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Norway spruce phenotype variability determined by needle anatomy in Bohemian Forest compared to other regions of the Czech Republic

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Abstract: Young trees (saplings) of the Norway spruce (*Picea abies* [L.] Karst.) regenerating populations were analysed on 7 plots in the Šumava Mts. (Bohemian Forest), on 5 plots in the Jeseníky Mts. (Eastern Sudetes), and 1 plot in the Krkonoše (Giant Mts.). All 13 plots were located at the forest altitudinal (vegetation) zones of natural *Picea abies* stands. Each selected tree was characterized by microscopic features of the first-year needles. The free-hand needle cross-sections were prepared from three needles of each tree and measured by digital microphotos. The following needle characteristics were measured: width, thickness, and vascular bundle diameter. Each population was described by variability of these parameters. Populations were classified based on the data set. Two artificially planted populations were most different. Populations resulting in natural stands have different phenotype variability, possibly as a result of the parent stand history: two extreme examples are Eustaška locality (Jeseníky Mts.) with no known disturbance, and Trojmezí locality (Šumava Mts.), where wind and bark beetle disturbances were repeatedly recorded.

Keywords: microscopy; needle size; *Picea abies*; populations; quantitative anatomy; regeneration

Norway spruce (*Picea abies* [L.] Karst.) is not only one of the most important commercial tree species of Europe but also in Central European conditions it represents a natural dominant of mountain forests in the proximity of the alpine forest limit (Bohn et al. 2003; Štefančík et al. 2018). As these forests are highly vulnerable to air-pollution impacts (e.g. Schulze et al. 1989; Cudlín et al. 2001; Vacek et al. 2019), disintegration of the tree layer of these forests occurred in many mountain ranges of the Czech Republic and in the other countries in Central Europe. Climate changes are added to this, which are the cause of the occurrence of the climatically extreme periods. These have resulted in the increase of drought stress, and possibly in a large area of the canopy disturbance, for example, as a result of the bark beetle gradation. For this reason, the regeneration of mountain *P. abies* forests in conditions of their natural occurrence is currently an urgent task, mainly under climate change (Frank et al. 2016). Different demands should be placed on the process of reforestation

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at these locations, since mountain forests do not have the commercial function – timber production as the only priority, while other forest functions become crucial. The need to establish highly stable forest stands having a similar structure to that of natural forests in analogical conditions is crucial. The stable forest ecosystems fulfil all forest functions (environmental services) required at exposed mountain locations in the best way.

The Šumava Mts. (Bohemian Forest) represent the most extensive mountain region with the largest area of natural *P. abies* forests in the Czech Republic. The *P. abies* forests there are under long-term pressure of acid deposition, climate change and bark beetle outbreak (Kindlmann et al. 2012). The stand disintegration results in large clear-cut areas with undesirable environmental consequences (e.g. Svoboda, Podrázský 2005). Regeneration of the new *P. abies* successive populations and their characterization has enormous importance.

Differentiation of the *P. abies* mature tree forms (phenotypes) has been used for a long time. It is based mainly on the appearance of tree crowns and branching (Samek 1964; Fanta 1974; Schmidt-Vogt 1977). The crown morphology together with growth properties is related to tree genetics (Caré et al. 2020). It is not possible to distinguish these phenotypes in young trees (seedlings, saplings), they are applicable in (sub)mature individuals only. Several phenotype traits including seedling growth and phenology were selected for the characterization of phenotype variability and phenotype-environment associations in the *P. abies* seedlings in Switzerland (Frank et al. 2016). Several studies bring a modern approach to distinguishing phenotypes on the basis of physiological traits (e.g. Hrivnák et al. 2022).

Relatively good knowledge is available in the field of variability of the population features along altitudinal and related climate gradients (Pacalaj et al. 2002; Gömöry et al. 2012; Romšáková et al. 2012; Frank et al. 2016; Hrivnák et al. 2019).

It is known from the field observations that needles of individual trees differ significantly (Matějka et al. 2014). Thus, an idea of describing the phenotype on the basis of microscopic needle features arose. The anatomical structure of *P. abies* needles is described in Figures 1 and 2. The needle anatomy is related to needle functions (Wang et al. 2021), e.g. parenchyma tissues are sites of photosynthesis, the vascular bundle is related to water supply and transport of photosynthesis products. The epidermis with the wax layer separates the inner space of the needle from its aerial environment and its substantial importance in polluted air is well known (Tuomisto, Neuvonen 1993). Physiological studies are concerned with subcellular structures in prevailing part (Kivimäenpää et al. 2004). The quantitative needle anatomy was also used in other woody species [e.g. by Viewegh and Cambalova (1993)].

The basic units forming the crown architecture are apical meristems from which terminal, lateral and secondary buds are formed (Hallé et al. 1978). The internal structure of the bud controls the shape and size of the newly formed shoot and thus the overall formation of the crown (Hallé et al. 1978). The final needle size depends on the exogenous conditions at the time of the formation of leaf primordia, mainly from the beginning of July to the middle of October of the growing season. The size of the newly formed shoots is determined by the number of needles arranged in a genetic spiral,

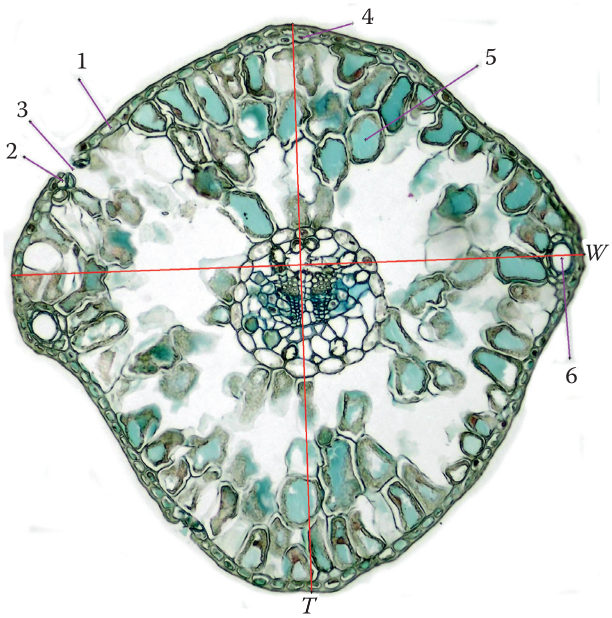

Figure 1. Example of coloured needle section of *Picea abies* [set of planted seedlings analysed by Matějka et al. (2014)], tree sample No. 12 with measured dimensions W = 0.822 mm, T = 0.821 mm, D_1 = 0.220 mm and D_2 = 0.215 mm; modified according to Bracegirdle and Miles (1971)

1 – epidermis with cuticle; 2 – guard cell; 3 – stoma (stomatal pore); 4 – sclerenchyma (hypodermis); 5 – mesophyll; 6 – resin canal; T – measured thickness; W - measured width

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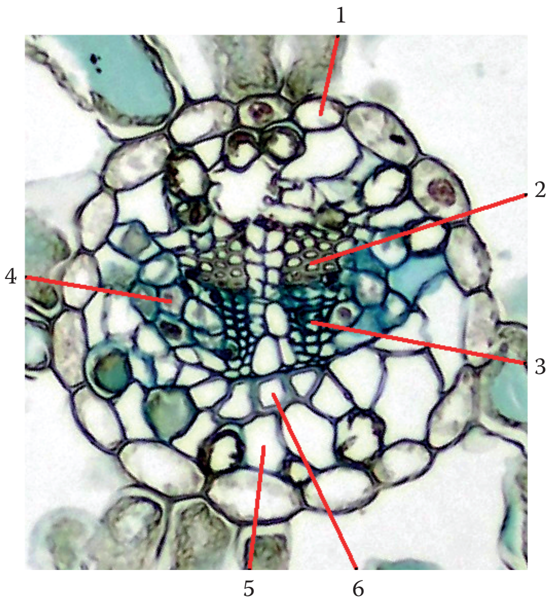

Figure 2. Detail of previous picture with vascular bundle

1 – endodermis; 2 – xylem; 3 – phloem; 4 – group of albuminous cells; 5 – pericycle (transfusion tissue); 6 – sclerenchyma fibres

on the one hand, and by the average length of the internodes [growth length/number of needles per growth (Gruber 1986, 1988)]. It is a principle of the relation between needle structure and phenotype based on the type of branching in the tree crown which was defined by Schmidt-Vogt (1977).

This paper was concerned with young populations only, since the needles of differently old trees can have different shape [compare other tree species such as *Picea rubens* (Ward 2005), and *Pseudotsuga menziesii* (Apple et al. 2002)]. Physiological features of needles are developing according to the tree age (Wieser et al. 2002).

The first study of microscopic phenotype properties of *P. abies* needles was conducted in the Krkonoše Mts. and Jeseníky Mts. (Matějka 2014; Matějka et al. 2014). The authors distinguished several basic types of needles on the basis of the needle width and thickness relation mainly.

The goal of this study is to describe *P. abies* population variability using quantitative microscopic features of needles in the young populations (regenerations) in the Šumava Mts. and compare it with findings from other mountain regions within the Czech Republic. Basic question is: Do natural populations differ substantially in the needle traits?

MATERIAL AND METHODS

Research plots

All plots are situated in localities within *Picea abies* natural distribution. They are listed in Table 1. Because regional differentiation of the *P. abies* genetic structure of a population can be environment-dependent (Di Pierro et al. 2017), all plots were selected in similar environmental conditions: mean air temperature varies between 2.0 °C and 4.5 °C, soils are Podzols or similar types (only plot RL is water-logged). Plots with natural regeneration were accompanied by two sites with planted *P. abies*.

Šumava Mts. In total 7 plots with long-term observations represent *P. abies* natural regeneration on or near mountain tops (Kindlmann et al. 2012; Matějka 2014) Boubín (Boub N and SW), Jezerní Mt. / Čertovo Lake (CL and CU), Plechý Mt. / Plešné Lake (RL, T and PJ4). These localities are under different bark beetle (*Ips typographus*) attack which results in the dieback of parent stands. The stand disintegration is reflected by a diffuse light increase (measured by means of the hemispherical photography) on the level of herb and shrub (regeneration) layers.

Jeseníky Mts. All 5 plots represent two pairs of stands with natural / artificial regeneration and one plot in natural forest (Matějka 2014).

Krkonoše Mts. One plot was selected for comparing the *P. abies* phenotype variability. It was permanent research plot No. 24 (TVP24), which was established in 1980 (Vacek, Matějka 2010). The genetic structure of the mature *P. abies* population was studied by Ivanek et al. (2009). The *P. abies* regeneration study was carried out after the tree layer destruction by wind (Matějka 2014). The first complex result evaluation in the young *P. abies* population was published by Matějka (2014) and Matějka et al. (2014).

Sampling and analyses

Seedling foliage was evaluated by a number of needle quantitative anatomic features that can be determined in a needle cross-section. The needles of young trees (saplings) of the height approximately between 50 cm and 150 cm were sampled during August and September from the sunny part of the crown. In total 25 to 50 trees were selected in a locality. The first-year needles were sampled in the field and they were fixed in an FAA solution: 5 mL of formaldehyde (36% aqueous solution) + 5 mL of glacial acetic acid + 90 mL of ethanol (50% mixture with water).

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Preparation of microscopic samples in the laboratory: each needle was transversally free-hand cut between 1/3 and 1/2 of the needle length (from the needle basis) in an approximate position of the maximum width. Three needles were cut from each sampled individual. The resultant preparations were photographed using the lens with 10× magnification by the Nikon Eclipse E200 microscope (Nikon, Japan) and Canon EOS 1100D digital camera (Canon, Japan). Total needle width (*W*) and thickness (*T*) were measured (Figure 1). Two (approximately) orthogonal diameters (D_1 and D_2) of the vascular bundle were also measured. All measurements were carried out using the PhotoOverlay software (www.infodatasys.cz/software/hlp_FotoOverlay/FotoOverlay.htm).

The vascular bundle area was calculated from two orthogonal diameters D_1 and D_2 following the Equation (1):

$$A_{ves} = \frac{\pi \times D_1 \times D_2}{4} \quad (1)$$

where:

A_{ves} – vascular bundle area;

D_1; D_2 – orthogonal diameters.

The vascular bundle proportion in the needle transection was evaluated using the Equation (2):

Table 1. Basic features of selected sites; diffuse light was measured through hemispherical photos application or approximated from canopy cover (~ values)

Locality (name)	Coordinates S-JTSK		Altitude	Mean temperature	FAZ	Parent stand	Origin of saplings	Year of sampling	No. of trees	Diffuse light
	X (m)	Y (m)	(m a.s.l.)	(°C)						(%)
Šumava Mts.										
Boub N (Boubín N)	1157320	803157	1 310	2.9	8	present		2021	31	32
Boub SW (Boubín SW)	1157504	803766	1 307	3.2	8	present		2021	31	34
CL (Čertovo jezero)	1132007	845019	1 043	4.5	6	present		2020	45	30
CU (Čertovo jezero)	1131127	845857	1 326	2.7	8	damaged by wind (2017)	natural regeneration	2019	47	93
RL (Rakouská louka)	1182111	804241	1 339	2.8	8	present and damaged		2014	25	~90
T (Trojmezí)	1182034	804437	1 343	2.7	8	dead by bark beetle (2005–2008)		2014	25	90
PJ4 (Plešné jezero)	1181892	802972	1 053	4.5	6/7	dead by bark beetle (2007–2008)		2018	48	87
Jeseníky Mts.										
E:P (Eustaška)	1069608	541520	1 196	3.0	8	present	natural regeneration	2013	20	–
A:P	1073535	545126	1 141	3.1	7/8	absent – clearcut	natural regeneration	2013	50	~100
A:U	1073798	544924	1 123	3.2	7/8		planted	2013	49	~85
DS:P	1068072	548642	1 329	2.2	8/9		natural regeneration	2013	50	~100
DS:U	1067983	548851	1 324	2.0	8/9		planted	2013	50	~100
Krkonoše Mts.										
TVP24	984119	639660	1 210	3.3	8	damaged by wind	natural regeneration	2014	49	~100

S-JTSK – triangulation system used for mapping in the Czech Republic; FAZ – forest altitudinal (vegetation) zone

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$$P_{ves} = \frac{A_{ves}}{\frac{\pi \times T \times W}{4}} \quad (2)$$

where:

P_{ves} – vascular bundle proportion in the needle transection (%);

T – needle thickness;

W – needle width.

Hierarchical classification of the studied populations was based on correlation dissimilarities in a similar way like in Matějka (2017), only dendrometric variables were replaced by the mean microscopic needle features for individual trees: needle width, needle thickness and vascular bundle area. Dissimilarities were calculated by the software CorrelDist [part of the DBreleve package, Matějka (2020)] and classification by the unweighted pair-group average algorithm was carried out in Statistica (Version 8, 2007). Single variable differences between localities were tested by single-factor ANOVA in the Statistica package.

RESULTS AND DISCUSSION

Descriptive statistics for four basic needle variables are summarized in Table 2.

The needles were highly variable both among individual trees and among needles in one tree (Figures 3–5). Regarding the previously published needle types (Matějka et al. 2014), inversion types (thickness > width) prevailed. They are followed by intermediate needles. Typical flat needles (thickness << width) were missing. Trees with the needles with sclerenchyma strips had minimal representation. Can it be assumed that these needles were not fully developed? The size of the cross-section area was variable (Figure 5). The combination width and thickness allows to distinguish the localities based on the probability ellipses (Figure 6). The highest needle thickness (Figure 7) was recorded in localities Boubín N, TVP24 (Krkonoše) and in two planted populations at Jeseníky (A:U and DS:U). The smallest needle thickness was observed on Jezerní Mt. in Šumava (plot CU). The needle width (Figure 8) brought a different picture: the gradient was from the smallest needles in localities Čertovo jezero – CL (Šumava) and Eustaška – E:P (Jeseníky) to the largest needles on TVP24 (Krkonoše). The vessel area (Figure 9) corresponded to the needle width – Pearson's correlation coefficient $r = 0.828$ ($\alpha < 0.0001$) had the highest value from all compared correlations.

Table 2. Descriptive statistics of the *Picea abies* needle size in selected sites

Locality	N	Needle thickness [T (mm)]			Needle width [W (mm)]			Vascular bundle area [A_{ves} (mm^2)]			Vascular bundle share on the needle transection [P_{ves} (%)]		
		mean	SD	CV (%)	mean	SD	CV (%)	mean	SD	CV (%)	mean	SD	CV (%)
All	520	1.121	0.158	14.1	0.638	0.118	18.5	0.0343	0.0107	31.2	6.03	0.73	12.1
Boub N	31	1.194	0.126	10.5	0.607	0.077	12.7	0.0351	0.0088	25.2	6.14	0.83	13.5
Boub SW	31	1.086	0.172	15.9	0.564	0.088	15.6	0.0295	0.0084	28.5	6.04	0.69	11.4
CL	45	0.991	0.171	17.3	0.509	0.071	13.9	0.0230	0.0070	30.4	5.76	0.72	12.5
CU	47	0.942	0.225	23.9	0.654	0.096	14.7	0.0286	0.0106	37.2	5.81	0.80	13.7
RL	25	1.101	0.121	11.0	0.697	0.098	14.0	0.0373	0.0102	27.4	6.11	0.78	12.8
T	25	1.129	0.138	12.3	0.708	0.149	21.1	0.0365	0.0115	31.6	5.74	0.68	11.8
PJ4	48	1.158	0.117	10.1	0.662	0.112	16.9	0.0365	0.0104	28.5	5.97	0.74	12.4
E:P	20	1.022	0.115	11.3	0.488	0.048	9.8	0.0236	0.0039	16.6	6.04	0.72	12.0
A:P	50	1.152	0.111	9.6	0.623	0.080	12.9	0.0347	0.0072	20.7	6.15	0.66	10.7
A:U	49	1.195	0.092	7.7	0.707	0.102	14.5	0.0393	0.0072	18.4	5.95	0.75	12.7
DS:P	50	1.122	0.104	9.2	0.582	0.059	10.2	0.0306	0.0053	17.3	5.97	0.66	11.0
DS:U	50	1.208	0.092	7.6	0.645	0.061	9.4	0.0379	0.0061	16.2	6.20	0.66	10.7
TVP24	49	1.205	0.104	8.6	0.772	0.118	15.3	0.0473	0.0119	25.2	6.41	0.66	10.3

N – number of trees; mean – arithmetic average; SD – standard deviation; CV – coefficient of variation

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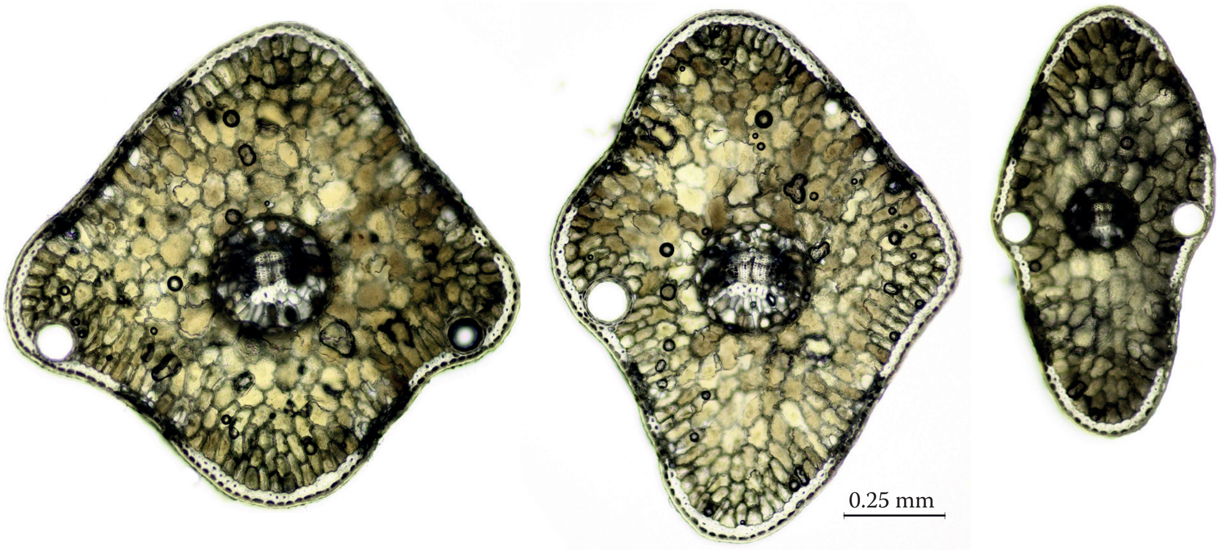

Figure 3. Example of three *Picea abies* needle cuts from tree sample No. 68 of the Trojmezí locality (Šumava); average values $T : W = 1.26$, $T \times W = 1.144\ \text{mm}^2$

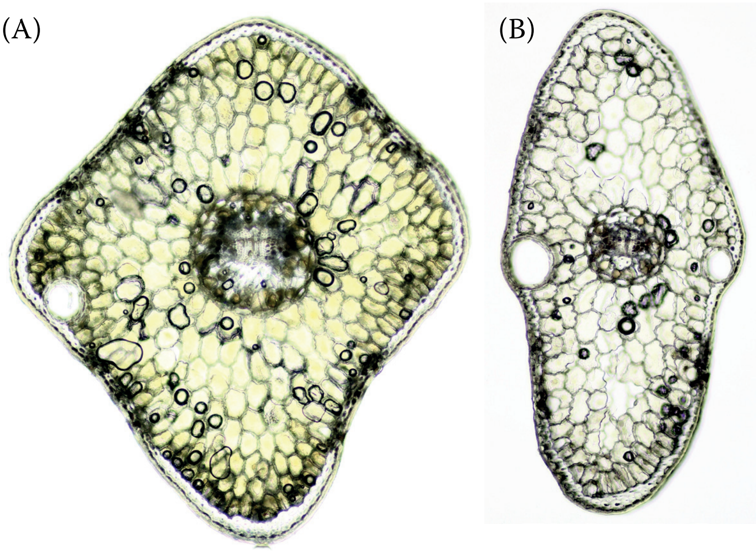

Figure 4. Gradient of *Picea abies* trees in the locality Trojmezí (Šumava) according to $T : W$ ratio on the needle cuts (A) tree sample 73 ($T : W = 1.14$); (B) tree sample 61 ($T : W = 2.09$)

Measure is the same as in Figure 3

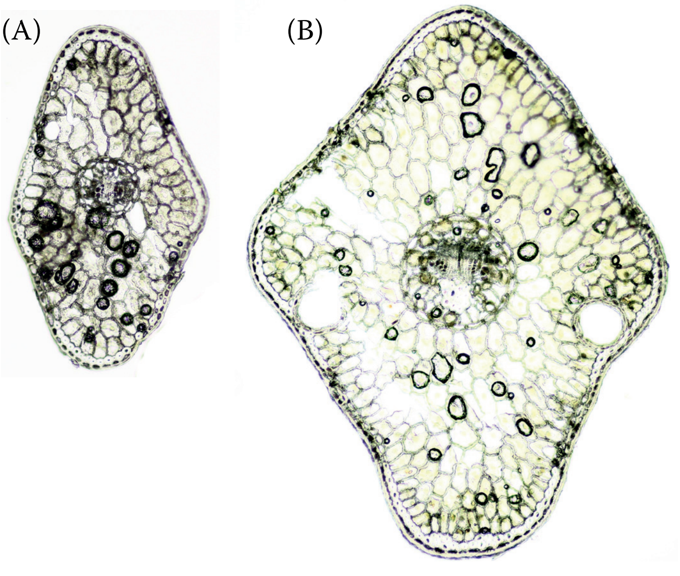

Figure 5. Gradient of *Picea abies* trees in the locality Trojmezí (Šumava) according to cut area ($T \times W$); (A) tree sample 66 ($T \times W = 0.446\ \text{mm}^2$), (B) tree sample 73 ($T \times W = 1.445\ \text{mm}^2$)

Measure is the same as in Figure 3

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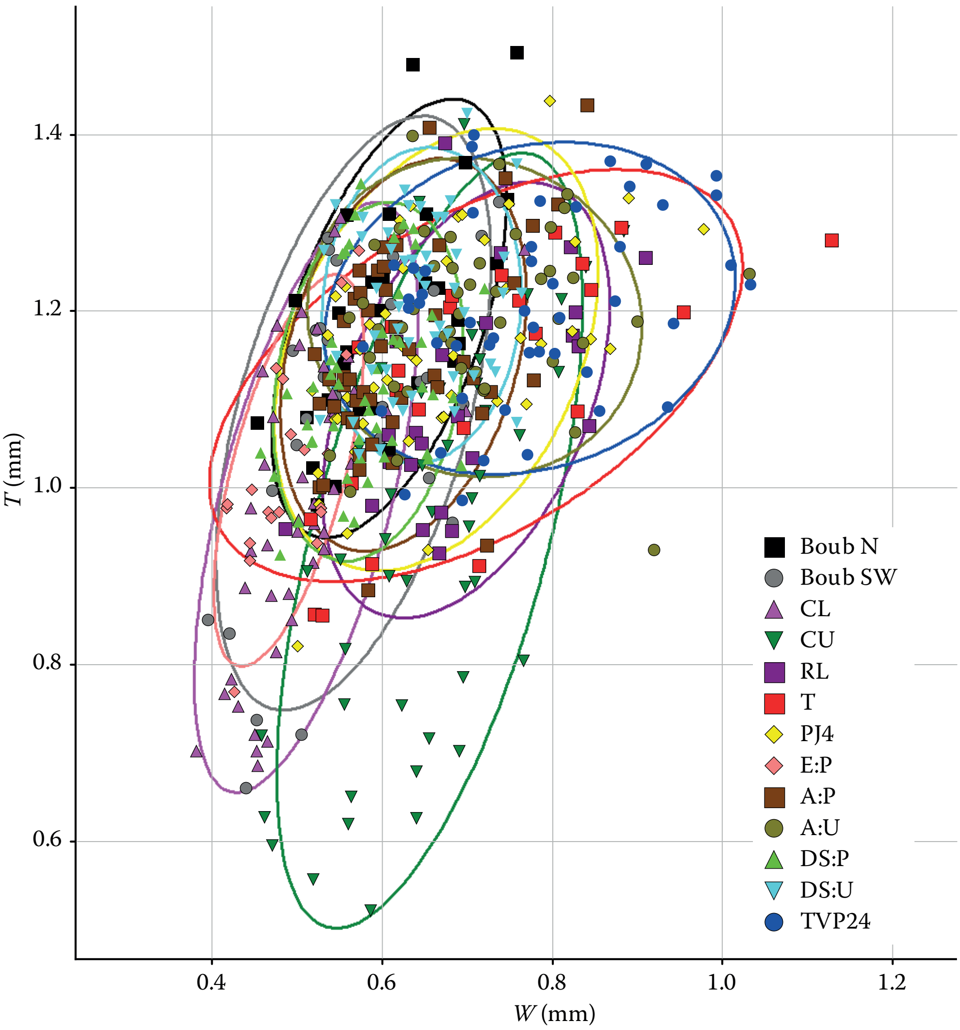

Figure 6. Average width (*W*) and thickness (*T*) of *Picea abies* needles on the transversal cuts; each value is a mean of three samples; distribution ellipses embrace 95% of respective points

The needle thickness shows the coefficient of variation (*CV*) between 7.6 and 23.9%. The lowest values were found in planted populations. The highest *CV* is at localities in the Šumava Mts. The *CV* of needle width is between 9.4% and 21.1%. The highest *CV* values are found in the Šumava Mts. again. The vascular bundle area is variable with the highest *CV* (16.2% to 31.6%). *CV* for the vascular bundle proportion varies between 10.3% and 13.7% (Table 2).

The population variability could be evaluated on the basis of variability of individual parameters (Figures 7–10) or using the size of distribution ellipses for needle width and thickness (Figure 6). The most variable population was found in Šumava, Trojmezí locality, where the original natural forest was located (Figure 4). The highest needle width variability was recorded there. The highest variability of the needle thickness was found at CU locality near the Jezerní Mt. top. The

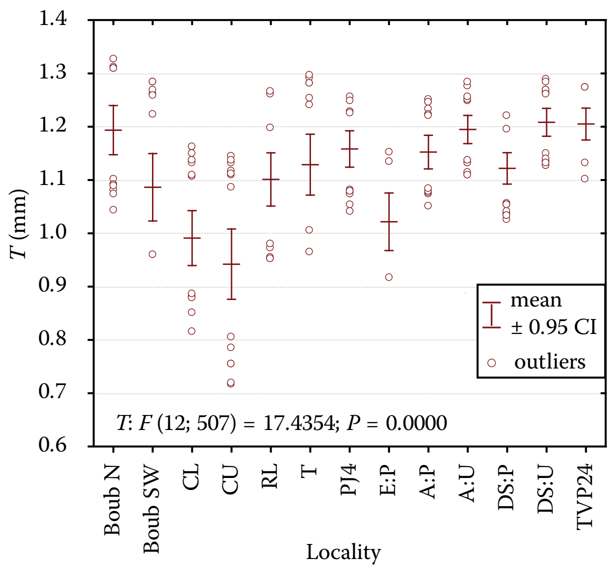

Figure 7. Box-plot for average thickness (*T*) of *Picea abies* needles on the transversal cuts according to the locality

CI – confidence interval

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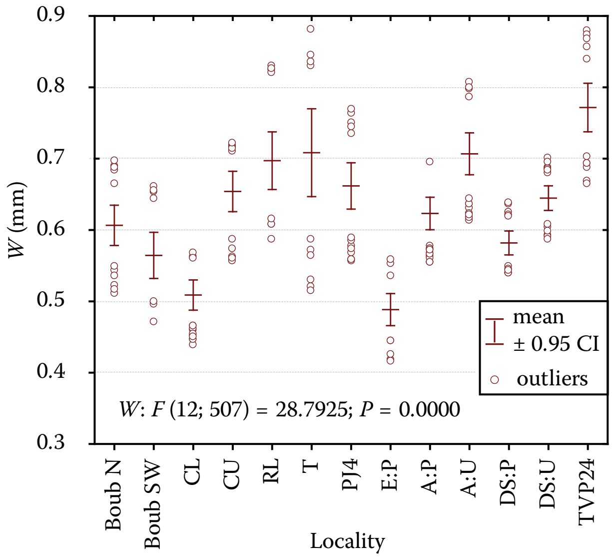

Figure 8. Box-plot for average width (W) of *Picea abies* needles on the transversal cuts according to the locality

CI – confidence interval

univariate ANOVA test for differences in means between localities (average data for individual trees were processed) was significant for each variable: needle width (error of the F-test $\alpha < 0.0001$; Figure 8), thickness ($\alpha < 0.0001$; Figure 8), vascular bundle area ($\alpha < 0.0001$; Figure 9) and vascular bundle area proportion in needle section area ($\alpha = 0.0007$; Figure 10).

Results of the population classification (Figure 11) pointed to strong dissimilarity of planted *P. abies* in the Jeseníky Mts. (localities A:U and DS:U). Populations from the Jeseníky Mts. (A:P and DS:P) and Krkonoše Mts. (TVP24) were in a separate cluster. All populations from Šumava were very similar (together with trees from the natural forest reserve Eustaška in the Jeseníky Mts.). These populations were separated into three subclusters:

(*i*) Localities with medium-wet to wet soils near the mountain tops, with stable *P. abies* stands (Boubín N, RL and E:P). *P. abies* was not planted there during history. Its long-term continuous existence in the tree layer is possible there.

(*ii*) Localities with periodically dried soils, where *P. abies* planting (partial at minimum) was possible during history (Boubín SW, CL, CU).

(*iii*) Two localities with extreme conditions near the Plešné Lake (T and PJ4) where historical stand damage was possible. Namely the plot T is very close to a locality where disturbances during history were studied (Janda et al. 2010; Čada, Svoboda 2011; Čada et al. 2013).

The study based on allozymes (Šnytr, Mánek 2010) revealed that the *P. abies* population from the Boubín Nature Reserve was the most distinct from all other populations studied. Because the precise location of the above-mentioned population is not known, it is not fully comparable with our results.

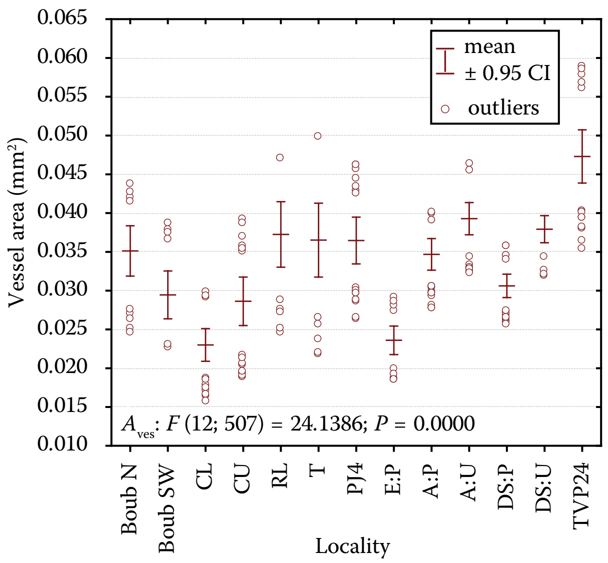

Figure 9. Box-plot for average vascular bundle area (A_{ves}) of *Picea abies* needles on the transversal cuts according to the locality

CI – confidence interval

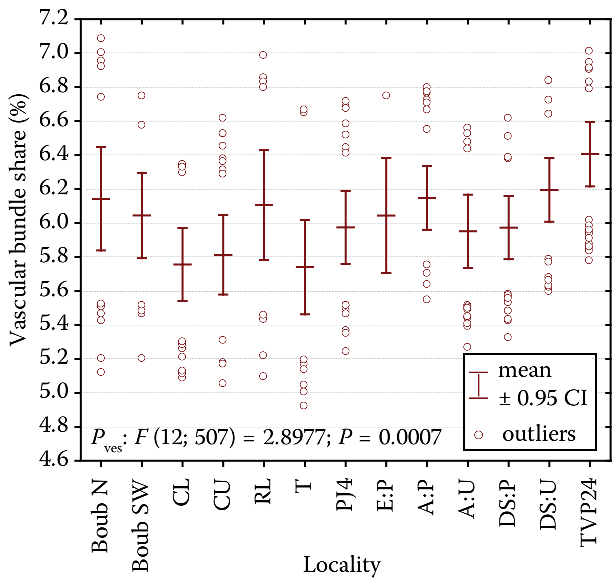

Figure 10. Box-plot for average vascular bundle share (P_{ves}) for *Picea abies* needles on the transversal cuts according to the locality

CI – confidence interval

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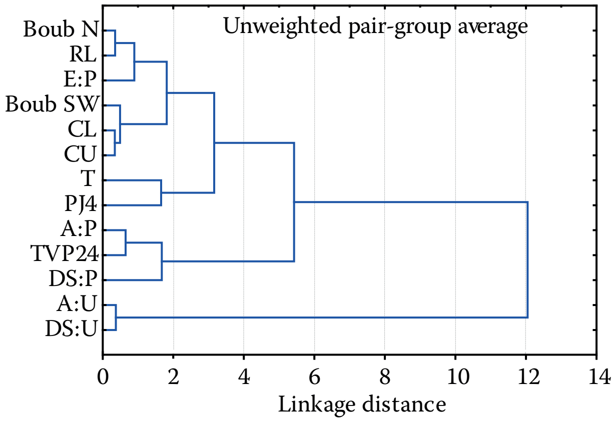

Figure 11. Unweighted pair-group average classification of *Picea abies* populations on selected localities on the base of correlation dissimilarities

The populations on Jezerní Mt. and Trojmezná Mt. had relatively low genetic (allozyme) diversity and high phenotype variability in probably comparable localities CU and T.

Neutral processes also affect genes controlling quantitative phenotypic traits such as growth and tree architecture in some species (Gömöry et al. 2013); so we can formulate a hypothesis about the relation between phenotype and genetic variability of populations.

In the Czech Republic, genetic differentiation of *P. abies* populations distributes it into two regions: south-western part represented by Šumava, Krušné hory Mts. and partly the Krkonoše Mts. belongs to the first region, Beskydy, Jeseníky and partly Krkonoše Mts. are in the second one (Tollefsrud et al. 2008; Figure 3). This difference can be a reason for phenotype variability among the studied localities.

Nevertheless, it is known that average air temperature explained the greatest amount of the *P. abies* among-population variation (Frank et al. 2016). Our data do not give evidence for it, because altitudinal and temperature variation among the studied localities is low.

Westergren et al. (2018) bring evidence for differentiation among populations according to the position in the species area. Our results are not comparable in this point of view because all our studied populations are relatively located in the centre of the species distribution area and within the altitudinal zone of the species optimal occurrence.

Planting material from forest nurseries is strictly selected that both growth and phenotype properties are influenced (e.g. Jurásek et al. 2009). It was reflected in distinct features of individuals from natural regeneration and planting in the Jeseníky Mts. All parameters of the needle size were higher in the planted trees.It indicates artificial selection of fast-growing trees which can lead to the instability of the resulting stand.

CONCLUSION

The quantitative needle anatomy could be used for the study of *P. abies* populations. It is relatively rapid and not so expensive as proper genetic [e.g. DNA or allozymes; Westergren et al. (2018)] studies. Basic traits for this purpose are microscopic needle width and thickness.

The method makes it possible to evaluate heterogeneous populations in the early (juvenile) phase of the individual's development in similar abiotic conditions on ecologically optimal habitats. It can then support other genetically determined functions and expand provenance attempts. The research confirmed the high variability of the needle features during the growth of coniferous populations, which adapt to specific habitat conditions for sun radiation which influences the growing crown.

The among-population variability of *P. abies* in the Czech Republic is high. Planted populations can be distinct by low variability of needle traits, as visible from two pairs of plots with natural and planted *P. abies*, because plants are strictly selected within several developmental stages (seed preparation, young plant selection in a nursery, selection before export from nursery to planting) including transplantation stress, and natural selection of plants on the site as adaptation to the environment.

Populations belonging to natural stands embrace the width spectrum of phenotype variability, possibly as a result of the parent stand state – compare two limit examples, Eustaška locality (Jeseníky Mts.) with no known disturbance and low variability, and Trojmezí locality (Šumava Mts.), where wind and bark beetle disturbances were recorded repeatedly and phenotype variability is high.

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