Evaluation of physiological and health state of Norway spruce plants with different growth rate at juvenile stage after outplanting at mountain locations

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ABSTRACT: Norway spruce (*Picea abies* [L.] Karst.) seedlings grown from seed originating from high mountain locations (8th forest altitudinal zone – Norway spruce vegetation zone 1,000–1,250 m a.s.l.) show higher growth variability than seedlings from populations adapted to more favorable conditions at a lower altitude a.s.l. Seedlings smaller than 8 cm in height were usually culled during sorting before transplanting (in common nursery practice) regardless of the fact whether it was not planting material from high mountain locations. This paper presents the results of the physiological and health state of 16 year old spruce stands established by outplanting of specifically sorted planting material (comprising also slowly growing seedlings) on the research plot Pláň (Krkonoše Mts). Differences among variants in water losses during drying were relatively small and statistically insignificant due to high individual variability; nevertheless, they indicate a certain positive trend in plants with slower growth dynamics in the nursery. Differences in chlorophyll fluorescence among the variants were statistically significant. The trend of higher frost hardiness in the "small" variant was obvious again. The health status results document the initial assumption of very good adaptation to adverse mountain conditions in trees grown from seedlings characterized by slow growth in a nursery. The results of evaluation of physiological parameters and health status confirm a hypothesis that plants with the initial slow growth are a stable component of the population spectrum of mountain spruce trees. The results document good preconditions for the establishment of vital and stable stands when the entire growth spectrum of planting stock and particularly of plants produced from originally slow-growing seedlings is utilized.

Keywords: health status; mountain locality; Norway spruce; physiological trait

Norway spruce (*Picea abies* [L.] Karst.) seedlings grown from seed originating from high mountain locations (8th forest altitudinal zone) show higher growth variability than seedlings from populations adapted to more favourable conditions at a lower altitude above sea level. Former legislation, which was still in force in the Czech Republic in the nineties of the last century (Departmental Standard ON 48 2211 1989), recognized as spruce standard seedlings plants of minimum shoot height 8 cm while nonstandard seedlings could be used only in valuable species and ecotypes of woody plants. Seedlings more than 10 cm in height were recommended for mechanized transplanting. It means that in forest nurseries seedlings smaller than 8 cm in height were usually culled during sorting before transplanting. This practice may cause the narrowing of the genetic spectrum, because was not use part of populations from high mountain locations for reforestation.

Seedlings with slow growth at a juvenile stage apparently represent a very valuable part of mountain populations with the best adaptation to extreme environmental conditions. They are probably individuals capable to survive extreme climatic fluctuations that may occur once over several tens of years (Lang 1989). This statement is also supported by the fact that the shoot height of spruce seedlings decreases with the increasing altitude of their origin (Modrzyński 1995; Kotrla 1998). It

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is assumed that in the process of adaptation to adverse conditions of the mountain environment the spruce populations acquire higher resistance at the expense of growth rate at a juvenile stage, i.e. in the first several years of age.

The deterioration of the condition of some young forest outplantings currently arouses a question whether sorting in a forest nursery did not cause the undesirable narrowing of the genetic spectrum of mountain spruce populations when individuals with the best adaptability to extreme mountain conditions were culled. Therefore detailed investigations of morphological, physiological and genetic traits of young spruces with known growth rate in a nursery and after outplanting are carried out in the framework of the grant project "Conservation of the stability and biodiversity of Norway spruce mountain populations". This paper presents the results of the health and physiological state of young spruce stands established by outplanting of specifically sorted planting material (comprising also slowly growing seedlings) on the Pláň research plot monitored in the long run in a model mountain area of the Krkonoše Mts.

MATERIAL AND METHODS

The research plot "Pláň" was established in 1994 on the northern slope of the Stoh ridge in the Krkonoše Mts. (forest stand group 73, forest site type group 8K, altitude 1,000–1,100 m a.s.l., clearcut area ca 2 ha in size). One of the objectives was to investigate the influence of specific sorting in a forest nursery on the growth and stability of outplantings of Norway spruce mountain populations. Plants grown from specifically sorted seedlings were outplanted. In 1992, before transplanting, two-year seedlings originating from the 8th forest altitudinal zone (FAZ) (designation of origin: B/SM/0001/22/8/TU) were divided into 3 size categories: smaller than 8 cm (the "small" variant), 8 to 15 cm ("intermediate medium") and 16 to 22 cm ("large"). The plants were cultivated under standard procedure for bare-rooted planting stock after sorting. The four-year plants (2 + 2) were set onto a mountain clearcut area. Each variant comprised 3 replications by 100 plants. In the proximity of research plot a part of the even-aged forest outplanting was demarcated as the control comparative material (planting stocks from common nursery practice). Height and diameter growth and health status of outplantings are evaluated regularly on this research plot. A more detailed evaluation of phenology and physiological state was done in spring 2009 and 2010.

Physiological characteristics (chlorophyll fluorescence, frost hardiness, resistance to desiccation) were determined in a laboratory of the Research Station in Opočno (Opočno RS). After plants transport to the laboratory, branch samples collected on 26 May 2009 on the Pláň research plot were put into water dipping their bases and covered by polyethylene sheet in order to ensure the water imbibition of branches in a moist environment. On the next day, annual shoots were gradually clipped off, weighed immediately and subsequently subjected to controlled desiccation in laboratory conditions. Water losses were determined after 15 minutes when mainly stomatal transpiration took place, then after 60, 180 and 240 minutes when water losses were caused mainly by cuticular transpiration. When the evaluation of water losses terminated, samples were dried at 80°C to constant weight and their dry matter and initial water content were determined. Fifteen branch samples from each variant were evaluated.

Other parts of branches were used to measure chlorophyll fluorescence and frost hardiness. Separate needles were severed from annual shoots, stuck on an adhesive tape on a pad and dark-adapted in a moist chamber at a laboratory temperature for 45 min at least. The basic characteristics of chlorophyll fluorescence and photosynthetic electron transport rate (ETR) were measured at increasing light intensity with an Imaging-PAM 2000 device (Heinz Walz GmbH, Effeltrrich, Germany). Measuring light of the intensity 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and saturation impulse of the intensity 2,400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 800 ms were applied for measurements. The basic measured characteristic of chlorophyll fluorescence was the maximum quantum yield of photosystem II photochemistry (F_v/F_m) calculated as the ratio of variable (F_v) to maximum (F_m) fluorescence. Variable fluorescence was obtained from a difference between the basic fluorescence of dark-adapted needles (F_0) and maximum fluorescence (F_m) after the radiation of a sample with an impulse of saturation light. The maximum quantum yield of photochemistry was computed from the formula $F_v/F_m = (F_m - F_0)/F_m$.

The remaining parts of twigs, from which a part of needles was severed, were put into polyethylene bags and subjected in a freezing box to a temperature of –20°C for 20 hours. After tempering to room temperature another measurement of chlorophyll fluorescence followed to determine the extent of damage caused by a freezing test. 15 samples from each variant were evaluated.

Table 1. The scale for phenological evaluation of young spruces

Degree of bud break	Bud state
0	dormant, buds are not swollen
1	swollen buds, translucent green needles
2	burst buds, needles begin to emerge from fascicles
3	emerged needles
4	beginning of shoot elongation growth
5	intensive elongation growth of young shoots

Bud break was evaluated once in the spring season (all buds) by the scale shown in Table 1. The evaluation comprised 70 to 100 spruces from each variant.

Health status was evaluated in autumn according to foliage percentage and frequency of occurrence of damage to stems and branches (injuries, breakages, deformations) in 70 to 100 spruces from each variant (Table 2). Foliage was evaluated visually to the nearest tens of percent.

Data from field and laboratory measurements were processed and statistically evaluated by the Excel and QC Expert software. Analysis of variance (two factors ANOVA) was used to test the differences due to height categories and freezing test on characteristic of chlorophyll fluorescence. The confidence interval at a 5% significance level is used for the representation of statistical significance in graphs.

RESULTS

Water losses during controlled desiccation

Water losses were determined in the course of desiccation of severed spruce annual shoots imbibed with water in laboratory conditions (21 ± 1°C, relative air humidity 50 ± 5%). Fig. 1 illustrates water content expressed in percent of the initial water content after 15 and 180 min of exposure. The graph shows the highest losses in spruces of the "large" variant, followed by "medium" variant and the smallest losses were in the "small" variant, during stomatal (the first 15 min) and cuticular (180 min) transpiration. Differences among variants were relatively small and statistically insignificant due to high individual variability; nevertheless, they indicate a certain positive trend in plants with slower growth dynamics in the nursery.

Chlorophyll fluorescence

Fig. 2 documents the variable to maximum fluorescence (F_v/F_m) ratio determined after the irradiation of a dark-adapted needle sample that represents the maximum quantum yield of photosystem II (PSII) photochemistry. It is documented in literature that the values of this characteristic in undamaged leaves of trees of the temperate zone are usually higher than 0.75. Hence all evaluated variants in fresh condition (before a freezing test) showed good condition and functionality of the assimilatory apparatus.

The exposure to freezing temperatures (–20°C for 20 h) caused partial damage to photosystem II, which resulted in a decrease in the F_v/F_m value. The most pronounced damage was found out in the "large" variant while the "small" variant showed the smallest damage (Fig. 2). The values of spruces from the "medium" variant were between those of the other two variants. The trend is the same as in water losses when the highest resistance was observed in plants from the "small" variant and the lowest resistance was in the "large" variant. Differences in chlorophyll fluorescence among the variants were statistically significant (Table 3). The trend of higher frost hardiness in the "small" variant was obvious again.

Table 2. Indexes for the evaluation of damage to spruce stem and branches

Type of damage	Description of damage	Index
Stem damage	no damage	0
	substitute shoots	1
	stem breakages	2
Branch damage	no damage	0
	moderate damage (small injuries, breakages of weak branches)	1
	medium damage (larger injuries, damage to thicker branches)	2
	great damage (tree stability is disturbed, deep injuries of stem)	3
	total crown devastation	4

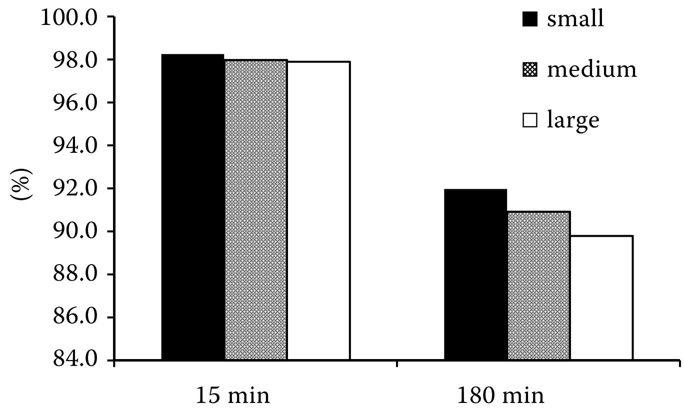

Fig. 1. Water losses (in % of the initial water content) after 15 and 180 min of desiccation. The whiskers show the confidence interval at a 5% significance level

Reaction of the assimilatory apparatus to increasing radiation intensity

At the increasing intensity of photosynthetically active radiation (PAR) it was evaluated the photosynthetic electron transport rate (ETR) indicating the speed of transport of electrons from photosystem II (PSII) and their utilization for further processes of photosynthesis.

In this characteristic very similar values were recorded in all three evaluated variants of fresh, unfrozen needle samples (Fig. 3). In samples subjected to a freezing test (–20°C for 20 h) pronounced disturbance of PSII photochemistry occurred, which resulted in a decrease in the values of ETR in the entire course of curves, i.e. at all intensities of photosynthetically active radiation. The lowest decrease was observed in the "small" variant and the highest decrease in the "large" variant. If the experimental variants were compared, also in this case the trend was identical to that of the other above-mentioned characteristics.

Bud break

The evaluation of buds break in spring 2010 are presented in Fig. 4. The frequency of spruces showed different degrees of bud break (the evaluation is described in the chapter Method). The highest proportion of later flushing trees was observed in the "small" variant.

Health status

The health status and frequency of spruce damage in the particular research variants were evaluated on Pláň research plot in the autumn season. Fig. 5 illustrates the average foliage percentage. Spruces grown from the smallest seedlings ("small" variant) that would be culled during standard sorting had the best foliage. The poorest foliage was observed on control plots in forest outplantings.

Damage to branches and stem was evaluated according to severity. Damage indexes are shown in Table 2 in the chapter Material and Methods.

The frequency of stem damage occurrence is illustrated in Fig. 6 and the frequency of branch damage occurrence is shown in Fig. 7. These results also document the very good condition of variants grown from the smallest seedlings (small). The most frequent damage was observed on the control plot in a forest outplanting.

The above results also document the initial assumption of very good adaptation to adverse

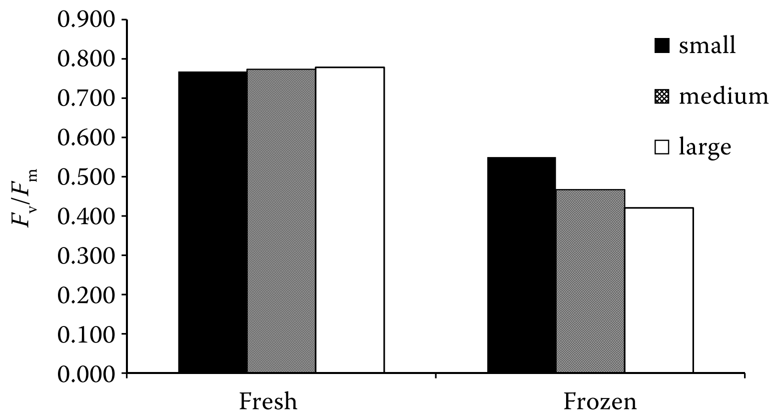

Fig. 2. Maximum quantum yield of chlorophyll fluorescence (F_v/F_m) in fresh samples of spruce needles and after their exposure to freezing temperatures. The whiskers show the confidence interval at a 5% significance level

Table 3. Analysis of variance for values of chlorophyll fluorescence (F_v/F_m) on research plot Pláň

Source of variance	Sums of squares	Mean squares	Degrees of freedom	Standard deviation	F-exp.	F-test	
Height categories	0.312	0.156	2	0.3952	9.4661	3.0300	Significant
Fresh/frozen	1.170	1.170	1	1.0816	70.9190	3.8769	Significant
Interaction	0.505	0.253	2	0.5027	15.3213	3.0300	Significant
Residue	4.355	0.016	264.000	0.128			
Sum	6.964	0.026	269.000	0.161			

mountain conditions in trees grown from seedlings characterized by slow growth in a nursery.

DISCUSSION

The overall evaluation of the physiological state of young spruces grown from seedlings with different growth rate in a forest nursery and planted to an extreme mountain clearcut area showed the highest water losses during controlled desiccation in laboratory conditions in spruces grown from the fastest growing seedlings ("large" variant). They were followed by spruces grown from mediocre seedlings ("medium" variant) and the lowest water losses were observed in spruces grown from small, slow-growing seedlings that would be culled by standard sorting ("small" variant). This trend was identical in the first 15 minutes (mostly stomatal transpiration) and also after 180 minutes (mostly cuticular transpiration). Even though differences in the results were not significant because of high individual variability, they document good water "management" in the variant grown from slower-growing seedlings. The values of the maximum quantum yield of fluorescence (F_v/F_m) in fresh samples hardly differed among the variants. They all indicated good condition and functionality of the assimilatory apparatus. After the exposure of branch samples to freezing temperatures the highest damage to the assimilatory apparatus (the highest drop in the values of F_v/F_m ratio) was found out in spruces of the "large" variant, followed by spruces of the "medium" variant and the smallest damage was observed in spruces of the "small" variant. This test also documents higher resistance to stresses in outplantings originating from slower-growing seedlings. The evaluation of the photosynthetic electron transport rate (ETR) at increasing radiation intensity showed a similar trend among the variants in samples in fresh condition and damage caused by freezing test increasing from "small" to "large" variants.

The observed differences among variants in all studied physiological characteristics were relatively small and statistically insignificant due to the high individual variability of trees. Nevertheless, these are important findings confirming an assumption that seedlings with slow juvenile growth represent a very valuable part of mountain spruce populations that should not be culled in nurseries.

Mountain populations of Norway spruce (*Picea abies* [L.] Karst.) show higher variability of seed and seedlings compared to spruce from lower lo-

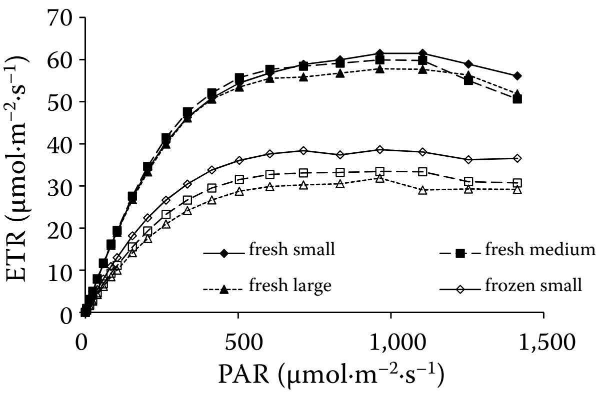

Fig. 3. Photosynthetic electron transport rate (ETR) at increasing radiation (PAR) intensity

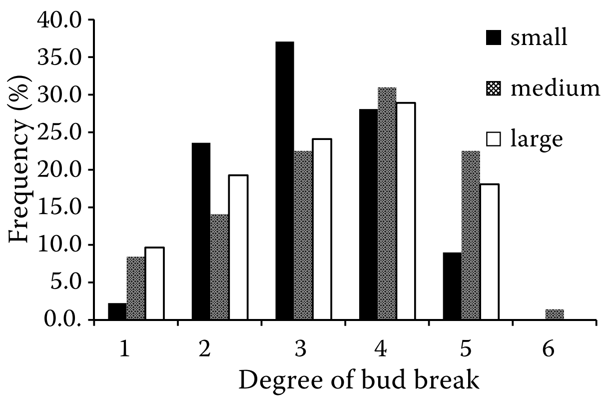

Fig. 4. Proportions of spruces with different degrees of bud break on the date of evaluation 1st June 2010 (a description of the particular degrees of bud break see Table 1

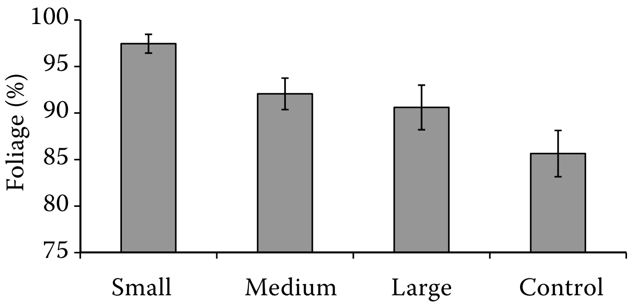

Fig. 5. Average foliage percentage in spruces on research plot Pláň. The whiskers show the confidence interval at a 5% significance level

cations (Kotrla 1998). Differences in growth rate and dynamics also exist in seedlings grown in constant conditions (Holzer 1984; Holzer et al. 1987). Differences in growth among spruce populations originating from different altitudes and grown in the same environment are most pronounced in the first years of seedling life (Holzer 1984; Quamarudin et al. 1995). The lower growth rate of spruce mountain populations is assumed to be connected with their increased adaptation to adverse mountainous conditions (Oleksyn et al. 1998). Other authors have also documented the relationship between growth and vulnerability to adverse effects. Bigras (2000) determined, on the basis of chlorophyll fluorescence measurements, higher vulnerability to the effect of elevated temperatures in fast-growing seedlings of *Picea glauca* compared to slow-growing ones. Below-average growth in young trees of Norway spruce with high tolerance to SO_2 was reported by Wolf (2001).

Higher frost hardiness in spruce populations originating from higher elevations or from more northern areas, compared to seedlings from lower locations or of more southern provenance, was described by Simpson (1994), Hawkins and Shewan (2000), Westin et al. (2000) and Sogaard (2008), while better drought resistance in these populations was reported by Modrzynski and Eriksson (2002). In two-month spruce seedlings of an ecotype adapted to higher altitude Valcu et al. (2008) found out a lower level of thermotolerance and a higher level of tolerance to oxidative stress compared to seedlings of an ecotype from lower altitude.

The hypothesis of the relationship between adaptability to adverse influences and growth rate of spruce was confirmed by our results demonstrating good drought resistance and frost hardiness in plants grown from originally slow-growing seedlings. After outplanting to extreme mountainous conditions the markedly better health status and higher growth rate were observed in the planting stock grown from "small" (slow-growing) seedlings than in originally fast-growing plants. The foliage percentage was highest in the originally small plants (Jurásek, Martincová 1996, 2001). Detailed evaluation confirmed their good physiological predispositions to resist adverse influences and climatic extremes occurring in mountain areas in longer time intervals. It agrees with conclusions of Bigler and Veblen (2009) that fast growth and larger size may appear as an advantage from the aspect of higher competitiveness and enhancement of short-term chances of plant establishment. However, fast growth and large size imply lower investments in defence, lower wood density and mechanical strength, which may lead to a decrease in longevity.

Different criteria of the sorting of seedlings and plants should be used in the production of planting stock for higher mountainous locations because the

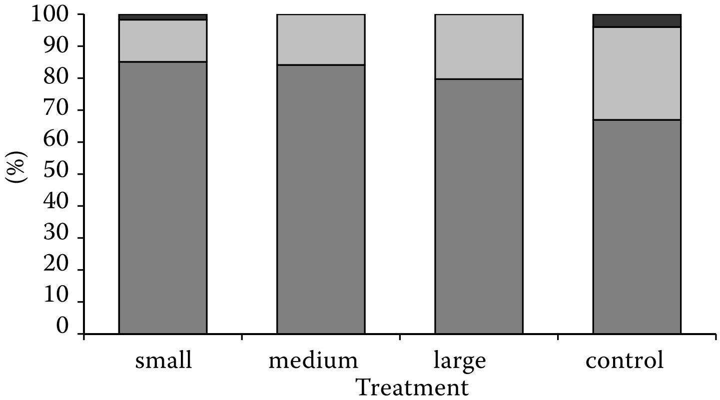

Fig. 6. Frequency of stem damage occurrence in the particular variants of spruce on research plot Pláň (a description of the particular degrees of bud break see Table 2)

■ – stem damage index 0

■ – stem damage index 1

■ – stem damage index 2

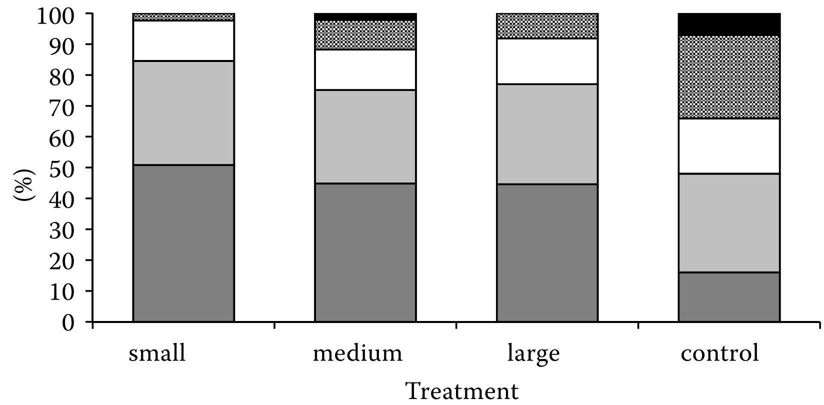

Fig. 7. Frequency of branch damage occurrence in the particular variants of spruce on research plot Pláň (a description of the particular degrees of bud break see Table 2 in the chapter Method)
■ – branch damage index 0
■ – branch damage index 1
□ – branch damage index 2
▩ – branch damage index 3
■ – branch damage index 4

culling of smaller, slow-growing plants may cause the narrowing of the genetic spectrum and discarding of just those plants that are best adapted to growth in extreme mountainous conditions (Holzer et al. 1987; Lang 1989; Jurásek, Martincová 1996, 2001). Neither did Kotrla (1998) consider as desirable the sorting out of small spruce plants and their culling or planting separately onto different plots because it may cause the pronounced narrowing of the genetic structure of progenies. But it should be defined precisely what seedlings are the real cull and in what seedlings slow growth may be connected with favourable genetic endowment for extreme conditions.

This latest knowledge has already been embodied in current legislation of the Czech Republic and in the Czech technical standard in force (ČSN 48 2115 1998) in which the size of seedlings used for transplanting or planting into containers is not defined any more. In plantable planting stock the current standard ČSN 48 2115 takes into account specificities of the growth of Norway spruce mountain populations while it is possible to increase the maximum age of planting stock from the 8^{th} and 9^{th} forest altitudinal zone by 1 year and the shoot height is not considered as the main morphological traits of quality.

CONCLUSION

The experiments demonstrated that the relatively small differences in physiological parameters, which were observed among the variants, markedly influenced the health status of trees after 16 years of growth on an extreme mountainous clearcut area. The results of evaluation of physiological parameters and health status confirm a hypothesis that plants with the initial slow growth are a stable component of the population spectrum of mountain spruce trees. The results document good preconditions for the establishment of vital and stable stands when the entire growth spectrum of planting stock and particularly of plants produced from originally slow-growing seedlings is utilized.

Knowledge of the growth of Norway spruce mountain populations documents that the growth dynamics of a part of the population with increased resistance to stress factors is manifested more pronouncedly in the second decennium after outplanting onto extreme mountain sites.

This is the reason why it will be useful and necessary to study and evaluate all other experimental plantings of Norway spruce in longer time series after outplanting.

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