JOURNAL OF FOREST SCIENCE, *60*, 2014 (3): 96–108

Phenotype features in juvenile populations of *Picea abies* and their growth

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ABSTRACT: The young populations of Norway spruce (*Picea abies*) can be evaluated in terms of both variability in the quantitative characteristics and share of different phenotypes according to the needle types. A set of two-years-old seedlings produced in the nursery and several populations of natural regeneration in the Krkonoše Mts. and the Krušné hory Mts. were evaluated using the needle anatomy, size and colour. The tree height growth was measured simultaneously. The growth was related to some needle features. Four basic anatomical types of needles were distinguished in the mountain Norway spruce seedlings: flat needles, intermediate needles, inversion type and sclerotized needles. The growth of natural regeneration populations is mainly correlated with the needle width and the length/width ratio. The population variability according to growth can be related to the variability of some phenotype characteristics. This relationship differs in natural regeneration and planting.

Keywords: anatomy; height growth; needle types; population variability; seedlings

The type differentiation of forms (phenotypes) of Norway spruce (*Picea abies* [L.] Karst.) mature trees has been used for a long time. It is based mainly on the appearance of tree crowns and branching (Samek 1964; Fanta 1974, 1976; Schmidt-Vogt 1977). Attention has also been paid to a change in branching and to the growth of secondary and/or tertiary crown structures in relation to the regeneration of a tree after an air-pollution stress (Cudlín et al. 1999, 2001). Nevertheless, methods of the type differentiation of young spruce trees up to the advance growth stage and/or small pole stage have not been known until now. Scientific publications on the phenotype of common tree species with high economic importance such as *P. abies* are scarce. For instance, Web of Science refers to only two papers using the keyword "phenotype" together with "*Picea abies*". The first paper (Popov 2013) deals with *Picea abies* and *Picea obovata* distinguishing. The second one (Danusevicius, Lindgren 2002) uses the spruce phenotypes for tree improvement (without concretization of phenotype features).

It is to assume that various morphological and anatomical characteristics of *Picea abies* develop in a similar way during ontogenesis, like in the other spruce species, e.g. *Picea rubens*, where a change in the needle cross-section was described in trees of different age (Ward 2005). Currently, research on population variability (not only in spruce) is directed at the investigation of genetic variability (Tollefsrud et al. 2008); however, the knowledge of phenotype variability is important particularly for its practical applications in the field of silviculture. This importance may be accentuated e.g. by testing the relation between the growth rate of trees and their quantitative phenotype features as will be shown below.

The type differentiation of young spruce trees appears to be important both in young stands, e.g. during the cleaning of advance growths, and in the production of planting material and its preparation in forest nurseries (Jurásek et al. 2009). Let's assume that different phenotypes differ in growth parameters and so they can create different functional components of a future stand. More exactly

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Supported by the Ministry of Agriculture of the Czech Republic, Project No. QI112A170.

expressed, different trees can have a different position within the spectrum of r- and K-growth strategies (MacArthur, Wilson 1967; Pianka 1970; Reznick et al. 2002). The species classification on the scale of sun demanding and shade-tolerant tree species is also disputable as advised by Kindlmann et al. (2012). Therefore a discussion about spruce trees with different growth strategy started: trees with "ruderal" strategy that are able to fill up the stand space thanks to their fast growth while trees with "climax" strategy are growing slowly but they create the stable frame of the stand that exhibits an increased resistance to (especially mechanical) disturbances. Different share of trees with different growth strategy was observed when the growth of populations from natural and artificial regeneration was compared (Matějka, Leugner 2013). From the aspect of the long-term stand dynamics, it is a different selection (r- or K-selection) according to the presence and/or absence of disturbance factors. As the frequency of disturbances (e.g. the occurrence of strong winds) increases with the altitude, it is possible to expect that a wider spectrum of trees according to their growth strategy should be represented in the population at higher altitudes [8th (spruce) or also 7th (spruce with beech) forest/vegetation/altitudinal zone – FAZ] compared to the population at lower altitudes where disturbances are not so frequent and the long-term dynamics of stands is usually governed by the gap model (so called small developmental cycle; cf. Kindlmann et al. 2012).

Phenotype variability is a reflection of genetic variability of the population, i.e. of genetic diversity. The spruce phenotype of extremely young plants (up to 3 years) is used for the breeding programmes (Danusevicius, Lindgren 2002). The phenotype variability is a feature related to the structure and dynamics of whole ecosystems (Wehenkel et al. 2010).

Phenotype variability within young populations is also reflected in variability of the growth rate of trees within the whole population. However, growth rate cannot be directly compared because the evaluated populations consist of trees of different size and usually of different age. This is the reason why a new method for the evaluation of growth variability under these conditions is proposed below.

The objective of this paper is to analyse needle morphology and anatomy of young spruce trees in order to distinguish the trees with different growth rates. Two types of populations were analysed:

– a set of seedlings grown in a forest nursery was an example of extremely young populations (2-years-old plants);
– advance growth (regeneration) in several localities in the Czech Republic – in the Krkonoše Mts. (Giant Mts.) and Krušné hory Mts. (Ore Mts.) was an example of young populations (about 10-years-old trees).

There arises a question about the relationship between growth rate and parameters of needles as the basic phenotype feature in young trees. These relationships were studied by means of models (regression models). The models describing the relation between age, tree size (mainly tree height) and (height) growth, known as growth models, are currently undergoing rapid development (Hasenauer 2006; Pretzsch 2009). Results are mostly applied to the analysis of production potentials of forest stands. This is the reason why these models are hardly applicable to the analysis of the growth of young populations of a regenerating stand. Blujdea et al. (2012) wrote in this sense: "The possibility of estimating young trees biomass is rather limited because forest yield tables are constructed starting from higher thresholds of proxy, such as diameter or height, and lack of availability of allometric equations". The growth models are similar to the allometric relations, which are based on growth theory (e.g. Pretzsch et al. 2013). This paper is a continuation of previous study (Matějka, Leugner 2013).

MATERIAL AND METHODS

Seedlings produced in a nursery. The planted material was collected for research on the structural characteristics of needles of Norway spruce with different growth strategy in 2012. Research material was sampled from the Vítkovice forest nursery (altitude 690 m a.s.l.) in the Krkonoše Mts. on May 9th, 2012. These were annual shoots of two-year *Picea abies* seedlings of the Krkonoše origin from the 8th FAZ (forest altitudinal zone) that represented the whole growth spectrum of this section (registration number of the section source: CZ-2-2A-SM-3044-22-8-H). The analyses were carried out in 90 trees from the nursery. The seedlings were planted in open beds on peat substrate with long-term effect of fertilizer. The following parameters of two-years-old seedlings were measured: height of the aboveground part, height in the preceding year (2010), root collar diameter, root length (length of the longest root; plants with one root prevailed), dry weight of the aboveground part and number of branches.

Regeneration in the Krkonoše Mts. The plots were localized in the area of the 8th FAZ in the eastern Krkonoše Mts., so that their natural conditions were as similar as possible. Six plots (3a, 5, 6, 7, 8, PRP 24) in total were identified for observations that were also used in the previous study (Matějka, Leugner 2013). A forest grew on all plots in the past; this was verified in stable cadastre maps. Plot 9 is practically identical with the long-term permanent research plot PRP 22, where various investigations have been conducted since 1980 (Vacek et al. 2007; Ivanek et al. 2009). The average tree height varies between 74 and 179 cm in natural regeneration, and between 107 and 296 cm in planted trees (Table 1).

Regeneration in the Krušné hory Mts. Five plots (Klínovec A, B, C; Špičák A, B) in total were identified in the area of the 8th and close 7th FAZ for the study of natural regeneration: three plots in Klínovec Mt. area and two plots on Božídarský Špičák Mt. Artificial regeneration (planting) was established in the closest proximity of the two plots and was also measured.

Vegetation conditions were documented on each plot by a phytocoenological relevé. In comparison with similar plots in the Krkonoše Mts., the Krušné hory Mts. localities are obviously areas with more fertile soils. It is documented by higher species richness and simultaneously by the recurrent presence of more demanding species (e.g. *Urtica dioica*, *Senecio ovatus*, *Oxalis acetosella*, *Mycelis muralis*) (Matějka 2012). Description and site characteristics of the particular research plots are given in Table 1. The average tree height was 92 to 118 cm in natural regeneration and 78 to 130 cm in planted trees (Table 1).

Field work. From July to September (2011 in the Krkonoše Mts., 2012 in the Krušné hory Mts.) randomly selected individuals in the regeneration on the plots were measured. The measured characteristics were total height of a tree in the given year (H with the index of the year of measurement) and height increment in the last six years (P with the index of the respective year). The heights of a tree in the particular preceding years were calculated additionally.

Twigs for the analysis of needle size and colour were taken from trees measured on some plots. These twigs were put into polyethylene bags to be transported and analysed in a laboratory.

Measurement of needle size. Needles of the last needle age class were removed from typical twigs. Twenty intact needles were placed on a transparent adhesive tape. This adhesive tape was placed on a transparent foil. The needle series prepared in this

Table 1. Basic features of selected plots and their localization (JTSK coordinates). The average tree height (mean ± standard deviation) in the year of measurement

Plot	Regeneration	JTSK-X (m)	JTSK-Y (m)	Altitude (m)	Temperature (°C)	Forest site type*	Trees measured	Tree height (cm)
Krkonoše Mts. (Giant Mts.)								
3a	natural	984,759	645,598	1,192	2.5	8N0	127	168.3 ± 52.8
5	natural	986,310	644,359	1,161	3.2	8K2/8N1/8N3	132	179.0 ± 67.9
6	planting	986,893	647,355	1,209	3.0	8K2	150	296.2 ± 58.6
7	natural and planting	983,740	638,618	1,295	3.0	9K1/8Z3	136	112.9 ± 37.3$^{1)}$
8	natural and planting	985,623	639,533	1,198	2.8	8K2/8K4	150	149.8 ± 42.8$^{2)}$
PRP 24	natural	984,119	639,660	1,210	3.3	8K4	187	73.7 ± 28.6$^{3)}$
Krušné hory Mts. (Ore Mts.)								
Klínovec A	natural	993,100	841,256	1,179	3.2	8Z4	80	94.4 ± 25.9
Klínovec B	natural and planting	993,451	841,052	1,146	3.5	8Z4	80	92.3 ± 26.2$^{4)}$
Klínovec C	natural	994,437	840,411	1,230	3.1	8Z4	80	96.2 ± 25.0
Špičák A	natural and planting	992,502	846,012	1,083	3.8	8A1 (7**)	80	112.7 ± 25.0$^{5)}$
Špičák B	natural	992,467	845,804	1,117	3.6	7Z8 (8**)	80	118.8 ± 29.0

JTSK coordinates – coordinates of the Uniform Trigonometric Cadastral Network, Temperature – average air temperatures during 1961–1990 were modelled using the PlotOA software (Matějka 2009), *nomenclature of the forest site types see e.g. Viewegh et al. (2003), **forest altitudinal vegetation zone according to the average temperature, $^{1)}$only natural regeneration, planted trees 130.2 ± 55.5 cm (133 trees), $^{2)}$only natural regeneration, planted trees 106.6 ± 21.7 cm (18 trees), $^{3)}$height in 2010, $^{4)}$only natural regeneration, planted trees 77.8 ± 12.7 cm (60 trees), $^{5)}$only natural regeneration, planted trees 129.9 ± 40.8 cm (60 trees) way were scanned at a resolution of 600 dpi. The images were stored as TIFF format files with lossless data compression. The scans were processed using the TopoL programme (TopoL Software s.r.o., Prague, Czech Republic) (www.topol.eu) in which the exact outline of each needle was manually determined using an appropriate magnification. On this basis the perimeter (*o*) and area (*p*) of each needle were calculated. Other software, e.g. FotoOverlay programme (IDS, Prague, Czech Republic) (www.infodatasys.cz/software/hlp_FotoOverlay/FotoOverlay.htm) (Matějka, Leugner 2013), can be used in a similar way. Applying these data, the length (*rl*) and average width (*rw*) of a needle were calculated as if these values corresponded to a rectangle of the identical perimeter and area – Equation 1:

$$rl = \frac{1}{4}\left(o + \sqrt{o^2 - 16p}\right) \qquad rw = \frac{1}{4}\left(o - \sqrt{o^2 - 16p}\right) \quad (1)$$

If the mean error of the point position on the needle circumference equals to one pixel size (Δ = 0.0423 mm), based on the law of the mean error accumulation, it is possible to estimate the mean error of the needle area (ε) as follows from Equation 2:

$$\varepsilon^2 = n\left(\frac{\partial P}{\partial x}\right)^2 \Delta^2 = n\left(\frac{l}{2n}\right)^2 \Delta^2 = \frac{l^2}{4n}\Delta^2 \qquad (2)$$

where:

n – number of break points on the needle circumference,
Δ – pixel size,
l – perimeter,
P – area,
x – the position of the circumference break point on an axis orthogonal to the circumference line.

The result of the example calculation is mean error $\varepsilon = 0.072\ \text{mm}^2$ (small needles, $n = 20$, $l = 15$ mm) to $\varepsilon = 0.164\ \text{mm}^2$ (large needles, $n = 15$, $l = 30$ mm).

Total length (*l*) and maximum width (*w*) of each needle were also measured, in both cases as the shortest line connecting two relevant points.

These measurements were done for a set of randomly selected trees and/or for trees in which other analysed characteristics were determined.

Mean weight of needles. At the locality of PRP 24 in the Krkonoše Mts., which was analysed in detail, twigs, or their parts with the first needle age class were air-dried. A hundred needles were counted off among these samples and they were weighed (weight M_{100}). The whole sample of needles was also used for a chemical analysis.

Nutrient status. Dried needles of the first needle age class from the locality of PRP 24 in the Krkonoše Mts. were subjected to a chemical analysis to determine the content of the elements N, P, K, Ca and Mg. The analyses were done in the Josef Tomáš Laboratory at Opočno using these methods: H_2SO_4/H_2O_2 mineralization, distillation method to determine total N, spectrophotometric determination of P, AAS to determine Ca and Mg, atomic emission spectrophotometry for K.

Needle colour. The scans were also used to read off the needle colour. Needle colour was measured in a set of 20 needles selected for measurement of their size. Damaged needles or needles with distinct colour change, mostly as a result of fungal infection, were excluded from the set. Measurements were done using the PlotOA software (IDS, Prague, Czech Republic) (Matějka 2009), where an average colour was determined in the surroundings of three points on each needle. The points were located randomly, approximately in the middle of the needle, in the upper and lower third of the needle while places with potential small local damage to the needle were avoided. The colour was determined in the proximity of 5 pixels (i.e. in a square of 11 × 11 pixels). Three colour components (R–G–B) were read off for red, green and blue colour.

Colour variability (var_{RGB}) of needles in trees in the population was evaluated by the expression (Eq. 3)

$$\text{var}_{RGB} = \text{STD(RGB\_R)}^2 + \text{STD(RGB\_G)}^2 + \text{STD(RGB\_B)}^2 \quad (3)$$

where:

STD(x) – standard deviation of variable x,
RGB_R, RGB_G, RGB_B – three colour components (one average value for one tree).

Anatomic features of needles in seedlings. Seedling foliage was evaluated by a number of quantitative anatomic features of needles that can be determined in a needle cross-section. For these purposes the needles sampled from fresh material were fixed in a FAA solution: 5 ml of formaldehyde (36% aqueous solution) + 5 ml of glacial acetic acid + 90 ml of ethanol (50% mixture with water). Four representative one-year needles were taken from each seedling for analysis. A block of standard length was cut out from their middle part; it was processed by the method of permanent preparations of the botanical microscopic technique – dehydration in an ethanol series, transfer to xylene and saturation with paraffin in a Shandon Citadel TM tissue processor – carousel type. The examined material was embedded into BIOPLAST EXTRA at a temperature of 58°C. Paraffin blocks were cross-cut in three to four series in the HM 325 rotary microtome (MICROM GmbH, Neuss, Germany). The section thickness was 4–5 μm. After deparaffinization, the samples were stained by the basic method of haematoxylin-eosin, malachite green – acid fuchsin and alcian blue-safranin. Stained preparations were encased into enthellan with xylene (Merck). The resultant preparations were photographed using the lens with 10- and 40-times magnification. Based on a digital image analysis, the total area of needle section, vascular cylinder, xylem, phloem and groups of albuminous cells and their mutual relations were determined. Total needle width and thickness were also measured. The means of the respective values from 3 to 4 needles were processed. All seedlings were not analysed microscopically because it was not possible to make suitable preparations from undamaged needles for all of them. The analyses were carried out in the laboratory of University of Ostrava by Václav Krpeš working group. Additional measurements and microphotos were done in IDS. Width and thickness of the needle were measured in the microphotos using the PhotoOverlay software (www.infodatasys.cz/software/hlp_FotoOverlay/FotoOverlay.htm).

Evaluation of population growth variability. Growth was modelled using a non-linear regression model (Eq. 4):

$$P_y = a \times h_{y-1}^{b} \tag{4}$$

where:

a, b – parameters,

h_y – height in the year y,

P_y – increment in the year y.

The model was fitted by the least-squares method in STATISTICA software (StatSoft, Inc., Tulsa, USA) (nonlinear estimation, Levenberg-Marquardt method; Hill, Lewicki 2007). Growth variability in each studied spruce population was evaluated on the basis of indefiniteness of the above-mentioned model, i.e. by means of the standard error of parameters a and b (SE_a and SE_b) and especially by the standard deviation of residuals (s_{resid}) calculated as a difference between measured increment and modelled increment.

Modelling was always performed for the year (y) preceding the year of measurement, i.e. for 2010 in the Krkonoše Mts. and 2011 in the Krušné hory Mts.

RESULTS AND DISCUSSION

Needle type differentiation

Spruce seedlings were analysed. The plants were small: average diameter of the root collar d = 1.29 ± 0.39 mm (mean ± standard deviation), average height of the seedling h = 7.47 ± 2.43 cm, height

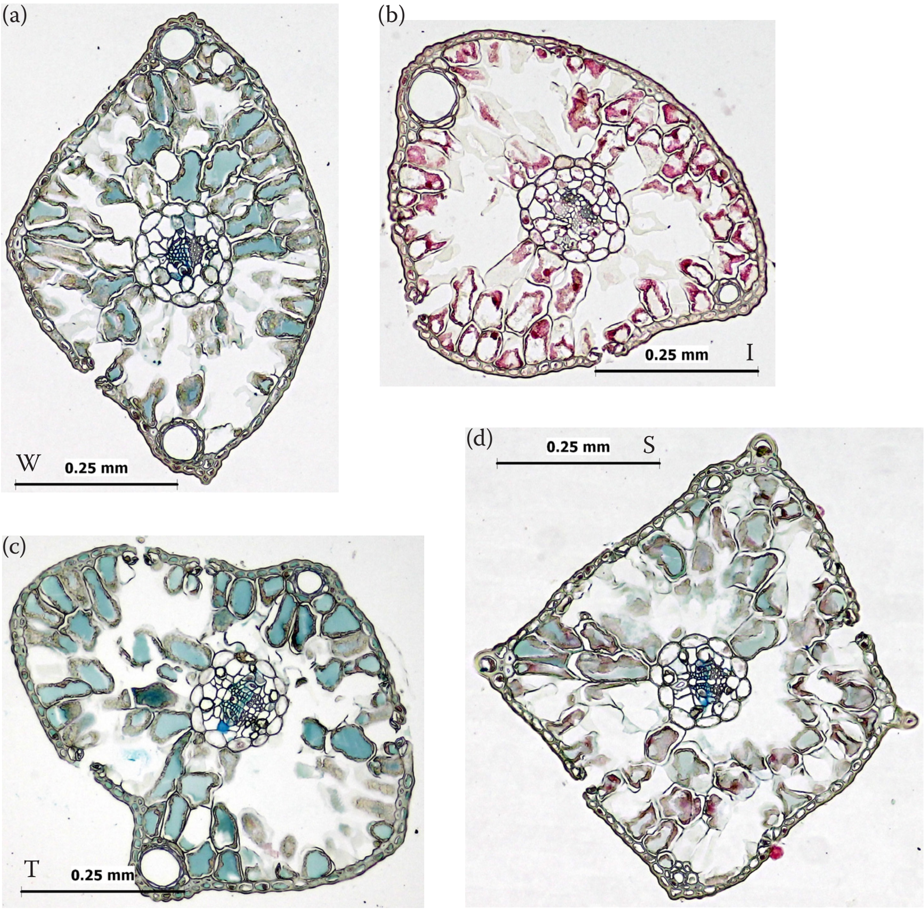

Fig. 1. Different types of cross-sections through young needles of *Picea abies*: (a) W – flat needle, (b) I – intermediate needle, (c) T – inversion needle, (d) S – sclerotized needle with surface strips

in the preceding year h_{2010} = 2.28 ± 0.82 cm, root length ROOT = 13.3 ± 4.7 cm, number of branches BRANCH = 1.1 ± 1.4, weight of aboveground part M_{AG} = 0.43 ± 0.30 g. For needle type differentiation, only those needles were selected that showed full development, which can be documented by the presence of two essential oil ducts. Four basic types of needles were distinguished using microscopic images of the cross-sections of needles (Fig. 1). The thickness (*mt*) to width (*mw*) ratio of a needle was used as the basic characteristic for this differentiation. Table 2 shows the percentage of the individuals according to needle-types in the analysed material:

- type W – flat needles: the width of the needle is distinctly larger that its thickness. Sclerotized strips on the surface may occur along the two lateral edges,
- type I – intermediate needles: the width and the thickness of the needle are approximately identical; sclerotized strips on the surface are missing,
- type T – inversion type: the width of the needle is smaller than its thickness; sclerotized strips on the surface are missing,
- type S – sclerotized needles with surface strips: the width and the thickness of the needle are approximately identical, there are four sclerotized strips. The needle has an approximately square cross-section.

Moderate sclerotization was observed in several needles of the preceding types.

Table 2. Counts of individuals according to the type of needles in the set of two-year mountain spruce seedlings grown in the Vítkovice nursery

Type of needle	*n*	Share (%)	d (mm)	h (cm)
W	16	42	1.4	8.4
(W)	4	11	1.4	7.8
I	9	24	1.6	9.5
(T)	1	3	1.6	11.0
T	1	3	2.3	12.0
W(s)	3	8	1.4	6.7
I(s)	1	3	1.4	6.0
S	3	8	1.3	8.2

types of needles were determined using the ratio microscopic thickness of needle/microscopic width of needle (*mt*/*mw*) where: type W (< 0.90), (W) (0.90–0.95), I (0.95–1.05), (T) (1.05–1.10) and T (> 1.10), W(s) – moderately sclerotized (< 0.90,) I(s) – moderately sclerotized (0.95–1.05); S – presence of sclerotized surface strips (see Fig. 1); *n* – number of individuals, d – average diameter of the root collar, h – average height of the seedling

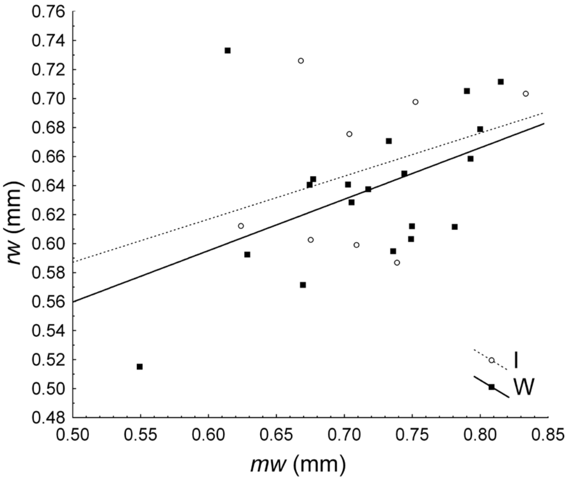

Fig. 2. The relation between microscopic width of needle (*mw*) and calculated average width of needle (*rw*) in samples of seedlings from the tree nursery is significant by the needle type – flat needle (W) (r = 0.48, P = 0.039) but insignificant by the type – intermediate needle (I) (r = 0.34, P = 0.41)

Although only a small number of needles was analysed (it was not possible to make an evaluable microscopic preparation for all needles) and the representation of some types is low (sometimes the type is represented by one seedling), the above-mentioned types seem to have different growth characteristics (Table 2). Seedlings with the inversion type of needles reach larger dimensions while seedlings with sclerotized needles were mostly smaller.

As the seedlings were grown in a forest nursery in uniform environmental conditions, it is to assume that the classification of seedlings according to the needle type is conditioned genetically. It does not depend e.g. on the insolation of the individual and given branch as it can be in the case of older trees, because only small plants (approximately 10 cm in height) from the nursery were analysed.

No similar typification has been done for trees from measured populations in the Krkonoše Mts. and the Krušné hory Mts. Nevertheless, all above-mentioned types were observed in the Krkonoše Mts. (e.g. on PRP 24) during the evaluation of other characteristics of this material. The variability of types in the Krušné hory Mts. was lower, sclerotized needles with surface strips were practically missing. A relation between the needle type and the phenotype according to branching (Fanta 1974; Schmidt-Vogt 1977) is possible, but unknown to date.

It is to note that the macroscopically determined width of the needle (*rw*) and the respective needle area correspond to the maximum photosynthetically active profile of the needle. However, such a width may differ and it usually differs from the width determined

Table 3. Modelling of the height increment of spruce populations from natural regeneration and planting based on the regression equation $P_y = a \times h^b_{y-1}$(data on all analysed localities)

Region	Regeneration	Year	*n*	*a*	*b*	P (cm)		
						(h = 50 cm)	(h = 100 cm)	(h = 150 cm)
Krkonoše Mts.	natural	2010	731	5.030	0.2697	14.4	17.4	19.4
	planting	2010	301	0.09451	1.131	7.9	17.3	27.3
Krušné hory Mts.	natural	2011	400	0.07746	1.095	5.6	12.0	18.7
	planting	2011	120	0.04376	1.292	6.9	16.8	28.4

n – number of measured trees, all regression coefficients are statistically significant on the level $\alpha < 2\%$, the last three columns show an average modelled height increment in trees of three selected height categories

by means of the microscopic cross-section of the needle (*mw*). This difference is related to the type of needle. The closest relationship of both parameters was found out in flat needles (type W) and in sclerotized needles (type S). On the other hand, a minimum relationship can be expected in inversion type T (Fig. 2). Both flat and inversion types are macroscopically near-to-identical.

Growth of populations of natural and artificial regeneration

The more distinctly exponential height growth of young populations from artificial regeneration was recorded both in the Krkonoše Mts. and in the Krušné hory Mts. (Table 3). The height growth of populations from natural regeneration is slower but they probably create more stable stands as already reported (Matějka, Leugner 2013).

Lower average (modelled) height increment in populations of natural regeneration was found out in the Krušné hory Mts. while the most obvious differences were in the smallest trees (model height of 50 cm). Height increment for the modelled category of the tallest trees (150 cm) was comparable in both mountain ranges. Differences between the two mountain areas cannot be interpreted directly because measurements were done in two climatically different years.

Table 4. The growth variability in the Norway spruce populations based on the regression $P_{2010} = a \times h^b_{2009}$

Regeneration	Locality	*n*	*a*	*b*	SE_a	SE_b	s_{resid}
Krkonoše Mts.							
Natural regeneration	3	127	0.4475*	0.768*	0.2008	0.0901	6.27
	5	132	1.0819*	0.565*	0.5156	0.0937	7.61
	7	135	3.3315*	0.418*	1.0988	0.0753	6.78
	8	147	2.4188	0.374*	1.2488	0.1067	5.65
	PRP 24	186	1.6287*	0.564*	0.4411	0.0647	5.69
Planting	6	150	5.8171	0.383*	2.9626	0.0947	12.50
	7	132	0.0045	1.677*	0.0029	0.1268	6.11
	8	18	0.4099	0.838	0.8272	0.4609	5.20
Krušné hory Mts.							
Natural regeneration	Klínovec A	80	0.0810	1.104*	0.0591	0.1658	3.46
	Klínovec B	80	0.0527	1.112*	0.0453	0.1914	3.06
	Klínovec C	80	0.0648	1.097*	0.0621	0.2150	3.60
	Špičák A	80	0.1289	0.979*	0.0912	0.1558	2.95
	Špičák B	80	0.1748	0.976*	0.1258	0.1594	4.28
Planting	Klínovec B	60	7.36	–0.026	14.521	0.4873	3.73
	Špičák B	60	0.2648	0.921*	0.1345	0.1097	4.49

n – number of measured trees; SE_a and SE_b – standard errors for parameters *a* and *b*; s_{resid} – standard deviation of residuals (measured increment – modelled increment), *parameters estimated at the level $\alpha \leq 5\%$

Population structure

Similarly like the variability of populations is different when the growth of their trees is compared, there also exist differences in the variability of foliage in these populations. When the populations from natural regeneration and the populations from artificial regeneration are compared in the Krušné hory Mts., the former show both lower growth variability (Table 4) and lower variability in the needle shape (given by the needle length/ width ratio (*rl*/*rw*), evaluated by both standard deviation and the total range of values (Table 6).

There exists a difference in the range of average needle width. The average needle width (*rw*) was larger or equal to 0.75 mm in one tree from natural regeneration (1.7% of the studied trees) while it was 5 trees (17% of the studied trees) in artificial regeneration in the Krušné hory Mts. The standard deviation of the needle width was higher than in the group of planted trees compared with the natural populations (Table 6). All these data indicate different phenotype composition of both types of populations. The variability of the evaluated populations can also be illustrated by variability of needle colour (Table 5) that is highest in the man-made population (planted trees), which can be caused by insufficient nutrition at extreme microsites.

Table 5. Variability in the needle colour (var$_{RGB}$) in the analysed localities

Regeneration	Locality	var$_{RGB}$
Natural regeneration	Krkonoše Mts. PRP 24	277.0
	Krušné hory Mts. Klínovec A	173.5
	Krušné hory Mts. Klínovec B	192.6
	Krušné hory Mts. Klínovec C	238.1
Planting	Krušné hory Mts. Klínovec B	316.9

The characteristics of needles on PRP 24 in the Krkonoše Mts., which were analysed in detail, were different. Needle length was distinctly larger than in the Krušné hory Mts. but needle width was smaller, so that a markedly different value of the shape index (*rl*/*rw*) was found out. It is also important that the value of the respective standard deviation was lower compared to the analysed localities in the Krušné hory Mts., which were described in the preceding paragraph, which does not correspond to lower growth variability in the Krkonoše Mts. (Table 4). Obviously, the variability of spruce populations according to growth and phenotype features is probably directly comparable only within one natural area. The needle length was one and only needle parameter measured in history. This length on PRP 24 is comparable with common data from the Krkonoše Mts. (Fanta 1974, 1976).

The structure of spruce population originating from natural regeneration depends on the structure of mature (parent) population, which can be illustrated by an example of the Krušné hory Mts. It was an area with distinct selection caused by the air-pollution stress. The local forests experienced a disastrous influence of acid deposition until the early nineties of the 20th century (e.g. Slodičák, Lomský 2008; Šrámek et al. 2008). The narrowing of phenotype variability of spruce natural regeneration can be explained by the narrowing of genetic variability of parent populations due to preceding long-term air-pollution stress. Therefore, the phenotype variability of populations originating from artificial regeneration (outplanting) may usually be higher than in natural regeneration. Higher variability of growth rate is compatible with this finding.

The difference in variability of features of spruce populations from the Krušné hory Mts. and the Krkonoše Mts. need not be caused by the mere narrowing of genetic variability as a consequence of the long-term air-pollution stress only. It may be a natural difference in *Picea abies* metapopulations in both mountain ranges resulting from the

Table 6. Variability in the quantitative morphological features of needles in selected populations of young Norway spruce in the Krkonoše and Krušné hory Mts.

Locality	Regeneration	*n*	*rl*		*rw*		*rl*/*rw*		
			mean	SD	mean	SD	mean	SD	max – min
Krkonoše Mts. PRP 24	natural	39	12.11	1.96	0.474	0.046	25.7	4.53	18.7
Krušné hory Mts. Klínovec C	natural	30	7.95	1.14	0.637	0.048	12.5	1.75	6.8
Krušné hory Mts. Klínovec B	natural	30	8.26	0.81	0.633	0.066	13.2	1.95	8.2
Krušné hory Mts. Klínovec B	planting	30	7.28	1.02	0.662	0.077	11.1	1.85	8.8

n – number of measured trees, *rl* – average length of needle, *rw* – average width of needle, SD – standard deviation spread of this species in the post-glacial period that is reflected in the genetic characteristics of this species. These are two geographically separated groups of populations when one of them was spreading from the south along the western mountain ranges of the Czech Republic (Šumava – Český les – Krušné hory) and the other group was coming to the Krkonoše Mts. across the Beskids Mts. and the Jeseníky Mts. (Tollefsrud et al. 2008). It was just in the area of the Jizerské hory Mts. and Krkonoše Mts. where both groups could come into contact, which could result in a local increase in the genetic diversity of *P. abies*. The specific position of the Jizerské hory – Krkonoše mountain range in connection with the spruce morphology was mentioned earlier (Fanta 1974). A study to verify such influences has to be done in the future.

Growth and other features of trees

The height of the studied youngest subjects, i.e. seedlings, is the most closely correlated with needle length *rl* ($P < 0.001$) and the correlation with needle thickness *mt* and with the area of needle cross-section (P_{TOT}) is also highly significant ($P < 0.001$). Correlation coefficients with root collar diameter indicate a similar group of features as significant. The size of seedlings (i.e. height in the

Table 7. Coefficients of correlation between the variables of the two-year-old seedlings of Norway spruce

Type	Variable	*n*	Mean	SD	d	h	h_{2010}	ROOT	BRANCH	M_{AG}
Needle biometry	*rl* (mm)	62	12.9	1.86	0.58*	0.73*	0.23	0.34*	0.49*	0.67*
	rw (mm)	62	0.60	0.06	0.42*	0.21	0.30*	0.30*	0.31*	0.42*
	rl/*rw*	62	20.6	2.9	0.31*	0.58*	0.03	0.15	0.32*	0.39*
Colour features	RGB_R	62	85.0	7.2	–0.40*	–0.20	–0.34*	–0.33*	–0.35*	–0.37*
	RGB_G	62	110.2	8.4	–0.49*	–0.28*	–0.37*	–0.47*	–0.40*	–0.43*
	RGB_B	62	66.3	6.7	–0.31*	–0.32*	–0.20	–0.13	–0.30*	–0.39*
	GRAY	62	261.6	19.6	–0.47*	–0.30*	–0.35*	–0.37*	–0.41*	–0.45*
	R_{ST}	62	0.62	0.01	0.02	0.23	–0.14	–0.05	0.00	0.10
	G_{ST}	62	0.42	0.01	–0.10	0.02	–0.06	–0.27*	–0.03	0.02
	B_{ST}	62	0.25	0.02	0.07	–0.12	0.11	0.23	0.02	–0.06
Micro-scopic features	P_{TOT} (mm^2)	34	0.239	0.062	0.58*	0.61*	0.36*	0.46*	0.42*	0.71*
	VSCB (μm^2)	34	20,796	4,964	0.41*	0.43*	0.34*	0.44*	0.26	0.52*
	XYLEM (μm^2)	34	1,260	400	0.03	–0.04	0.22	–0.01	–0.02	0.14
	PHLOEM (μm^2)	34	894	295	0.42*	0.39*	0.43*	0.24	0.25	0.54*
	ALBUMIN (μm^2)	34	1,809	531	0.05	–0.01	0.02	0.13	–0.00	0.10
	VSCB/TOT	34	0.088	0.013	–0.32	–0.37*	–0.08	–0.06	–0.32	–0.39*
	XYL/VSCB	34	0.062	0.019	–0.44*	–0.53*	–0.13	–0.48*	–0.37*	–0.41*
	PHLOEM/VSCB	34	0.043	0.009	0.17	0.10	0.23	–0.12	0.03	0.22
	mw (mm)	32	0.707	0.070	0.30	0.38*	0.47*	0.28	0.22	0.43*
	mt (mm)	32	0.626	0.103	0.60*	0.62*	0.15	0.33	0.36*	0.67*
	mt/*mw*	32	0.886	0.129	0.53*	0.47*	–0.13	0.21	0.28	0.50*

rl – average length of needle, *rw* – average width of needle, *rl*/*rw* – needle length/width ratio, RGB_R – colour components for red, RGB_G – colour components for green, RGB_B – colour components for blue, GRAY – calculated grey grade (GRAY = RGB_R+RGB_G+RGB_B); R_{ST}, G_{ST}, B_{ST} – standardized colour components, R_{ST} = RGB_R/GRAY, etc.; P_{TOT} – needle cross-section area, VSCB – vascular bundle area, XYLEM – xylem area, FLOEM – phloem area, ALBUMIN – area of albuminous cells, VSCB/TOT – ratio of vascular bundle area and total needle cross-section area , XYL/VSCB – xylem area and vascular bundle area ratio, PHLOEM/VSCB – phloem area and vascular bundle area ratio, *mw* – microscopic width of needle, *mt* – microscopic thickness of needle, *mt*/*mw* – microscopic thickness of needle and microscopic width of needle ratio; d – average diameter of the root collar, h – average height of the seedling, h_{2010} – height in the preceding year 2010, ROOT – root length, BRANCH – number of branches, M_{AG} – weight of the aboveground part), SD – standard deviations of the variables, *n* – number of evaluated pairs of values; *significant on the level $\alpha \leq 5$ %

preceding year h_{2010}) is highly associated with needle width *mw* (P = 0.007) and with phloem area (P = 0.010). Obviously, growth may be influenced by different parameters to a different extent particularly in the first years of the tree life. Mainly needle colour (particularly RGB_G, $P < 0.001$), needle section area P_{TOT} (P = 0.007) and vascular bundle area VSCB (P = 0.010) are important for the root length. The shoot weight of a tree correlates most closely with the needle section area (P_{TOT}; $P < 0.001$; Table 7).

If a relationship between the studied characteristics of recruits and their growth is sought, coefficients of correlation between these characteristics and deviations of the measured increment and modelled value of increment $P_y = a \times h^b_{y-1}$ (separately for each evaluated locality) can be used. The highest absolute value of correlation coefficient was calculated for the needle shape coefficient *rl*/*rw* for PRP 24 locality (Fig. 3) in the Krkonoše Mts.The slimmer the needle, the faster growth of a tree was found. It is also interesting that higher growth rate is related with increased variability of needle width (significantly for the standard deviation of *rw* values).

From the aspect of nutrition, positive correlation coefficients were calculated with nitrogen and potassium content (Table 8). It is necessary to note that three elements show the content lower than the limit. The limits (Boháčová 2004) are 1.2% for nitrogen (49% of trees exhibited N shortage), 0.1% for phosphorus (77% of trees exhibited P shortage) and 0.06% for magnesium (38% of trees exhibited Mg shortage). The contents of potassium and calcium do not decrease below the limits of 0.35 and 0.15%, respectively, in all the trees analysed. This situation is in accordance with the extreme conditions of the site. The naturally regenerated trees are frequently growing on specific microsites such as coarse woody debris, where the availability of some nutrients can be limited. This situation gradually changes as the root system of the tree is expanding.

There also arises a question whether the increased growth of some trees was caused by better availability of the elements at the given microsite or whether higher uptake was caused by the higher ability of faster-growing trees (with more developed root system)

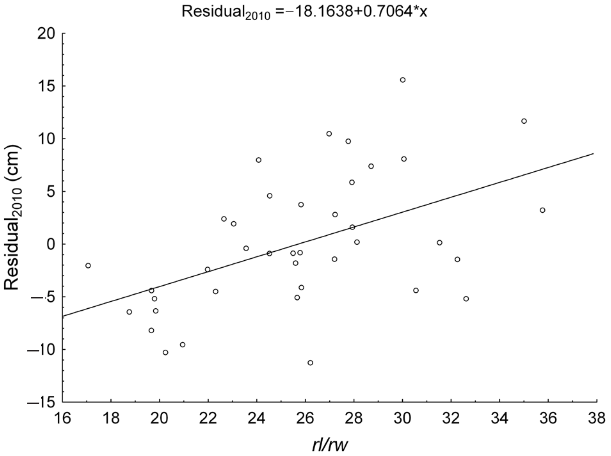

Fig. 3. Linear regression of a difference between measured and modelled height increment (residual) on the shape index of needle – needle length/width ratio (*rl*/*rw*) in PRP 24 locality (Krkonoše Mts.) in 2010 (r = 0.5065; P = 0.0010), a total of 39 trees were analysed

Table 8. Coefficients of correlation between growth in 2010 (residual$_{2010}$ – equal to a difference between measured height increment and modelled increment $P_{2010} = a \times h^b_{2009}$ and other selected characteristics of the young Norway spruce on PRP 24 (Krkonoše Mts.)

Type	Variable	mean	SD	Residual$_{2010}$
Colour features	GRAY	236.0	18.4	–0.18
	RGB_R	112.7	5.7	–0.44*
	RGB_G	125.0	4.5	–0.33*
	RGB_B	17.3	8.2	0.48*
Weight	M100_G	0.244	0.081	0.25
Content (%)	N	1.19	0.16	0.37*
	P	0.089	0.020	0.13
	K	0.476	0.072	0.33*
	Ca	0.356	0.108	0.19
	Mg	0.069	0.023	–0.09
Needle biometry (mm)	T	0.55	0.09	0.17
	W	1.12	0.12	–0.23
	L	12.2	2.0	0.39*
	rw	0.47	0.05	–0.22
	rl	12.1	1.96	0.40*
	rl/*rw*	25.7	4.5	0.51*
Statistics	STD(T)			0.02
	STD(W)			0.31
	STD(L)			0.08
	STD(*rw*)			0.33*
	STD(*rl*)			0.08
	n			39

GRAY – calculated grey grade (GRAY = RGB_R+RGB_G+RGB_B), RGB_R – colour components for red, RGB_G – colour components for green, RGB_B – colour components for blue, M100_G – weight of 100 needles (g), T – macroscopic thickness of needle, W – macroscopic width of needle, L – directly measured length of needle, *rw* – calculated average width of needle, *rl* – calculated average length of needle, *rl*/*rw* – needle length/width ratio, STD(T) – standard deviation of T, etc.; *significant on the level α ≤ 5 %, in total 39 trees were analysed to take up these elements. It is to assume that faster-growing trees that have longer needles may exhibit the greater elongation growth of roots at the same time. They can take up nutrients from the larger volume of soil while the trees with shorter roots take up fewer nutrients. After a longer time of the root system development (at the time of the closed canopy of the tree layer) this imbalance may be equalized and both slow- and fast-growing trees are able to take up the relatively identical amount of nutrients. However, this hypothesis should be verified by further long-term investigations and/or a planting experiment with different needle-type trees under controlled conditions.

Unlike the Krkonoše Mts., where high variability of needle shape was observed, the situation in the Krušné hory Mts. was different (Table 9). Only at the locality with natural regeneration there existed a relation between the deviation from the modelled height increment and one of the studied parameters of a tree, which was either needle width *rw* or needle length *rl*.

If the population variability in the average size of needles is higher, then the correlation between the size of needles and the height increment will be decreased. It was observed in the Klínovec B locality by the needle width (correlation *rw* × residual_{2011}; $r = 0.07$, Table 9). An artificial population can show increased variability in the needle size, because this population is often composed of different phenotypes (i.e. from distinct regions or different site types). It leads to a decreased strength of the relationship between growth and needle size.

Table 9. Coefficients of correlation between growth in 2011 (residual_{2011} – equal to a difference between measured height increment and modelled increment $P_{2011} = a \times \text{h}^{b}_{2010}$ and other selected characteristics of the young Norway spruce from the Krušné hory Mts.)

Variable	Residual_{2011}		
	Klínovec C	Klínovec B	Klínovec B
Regeneration	natural	natural	planting
RGB_R	–0.18	–0.16	–0.19
RGB_G	–0.34	–0.16	–0.18
RGB_B	0.03	–0.08	–0.14
GRAY	–0.19	–0.15	–0.20
rw	0.46*	0.07	0.13
rl	0.35	0.39*	0.13
rl/*rw*	0.11	0.18	0.03

RGB_R – colour components for red, RGB_G – colour components for green, RGB_B – colour components for blue, GRAY – calculated grey grade (GRAY = RGB_R+RGB_G+RGB_B), *rw* – calculated average width of needle, *rl* – calculated average length of needle, *rl*/*rw* – needle length/width ratio, *significant on the level $\alpha \leq 5$ %, in total 30 randomly selected trees were analysed in each locality

The importance of the needle length to width ratio is increasing from the youngest (seedlings) to older trees (regeneration) at the cost of the needle length. It remains a relatively good indicator of height increment. The situation may be different in populations with narrowed phenotype variability as it was probably the case in the Krušné hory Mts.

CONCLUSIONS

The structure of *Picea abies* populations from natural and artificial regeneration is distinctly different. These differences are reflected not only in phenotype variability of the populations but also in the growth rate of their trees.

The evaluation of phenotype variability of young spruce populations cannot be done without knowledge of how different types (phenotypes, types according to selection and growth strategies, etc.) participate in forest ecosystem dynamics. The share of these types in the populations is based on geographic and natural conditions (the spread of metapopulations with different phenotype composition in the post-glacial period). The influence of anthropic activities affects the structure of local populations (direct influence – typically silvicultural practices; indirect influence – for instance, selection as a consequence of air-pollution stress). The importance of the transfer of planting material from region to region is not negligible. In the mountainous environment it is also necessary to consider the spread of genotypes (phenotypes) typical of a given altitudinal zone to other zones (both upward and downward). What variability (diversity) of phenotypes (genotypes) should be optimal in young spruce populations cannot be recommended explicitly. There are man-made spruce populations established by outplanting in mountain conditions that have either wider or narrower variability in comparison with similar populations from spontaneous regeneration. For further development of man-made spruce stands, the representation of different types (phenotypes, growth types) in the population is essential so that it will have sufficient plasticity and will be resistant to potential stress (e.g. climate change, incidence of pests). Nevertheless, an excessive increase in this variability will apparently imply in the future a potential threat to or even mortality of a higher percentage of trees. Diversity of types in man-made (outplanted) populations should be compared with analogical diversity in young populations from natural regeneration that originate from the local potential of natural populations and are modified by local natural conditions and their variability in the past. Nevertheless, such natural populations are rare.

It has been proved that the dynamics of young spruce growth is related with some quantitative characteristics of needles, mainly with the needle length to width ratio or with the separately evaluated needle length that is more significant in the youngest trees (seedlings). The importance of needle width probably increases as the tree grows older. To maintain the stability of future forest stands, the plants with short needles should not be discarded from the produced planting stock although they exhibited a smaller height. To maintain sufficient diversity in man-made populations the specific non-discarding of plants of some types according to the characteristic needle section should be taken into account.

References

Blujdea V.N.B., Pilli R., Dutca I., Ciuvat L., Abrudan I.V. (2012): Allometric biomass equations for young broadleaved trees in plantations in Romania. Forest Ecology and Management, ***264***: 172–184.

Cudlín P., Novotný R., Chmelíková E. (1999): Recognition of stages of montane Norway spruce response to multiple stress impact using crown and branch structure transformation analysis. Phyton, ***39***: 149–153.

Cudlín P., Novotný R., Moravec I., Chmelíková E. (2001): Retrospective evaluation of the response of montane forest ecosystems to multiple stress. Ekológia (Bratislava), ***20***: 108–124.

Danusevicius D., Lindgren D. (2002): Efficiency of selection based on phenotype, clone and progeny testing in long-term breeding. Silvae Genetica, ***51***: 19–26.

Fanta J. (1974): Morphologische Variabilität der Fichte und Grundzüge der genetischen Rekonstruction der Gebirgsfichtenwälder im Krkonoše Nationalpark (ČSSR). Archiv für Naturschutz und Landschaftsforschung, ***14***: 179–200.

Fanta J. (1976): Morfologická proměnlivost původních populací smrku v Krkonoších. [Morphological variability of autochthonous populations of Norway spruce in the Krkonoše Mountains (ČSSR).] Lesnictví, ***22***: 415–434.

Boháčová L., Buriánek V., Fabiánek P., Hejdová J., Kapitola P., Lachmanová Z., Lomský B., Novotný R., Šrámek V., Uhlířová H. (2004): Monitoring zdravotního stavu lesa v České republice. Ročenka programu Forest Focus – Data 2004. [Forest Condition Monitoring in the Czech Republic. Annual Report Forest Focus – Data 2004.] Jíloviště-Strnady, VÚLHM: 431.

Hasenauer H. (ed.) (2006): Sustainable Forest Management. Growth Models for Europe. Berlin, Springer: 398.

Hill T., Lewicki P. (2007): STATISTICS: Methods and Applications. Available at http://www.statsoft.com/textbook/

Ivanek O., Matějka K., Novotný P. (2009): Genetická struktura dvou částí porostu smrku ztepilého při horní hranici lesa na území KRNAP. [Genetic structure of two parts of Norway spruce stand near the alpine forest limit in the Krkonoše National Park.] Zprávy lesnického výzkumu, ***54***: 300–306.

Jurásek A., Leugner J., Martincová J. (2009): Effect of initial height of seedlings on the growth of planting material of Norway spruce (*Picea abies* [L.] Karst.) in mountain conditions. Journal of Forest Science, ***55***: 112–118.

Kindlmann P., Matějka K., Doležal P. (2012): Lesy Šumavy, lýkožrout a ochrana přírody. [Forests of the Šumava Mts., Bark Beetle, and Nature Conservation.] Praha, Karolinum: 326.

MacArthur R.H., Wilson E.O. (1967): The Theory of Island Biogeography. Princeton, Princeton University Press: 224.

Matějka K. (2009): Nápověda k programu PlotOA. Plotting of ordination diagrams and cartograms. Available at http://www.infodatasys.cz/software/hlp_PlotOA/plotoa.htm

Matějka K. (2012): Zpráva spoluřešitele projektu QI112A170 za rok 2012. Možnosti cíleného pěstování a využití geneticky hodnotných částí populací sadebního materiálu smrku ztepilého s klimaxovou strategií růstu pro horské oblasti. [The associate's final report on the project QI112A170, year 2012. Options for cultivation and use of genetically valuable part of the Norway spruce population with the climax growth strategy for mountain areas.] Available at http://www.infodatasys.cz/proj005/QI112A170_results2012.pdf

Matějka K., Leugner J. (2013): Growth of Norway spruce (*Picea abies* [L.] Karsten) from artificial and natural regeneration in the Krkonoše Mts. and air temperature variability. Journal of Forest Science, ***59***: 150–158.

Pianka E.R. (1970): On *r*- and *K*-selection. American Naturalist, ***104***: 592–597.

Popov P.P. (2013): Structure and differentiation of spruce populations in the Komi Republic. Russian Journal of Ecology, ***44***: 193–198.

Pretzsch H. (2009): Forest Dynamics, Growth and Yield. Berlin, Springer: 664.

Pretzsch H., Dauber E., Biber P. (2013): Species-specific and ontogeny-related stem allometry of European forest trees: evidence from extensive stem analyses. Forest Science, ***59***: 290–302.

Reznick D., Bryant M.J., Bashey F. (2002): *r*- and *K*-selection revisited: the role of population regulation in life-history evolution. Ecology, ***83***: 1509–1520.

Samek V. (1964): Metodika výzkumu morfologické proměnlivosti smrku z hlediska fytogeografického. [Research methodology of morphological variability of spruce – phytogeography.] Zprávy lesnického výzkumu, ***10***: 18–25.

Slodičák M., Lomský B. (2008): Vývoj poškození lesních porostů v Krušných horách. [Development of the damage of forest stands in the Krušné hory Mts.] In: Slodičák M. et al. (eds): Lesnické hospodaření v Krušných horách. Hradec Králové, Lesy České republiky: 13–28.

Schmidt-Vogt H. (1977): Die Fichte. Ein Handbuch in zwei Bänden. Hamburg, Berlin, Verlag Paul Parey: 647.

Šrámek V., Hadaš P., Lachmanová Z., Fadrhonsová V., Vortelová L., Lomský B., Kulhavý J. (2008): Imisní zatížení Krušných hor. [Air pollution in the Krušné hory Mts.] In: Slodičák M. et al. (eds): Lesnické hospodaření v Krušných horách. Hradec Králové, Lesy České republiky: 45–70.

Tollefsrud M.M., Kissling R., Gugerli F., Johnsen O., Skroppa T., Cheddadi R., van der Knaap W.O., Latalowa M., Terhürne-Berson R., Litt T., Geburek T., Brochmann C., Sperise C. (2008): Genetic consequences of glacial survival and postglacial colonization in Norway spruce: combined analysis of mitochondrial DNA and fossil pollen. Molecular Ecology, ***17***: 4134–4150.

Viewegh J., Kusbach A., Mikeska M. (2003): Czech forest ecosystem classification. Journal of Forest Science, ***49***: 85–93.

Ward M. H. (2005): Age-related Trends in Red Spruce Needle Anatomy and Their Relationship to Declining Productivity. [Ph.D. Thesis.] Orono, University of Maine: 110.

Wehenkel C., Corral-Rivas J.J., Castellanos-Bocaz H.A. (2010): Is there selection by species diversity in *Picea abies* L.? Plant Ecology, ***208***: 47–54.

Received for publication November 5, 2013
Accepted after corrections February 20, 2014

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