) and the recommendations of KLEIN & PAULUS 1995 respectively. In the case of the spruces the sampling of needle material was performed twice a year (September and October) from the 7th whorl southern aspect. The sampling of needle material from the upper lightcrown of the pines was carried out four times per year (March, July/August, September, November). The first three needle years were analysed by needle age classes. Analytical methods are summarized in WIENHAUS & al. 2004 (unpublished). Statistical calculations (variance and correlation analyses) were carried out using the program SPSS 8.0 or 11.0/12.0 (SPSS Inc. Chicago, USA). Additional data concerning the crown condition (defoliation classes, BMVEL 2001) were available for every sample tree (Table 4).

## Results

### Needle dry matter

During the investigation from 1998 to 2001 significant and very significant differences of the dry weight of 100 needles were determined between the two spruce trial areas. It can be assumed that these differences have been mainly caused by the different weather conditions of the two locations. No significant differences were found between the vitality groups.

In contrast to the tree species spruce, pines are not characterized by a regular structure of crowns and branches. Shoot length, needle length and 100 needle dry weights depend strongly on the fact as to how they are exposed within the pine crown and decrease with a declining branch order. No significant differences of needle dry weights could be determined between the two tree groups ET and AT examined.

### Needle water content

The needle water content is substantially coinfluenced by the current weather situation before and during the vegetation period and the sampling. Therefore, this parameter is a good indicator for the water supply of the trees. A differentiation of the needle water content between the two vitality groups 1 and 2 (spruce) and the tree collectives ET and AT (pine), respectively, could not be ascertained.

Table 4. Annual needle loss, average needle loss over the examination period and classification of the sample trees according to defoliation classes.

| | 1998 | 1999 | 2000 | 2001 | average | 1998 | 1999 | 2000 | 2001 | average |
|---|---|---|---|---|---|---|---|---|---|---|
| SITE NORWAY SPRUCE | Vitality group 1 | | | | | Vitality group 2 | | | | |
| Tharandter Wald (TW) | | | | | | | | | | |
| Needle loss [%] | 7 | 5 | 9 | 9 | 7 | 17 | 10 | 15 | 16 | 14 |
| Defoliation class [0/1/2] | 9/1/0 | 10/0/0 | 10/0/0 | 8/2/0 | | 2/7/1 | 8/2/0 | 3/7/0 | 3/7/0 | |
| Oberbärenburg (OB) | | | | | | | | | | |
| Needle loss [%] | 12 | 12 | 11 | 9 | 11 | 23 | 22 | 23 | 19 | 21 |
| Defoliation class [0/1/2] | 4/6/0 | 8/2/0 | 7/3/0 | 8/2/0 | | 0/9/1 | 0/9/1 | 0/8/2 | 2/7/1 | |
| | 1998 | 1999 | 2000 | 2001 | average | 1998 | 1999 | 2000 | 2001 | average |
| SITE SCOTS PINE | Vitality group ET | | | | | Vitality group AT | | | | |
| Groß Schönebeck (GR) | | | | | | | | | | |
| Needle loss [%] | 18 | 17 | 14 | 13 | 16 | 19 | 18 | 14 | 16 | 17 |
| Defoliation class [0/1/2] | 2/7/1 | 1/9/0 | 4/6/0 | 4/6/0 | | 1/9/0 | 1/9/0 | 4/6/0 | 4/6/0 | |
| Kahlenberg (KA) | | | | | | | | | | |
| Needle loss [%] | 10 | 11 | 10 | 9 | 10 | 16 | 17 | 17 | 18 | 17 |
| Defoliation class [0/1/2] | 8/2/0 | 7/3/0 | 7/3/0 | 8/2/0 | | 2/7/1 | 2/8/0 | 3/6/1 | 3/6/1 | |
| Lichterfelde (LI) | | | | | | | | | | |
| Needle loss [%] | 9 | 15 | 18 | 17 | 14 | 16 | 18 | 17 | 18 | 17 |
| Defoliation class [0/1/2] | 7/3/0 | 3/7/0 | 3/6/1 | 4/5/1 | | 3/7/0 | 2/8/0 | 10/0/0 | 2/7/1 | |

Total chlorophyll and total carotinoid content

In the case of the tree species spruce the two vitality groups could be clearly distinguished by their chlorophyll contents (Fig. 1a) and also by their chlorophyll a/b and total chlorophyll/carotinoid ratios (regarding the latter depending on the location), particularly for the 3rd needle year. Because of their tree specific reference the two pigment ratios have the advantage of a low fluctuation margin within the clusters of trees to be compared. This differentiation gets particularly clear for the example of the Tharandter Wald.

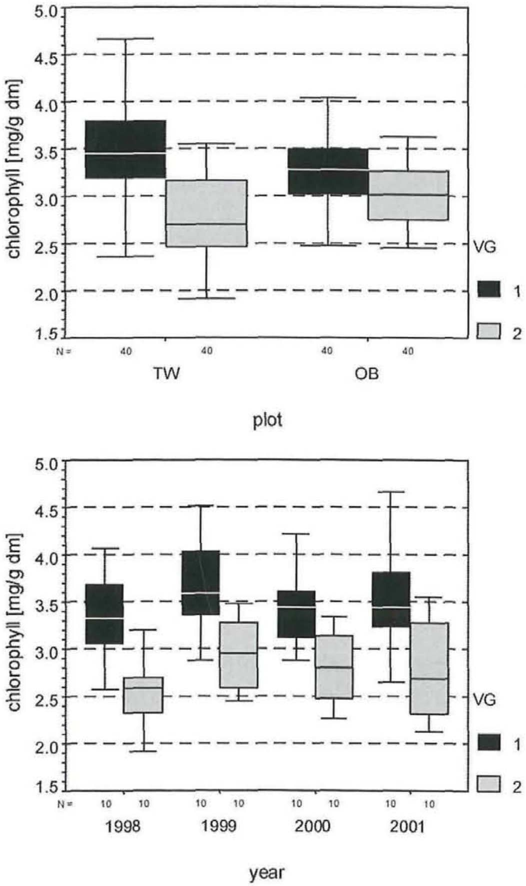



Fig. 1a. Total chlorophyll content of spruce trial areas in the 3rd needle year in September (upper fig.) and total chlorophyll content of the trial area Tharandter Wald (lower fig.), distinguished by vitality groups (VG) (study years 1998-2001).

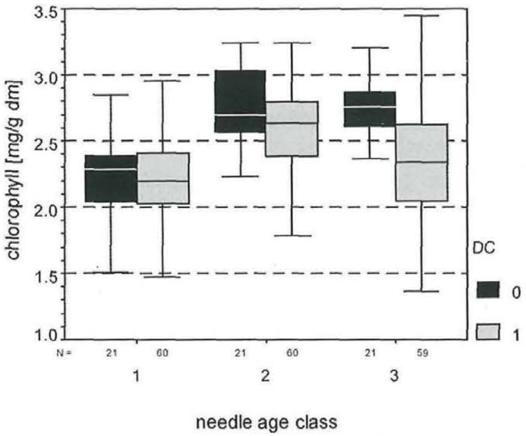



Fig. 1b. Total chlorophyll content (needle years 1-3, September) of pines in trial area Groß Schönebeck, distinguished by annual defoliation class (DC) (summary of study years 1998-2001).

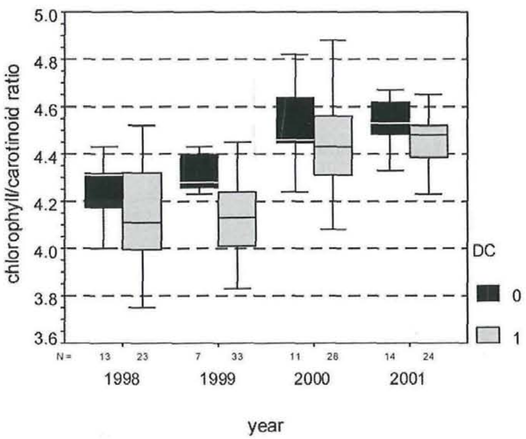



Fig. 2. Total chlorophyll/carotinoid ratio of the 3rd needle year of pines in trial areas Groß Schönebeck and Lichterfelde, distinguished by study years and annual defoliation class (DC).

No significant differences were obvious between the two vitality groups ET and AT of the examined pines regarding their chlorophyll contents. The statistical comparison between the two defoliation classes 0 and 1 showed significant differences only for the 3rd needle year and only for the pines of the trial area GR (p = 0.05). Changes of the pigment contents are not to be expected for the 1st needle year given the fact that the optimum photosynthesis conditions will prevail. Slight differences existed also in the 2nd year needles (Fig. 1b).

Clearer differences between the two defoliation classes were apparent regarding the chlorophyll/carotinoid ratios of the two permanent plots GR and LI for the 3rd needle year, in which pines of the defoliation class 0 appeared to have higher ratios (Fig. 2).

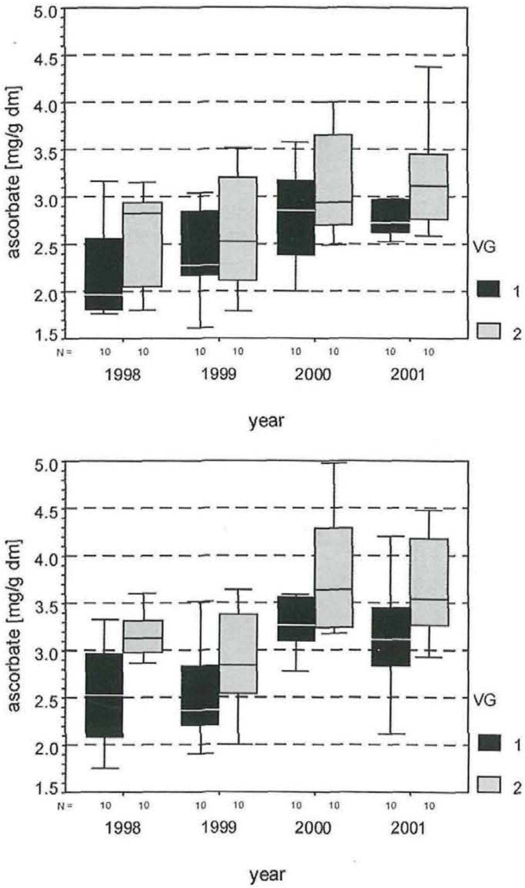



Fig. 3a. Total ascorbate content of spruce needles (3rd needle year, October) in the trial areas Tharandter Wald (upper fig.) and Oberbärenburg (lower fig.), distinguished by vitality groups (VG) (study years 1998-2001).

Total ascorbate content

Analysing the total ascorbate content of spruce needles a differentiation can be carried out both between the examined areas and between the two vitality groups. The main reasons for this observation might be increased ozone load and/or a low magnesium availability in the upper mountain regions. Therefore, total ascorbate is a suitable biomarker for vitality assessment. A differentiation is also possible between the vitality groups within one plot (Fig. 3a), particularly in Oberbärenburg. Since the differentiation of the vitality groups is less clear in Tharandter Wald, it can be assumed that there might be a correlation between ascorbate con

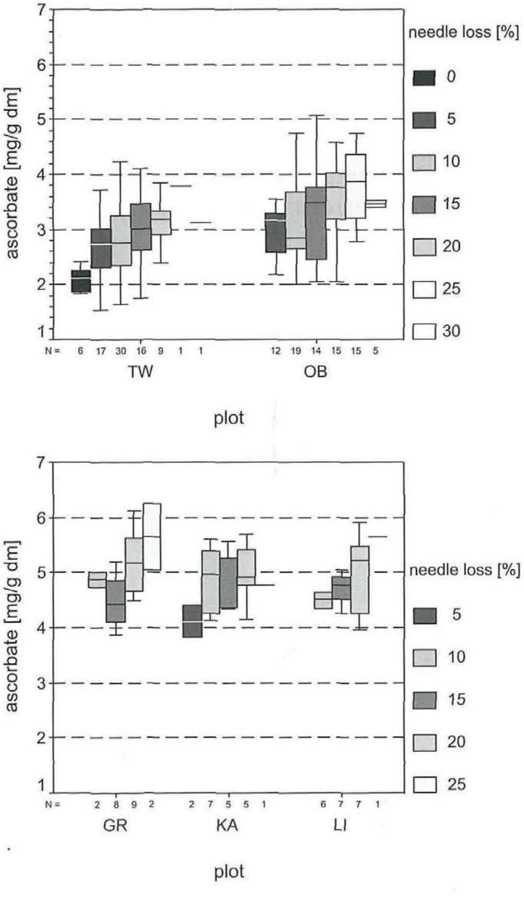



Fig. 3b. Comparison of the total ascorbate content of spruce needles in September 1998-2001 (upper fig.) and of pine needles (3rd needle year) in March 2000 (lower fig.) for different trial areas, distinguished by needle loss of the current (spruce) or previous (pine) vegetation period, respectively.

tent and needle loss or defoliation class, respectively (Table 4). This correlation is proved by a clear increase of the total ascorbate content with an increasing needle loss (Fig. 3b).

In the case of the pines significant differences were determined in the ascorbate content of the different defoliation classes in March, when the needle loss of the previous year was taken into consideration. With an increasing needle loss the remaining needles of the 2nd needle year showed in general higher ascorbate contents (Fig. 3b). However, there was a high deviation concerning the needles of an individual tree.

Soluble carbohydrates

Using the biomarker „soluble carbohydrates" a clear differentiation by trial area is remarkable in case of the spruces. A difference between the two vitality groups could not be detected. This proves that the content of soluble carbohydrates does not reflect the crown thinning of the examined spruces with respect to the defoliation classes 0 and 1. The distinction by sites can be explained by different ozone loads. Hence, the biomarker „soluble carbohydrates" is suitable for the differentiation by site partly and significantly, but not by vitality groups (Fig. 4a).

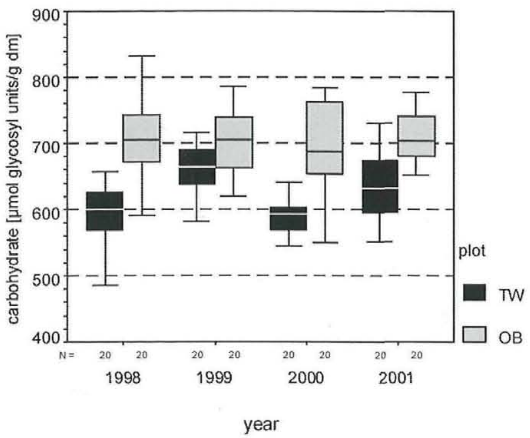



Fig. 4a. Soluble carbohydrate content of spruce needles (3rd needle year) in October for different years, distinguished by trial areas.

The total carbohydrate content of the pines of vitality group AT was lower compared to the pines of vitality group ET over the whole study in all three needle year classes. The greatest variations between the 2 groups was apparent on trial area LI. Particularly in September the differences were significant in all 4 years (except for: 2nd needle year 2000 and 2001). In the dry year 1999 the differences between the two tree groups were highly significant (t-test for independent samples; p = 0.001). Correlating to the increased carbohydrate contents also the osmolality of the needle pressing sap of the pines of vitality group ET were increased over the entire study period (Fig. 4b).

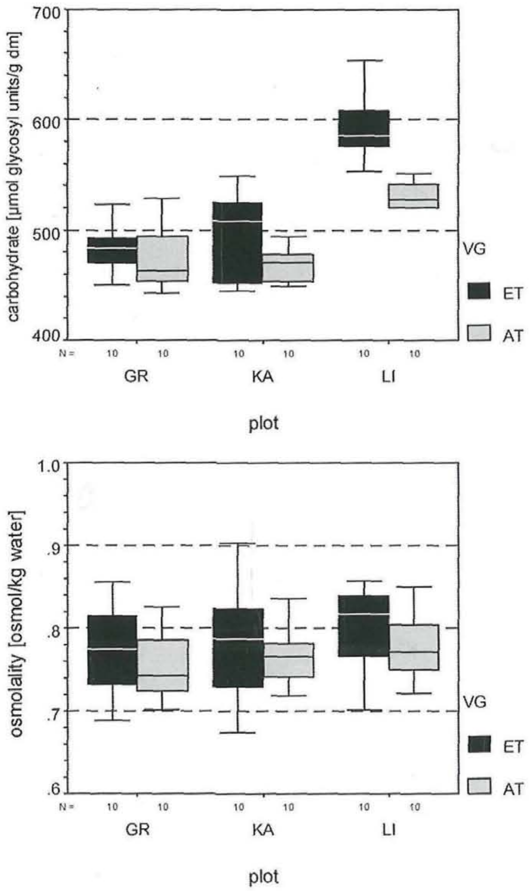



Fig. 4b. Soluble carbohydrate content (upper fig.) and osmolality of the pine needle pressing sap (lower fig.) of the 1st needle year in 1999 for different trial areas, distinguished by vitality groups (VG).

Starch

No statistically significant differences were determined in the starch content of spruce needles due to the large variation coefficients. However, in general the residual starch contents of the needles of vitality group 2 were higher than those of vitality group 1, the greatest differences concerning the 1st needle year class.

In pine there were no differences regarding the starch content of needles between vitality groups or regarding defoliation classes, respectively.

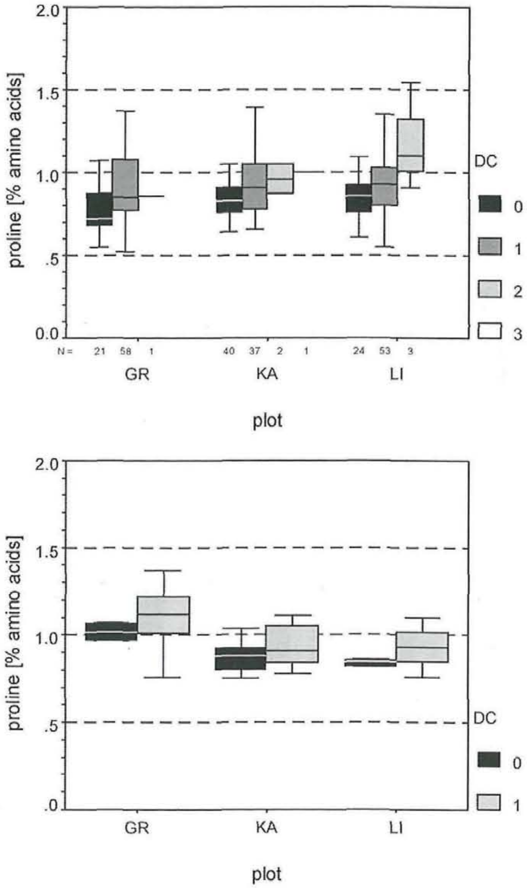



Fig. 5. Comparison of the average proline content (referring to total amino acid content) of the 2nd needle year of pine for different trial areas in September over the whole 4-year examination period (upper fig.) and in September 1999 (lower fig.), distinguished by defoliation class (DC).

## Soluble amino acids

The analysis of the soluble amino acid contents in spruce resulted in higher values for the 2nd and 3rd needle at the location Tharandter Wald over the whole

4-year examination period. This was more evident in October than in September. These differences are significant or generally higher despite the great variation coefficients. Although there is a strong location-dependent deviation between the individual trees, a differentiation between the trial areas is possible. No significant differences were ascertained regarding the two vitality groups.

Concerning the pine needles of the 1st and 2nd needle year of vitality group ET contents of soluble amino acids were generally higher only in August and September of the dry year 1999. There were no significant differences between the two vitality groups.

Proline

Proline is not suitable to differentiate between spruces of defoliation class 0 and 1. The application of proline as a biomarker for this tree species is only appropriate for a differentiation of more or less damaged trees or if an acute dry-stress-situation has to be assessed.

During the entire examination period the proline content (relating to-% soluble amino acids) of pines were higher in thinner crowns. In September the differences between the two defoliation classes 0 and 1 reached the level of significance (t-test for dependent samples only for the defoliation classes 0 and 1 (Fig. 5).

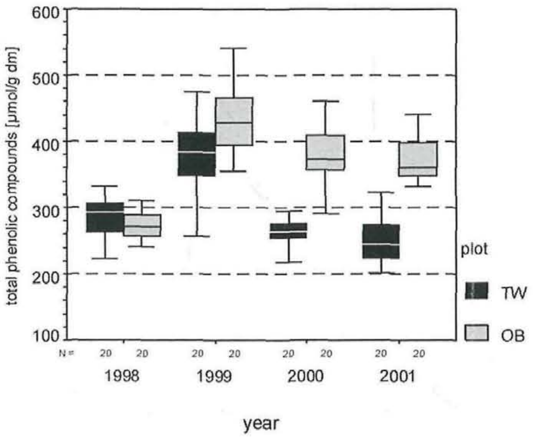



Fig. 6a. Comparison of the total content of phenolic compounds in 3rd needle age class of spruces in October distinguished·by trial areas and study years.

Phenolic compounds

A typical characteristic of conifer needles is their high content of phenolic compounds and their metabolic precursor stages. Although the genetic variability is well known from a variety of previous investigations, these substances were included in the examination program, since they are essential components of the non-specific and quantitative defense system of conifers.

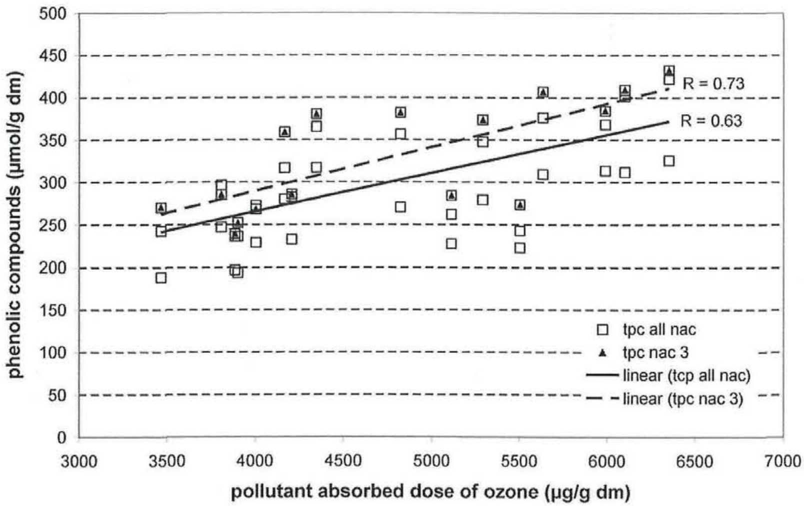



Fig. 6b. Correlations between ozone intake (PAD) from April 1st until sampling and the content of total phenolic compounds (tpc) of all needle age class (nac) / of the 3rd needle age class (nac 3) of spruce needles.

The spruce needle samples a tendency of increasing total phenol content from vitality group 1 to vitality group 2 becomes obvious, referring to needle age classes of the two trial areas. Likewise, significant differences between the two trial areas were also apparent as for the contents of total phenolic compounds (Fig. 6a).

Therefore, the biomarker „phenolic compounds“ is suitable for the assessment of vitality. It shows a direct linear correlation to the ozone pollutant absorbed dose (PAD) (Fig. 6b). The spruce needles of the trial area Oberbärenburg being subjects higher ozone loads, have higher total phenolic compounds values than these of the trial area Tharandter Wald with lower ozone loads. In general, the total phenols in healthy spruce as an antioxidative defense system are initially increased as oxidant stress.

Regarding pines, there are two chemical types (phenol type I and II) concerning the occurrence of phenolic compounds (KÄTZEL & LÖFFLER, in preparation). Therefore, a detailed comparison of the two vitality groups can only be carried out by the respecting chemical types. In general, the contents of total phenolic compounds increased with an increasing crown thinning. The total phenol content of the trees of phenol type I increased significantly already for a needle loss of 0-

15 % (p = 0.05). On trial area GR the needles of the 2nd year age class of phenol type II pines with a needle loss of 20-25 % were significantly different from the trees with a needle loss smaller than 15 % (p = 0.05). Likewise on trial area LI the highest total phenol content was verified for pines with a needle loss of 25 %, which were thus significantly different from the trees with a needle loss smaller than 15 % (and 35 %) (Fig. 7). However, the pines on trial area KA did not follow a clear trend. The results prove a close correlation between total phenolic compounds and ozone loads (AOT 40 values) for pine needles of all three needle age classes. This is comparable to the results of the spruce needles. As an example the results for the trial area GR (investigation from 1995 to 2003) are displayed in figure 8.

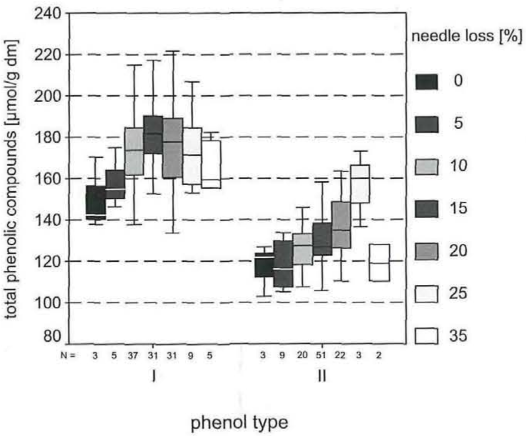



Fig. 7. Comparison of content of total phenolic compounds of the 2nd needle year age of pines with different relative needle loss in the trial area Lichterfelde, distinguished by the phenol type.

In addition to higher total phenol contents there were also higher contents of ortho dihydroxyphenols determined for pines from vitality group AT. In 2000 the differences between the two vitality groups were significant (p = 0.05). A similar trend was obvious regarding the 1st needle age class on all three trial areas in November 1999 (Fig. 9).

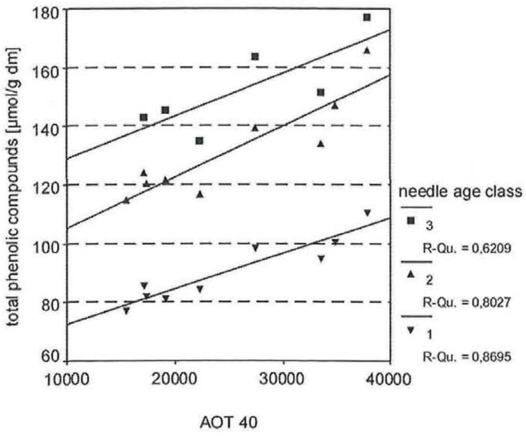
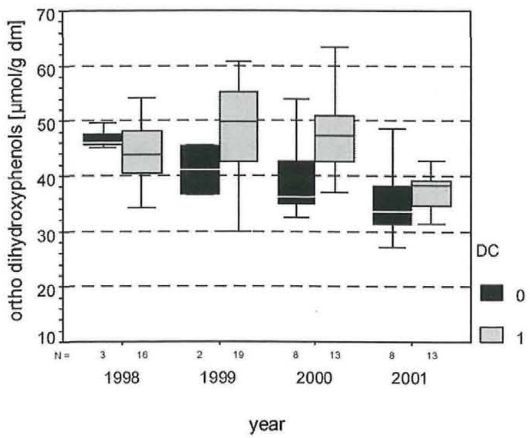



Fig. 8. Correlation between accumulated ozone exposure over a threshold of 40 ppb (AOT 40) and average content of phenolic compounds in pine needles (examination period 1995-2003, sampling month August, n = 8 trees/year).

CAPE index (nutrient index)

Regarding spruces the risk index according to CAPE & al. 1990 was determined based on the element contents for the 3rd needle age class. This index characterizes the status of the most important nutrient elements of the tree species spruce. The CAPE index allows a clear distinction between the two trial areas (Fig. 10). Unambiguous differences between the vitality groups could not be found.



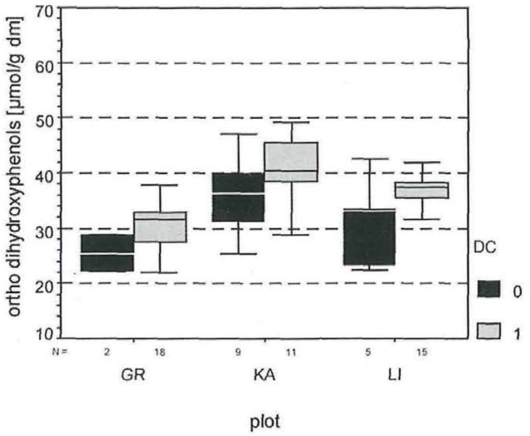



Fig. 9. Content of ortho dihydroxyphenols distinguished by defoliation class (DC) for the 2nd needle age class of pine trees on trial area Groß Schönebeck in July/August of all 4 study years (left) and the 1st needle age class of trees in November 1999 (right).

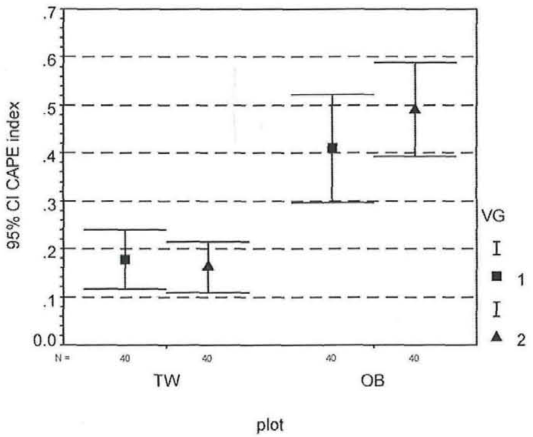



Fig. 10. CAPE index of spruce for different trial areas distinguished by vitality groups (VG).

## Discussion

The forest monitoring as an integral part of the environmental policies extension provides important information on the success of actions for pure air conservation policy and Forest Conversion Program or information about further risk factors. The additional use of biomarkers in the forest monitoring program is recommendable for objective and precise information about potential risks, vitality conditions and cause-effect chain is needed at an early time, which cannot be provided by the currently used methods of forest condition monitoring. Since the suitability of crown condition parameters for the vitality assessment is increasingly discussed controversially (ULRICH 1991, REHFUESS 1992, WIENHAUS & LIEBOLD 1993, ELLENBERG 1994, 1996, GRUBER 2004), the objective of this study was to examine, as to how far a specific biomarker-spectrum corresponds to the results of the crown condition evaluation. Such level-transferring connections were only expected as exceptions in minor vitality differences between the tree groups due to the physiology of needle fall (overview: GRUBER 1993). It is well known that the needle dropping takes place at the end of an active physiological adaptation process, as regards predominantly older and less metabolically active needles. Therefore, it can be assumed that the remaining needles of trees with heavier crown thinning are particularly metabolically active at least under eustress conditions. Consequently, correlations to the crown transparency have to be expected for older needle age classes on the one hand, which fall off next, and on the other hand for younger remaining needles which have to compensate for the needle loss by an increased metabolic activity.

In the early stage of damage the biomarkers needle dry weight, water ontent and starch did not allow a differentiation between the vitality groups for any of the examined tree species. In addition, the biomarkers carbohydrate and amino acid content were not suitable for the distinction of vitality groups in spruces.

However, in spruce needles the following biomarkers can be applied for the differentiation of vitality groups: total chlorophyll content, the ratios of chlorophyll a/b and the chlorophyll/carotinoid ratio, total ascorbate content, total phenolic compounds and CAPE index. The differences are particularly remarkable in the 3rd needle age class. Slight damaged spruces (vitality group 2), with a slight needle loss, are characterized by higher ascorbate contents, phenol contents and CAPE index-values as well as lower chlorophyll contents.

In the Scots pine needles slight differences between the two vitality groups were determined regarding the carbohydrate contents, the chlorophyll contents (3rd needle age class trial area GR, study years 1998, 2001) and the ascorbate contents (2nd needle age class, March 2000). The elite pines (vitality group ET) oppeared to generally or partly have significantly higher total carbohydrate and protein contents as well as higher clorophyll/carotinoid ratios than the comparison group.

On the other hand, the needles of slight damaged trees (vitality group AT) tended to have higher contents of ascorbate, proline, total phenolic compounds and ortho dihydroxyphenols. Since the visual defoliation class-response relates to needle discolorations or needle loss of the trees, it should be examined, whether and when the different classes are reflected by the pigment contents.

The results of this study have shown that biomarkers allow a differentiated assessment of the vitality condition of trees having not visible signs of damage, i.e. within the defoliation classes 0 and 1. Therefore, the application of biomarkers opens the possibility of optimizing and quantifying the assessment of the vitality condition of trees/tree groups already for latent changes and chronic loads of not accumulative pollutants. However, the assessment of the vitality of an area or specific sample trees out of a tree population is only conducive if several biomarkers are examined. Therefore, it is necessary to select those biomarkers of the variety of specific biomarkers, that have the greatest possible relevance. In order to select the most relevant biomarkers correlations to location conditions have to be investigated. The investigation of biochemical indicator systems for selected trees, which represent a tree population, makes a clearer and better assessment of the vitality of trees/tree groups possible than can be achieve by defoliation class evaluations, which cover very wide ranges. In addition, a long-term biomarker-based observation allows a differentiation, whether the needle loss of a tree is due to an adaptation reaction or a damage. In general, only a long-term evaluation of the selected biochemical parameters, which were presented for the tree species spruce and pine in this study, is meaningful or recommendable in addition to the defoliation class evaluation.

Tree-specific differences for the most expressive biomarkers can be explained primarily by the different ecosystem influence factors as the locations (e.g. weather, air pollution patterns). As has been shown by the analysis of the contents of phenolic substances in case of pine and the dispersion of the amino acid values in case of spruce have also genetical factors have to be taken into account for the selection of biomarkers for the vitality assessment.

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