

Evaluation of the growth and health status of selected clone mixtures in comparison with ordinary planting stock

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ABSTRACT: The present paper compares the growth of parent trees and potentially stress-tolerant mixtures of clones of Norway spruce (*Picea abies* [L.] Karst.) progenies coming from a specific locality near the Černá hora peat bog in the Krkonoše Mts. Growth was studied in generative ortet plantations in Trutnov locality and in a mountain ortet plantation Lesní bouda, in the 1st generation clone plantation Benecko and in the 2nd generation clone plantation in the Černožská rašelina locality. In the latter locality chlorophyll fluorescence and water losses during controlled desiccation were also measured in selected clones compared to control (generatively propagated) spruces. Partial data acquired until now prove the good growth dynamics and physiological state of some clones in extreme climatic conditions indicating that cuttings were taken from vital parent trees growing in exposed mountain localities. Growth relations among the clones were identical in all evaluated localities. The growth of the 2nd generation clone plantation has been markedly influenced by plantation and specific site conditions until now. The mutual interaction of clone growth and site conditions can change in time and therefore the study of clone plantations will continue in the years to come.

Keywords: chlorophyll fluorescence; clonal propagation; growth; mother plantations; mountain conditions; Norway spruce; water losses

Stabilization of forest functions is the main objective of the present forest management in mountain areas. Norway spruce (*Picea abies* [L.] Karst.) has an irreplaceable (stand-forming) function in forest ecosystems at higher mountain locations; therefore it is desirable to assess real potentials of this tree species in order to increase the tolerance of newly established plantations. Development of forest systems at high altitudes is limited by a combination of environmental factors. Besides these natural limitations high mountains are especially sensitive to air pollution that can have very negative effects on already damaged forest stands (GRILL et al. 2005).

The selection of planting stock genetically best adapted to the given conditions is a crucial issue for

reforestation of high-elevation localities (HOLZER et al. 1991). One of the possibilities of increasing the stability of future plantations is to use spruce trees with higher stress tolerance. This is the reason why a great attention has been paid to progenies of the most vital spruces from remnants of indigenous stands in the Krkonoše model mountain area.

The objective of the present paper is to inform about the results of our research on the use of potentially stress-tolerant progenies of Norway spruce in forest regeneration in mountain localities.

These clone mixtures from Norway spruce mountain populations were gradually produced in the framework of long-term programmes using the clonal propagation (JURÁSEK et al. 1994); their re-

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alization started in the eighties, at the time of the culmination of air-pollution disaster. In that period, within the programme of the gene conservation of indigenous forest tree species in the Krkonoše Mts. (SCHWARZ 1996; SCHWARZ, VAŠINA 1997) relatively tolerant individuals that survived in disintegrating forest stands were selected. Our previous activities (Ministry of Agriculture of the Czech Republic Project MZe QD1274 “Stress-tolerant Clone Mixtures for Mountain Areas”) in the Krkonoše model mountain area were aimed at the establishment of a series of ortet plantations and clone plantations of spruce coming from indigenous or potentially stress-tolerant trees (JURÁSEK, MARTINCOVÁ 2005). Further selection was done during the collection of cuttings from vital trees in the 1st generation clone plantation. That means *in situ* double selection was done in these rooted cuttings of the 2nd generation. The selection of individuals for further growing was performed on the basis of the complex evaluation of parent trees (the health status was the main criterion, and both the individuals with intensive growth dynamics and the slow-growing individuals were selected for a subsequent mixture of clones). After their growing in a nursery they were outplanted in exposed locations where their observation continues and their growth and health status are compared with the ordinary planting stock of generative origin. The objective of these experiments is to evaluate possibilities of natural selection of tolerant clones by situating ortet plantations and clone plantations into extreme mountain conditions.

MATERIAL AND METHODS

Growth and health status were evaluated in parent trees in generatively established ortet plantations – research plots (RP) in favourable conditions in the Trutnov area (Trutnov RP) and in rather extreme mountainous conditions in the Krkonoše Mts. area (Lesní bouda RP). Their vegetative progenies – clones were evaluated in a clone plantation in the Benecko area and in the 2nd generation rooted cut-

tings (coming from the clone plantation on Benecko RP and outplanted in the extreme mountain locality Černohorská rašelina). A description of the plots is shown in Table 1.

We studied the progenies of spruces coming from the area of the Černohorská rašelina locality, i.e. such progenies that were potentially best adapted to specific local conditions. A detailed evaluation was done in the half-sib progeny of tree No. 8 from this locality (designated as cr8). Total number of planting stock outplanted on RP was 900. The clones that had a high number of individuals in all studied localities were selected within this progeny. The evaluation of spruce growth in clone plantations (RP) was based on the measurement of height and diameter growth. Diameter growth in young plantations was assessed by measuring root collar diameters. Shape irregularities, coloration changes and needle loss (defoliation) and potential damage to shoots were recorded at the same time.

The physiological state of selected clones was evaluated in a laboratory in samples of branches collected in the 2nd generation clone plantation on Černohorská rašelina RP. Branches were taken from the 2nd whorl from above in rooted cuttings and control plants grown by a routine method. The samples were put into a cooling box in the field and subsequently transported to a laboratory for evaluation. In the laboratory the branch bases were put into water, covered and sealed with black polyethylene foil in order to maintain high atmospheric humidity and let soak water overnight at a room temperature. On the next day they were exposed to light (covered with transparent foil) minimally for one hour to induce stomatal opening. Parts of annual shoots were then used for the evaluation of water losses. Single needles were taken from the remaining parts of branches to measure chlorophyll fluorescence. Needles were stuck onto cellotape strips on paper pads and before the measurements started, they were let adapt themselves to darkness in moist dark chambers minimally for 30 minutes. After the green dark-adapted tissues were illuminated, the intensity

Table 1. Description of research plots (RP)

Research plot	Type	Altitude (m a.s.l.)	Years of foundations
Lesní bouda	ortet mixture	1,080	1989
Trutnov		520	1990
Benecko	clone plantation 1 st generation	750	1997
Černohorská rašelina	clone plantation 2 nd generation	1,180–1,200	2004
			2005

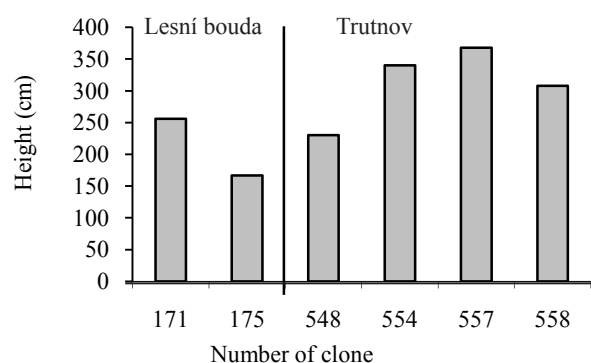


Fig. 1. Shoot height of parent spruces in generative mother plantations 12 years after outplanting

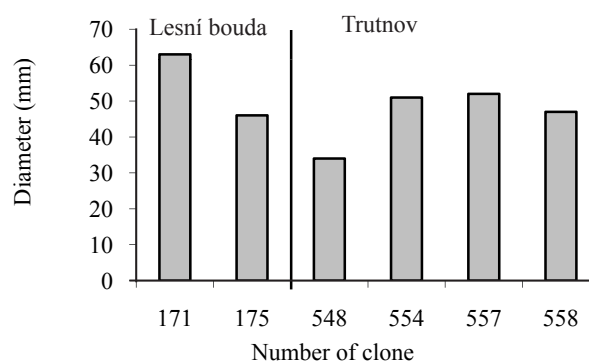


Fig. 2. Stem diameter of parent spruces in generative mother plantations 12 years after outplanting

of their fluorescence changed in a typical way indicating the state of the photosynthetic apparatus (MOHAMMED et al. 1995).

Chlorophyll fluorescence was measured with an Imaging-PAM 2000 device (Heinz Walz GmbH). Three needles from each branch were evaluated. In dark-adapted needle samples the basic characteristics of fluorescence were measured: F_o – minimal fluorescence and F_m – maximal fluorescence after a strong flash of light; from these variables the maximal quantum yield of fluorescence $(F_m - F_o)/F_m$ designated as F_v/F_m was computed, representing the maximal photochemical efficiency of photosystem II. This characteristic is used most frequently to assess the state of assimilatory organs (MAXWELL, JOHNSON 2000). A more detailed description of the above-mentioned basic variables was published in a number of theoretical papers (e.g. MAXWELL, JOHNSON 2000; LICHTENTHALER et al. 2005; RITCHIE, LANDIS 2005). Measuring light of the intensity $3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and saturation pulse of the intensity $2,400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with the duration of 800 ms were used for measurements in our laboratory.

The ability to resist drought was evaluated by repeated weighing of annual shoots in the course of controlled desiccation in laboratory conditions (SLAVÍK et al. 1965). Water content was expressed as % of the initial water content in saturation state.

Data were processed by Excel and QC Expert programmes. Analysis of variance (ANOVA) was used

to test the differences due to provenance of clones within in all studied characteristics.

Subsequently, paired comparisons of pairs of the clone progenies were done by Scheffé's test. Observed significant differences among the variants are documented in graphs of the particular characteristics (different letters show significant differences).

RESULTS

Comparison of the growth of parent trees and clones of the 1st and 2nd generation

Research plots were evaluated in the intervals of several years. So data acquired in plantations of different age growing in different natural conditions are available. The objective is not to compare the absolute values of reached height or stem diameter but to compare the relations among the clones or to compare the clone stock with ordinary generatively propagated plants.

Figs. 1 and 2 illustrate the height and diameter of parent trees in ortet plantations on Lesní bouda and Trutnov RPs 12 years after outplanting. Their evaluation must consider highly different growth conditions in the particular mother plantations (foothill and mountain sites). The presented values are mainly applicable to evaluate their vegetative progenies in clone plantations. The graphs document

Table 2. Analysis of variance for root collar diameter on Černohorská rašelina RP

Sums of squares	Degrees of freedom	Mean squares	F_{exp}
Variants (clones) $S_a = 809.5$	6	6,613	25,536
Error $S_r = 3,032.6$	574	5,229	
Total $S_c = 3,842.0$	580		

Conclusion of test: effect is statistically significant at the $\alpha = 0.05$ level

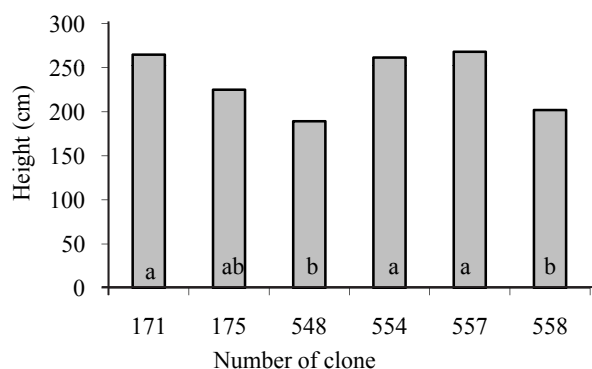


Fig. 3. Average shoot height of vegetative progenies of spruce (1st generation clones) in Benecko locality 9 years after outplanting – different letters in columns indicate statistically significant differences (5% significance level)

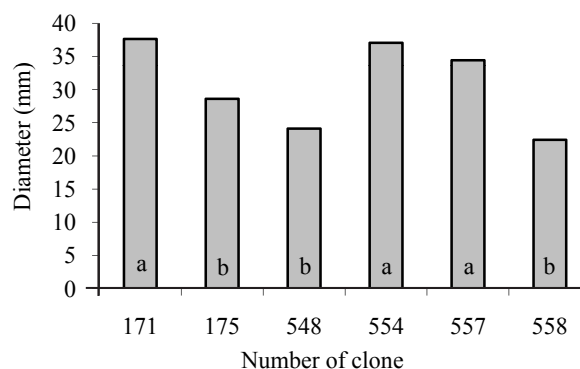


Fig. 4. Average stem diameter of vegetative progenies of spruce (1st generation clones) in Benecko locality 9 years after outplanting – different letters in columns indicate statistically significant differences (5% significance level)

excellent growth of tree No. 171 in Lesní bouda ortet plantation. The growth of tree No. 548 is obviously worse compared to the other trees in Trutnov ortet plantation.

A similar trend was observed in the clone plantation on Benecko RP (Figs. 3 and 4), where columns represent the average values of vegetative progenies (clones) of the above-described trees. All trees grow there in relatively identical conditions of one locality. Obviously, the growth of clone 171 is also very good in this locality while clone 548 is lagging behind.

The analysis of variance for morphological traits and the values of chlorophyll fluorescence of trees growing on Černohorská rašelina RP indicates high statistical significance of the influence of provenance of particular variants (clones) (Table 2).

Dispositions to the growth rate of particular clones were maintained to a large extent also in the 2nd generation clone plantation on Černohorská rašelina

RP (Figs. 5 and 6). The evaluation of morphological traits of the clone plantation in this specific locality showed very good growth of some clones originally coming from this locality, especially of clone No. 171. The worst growth was observed in the progeny of clone No. 548 again.

A comparison of the growth of rooted cuttings (2nd generation clones) and control planting stock produced by a routine method shows the relatively good growth of generatively propagated plants for the time being. The health status (defoliation was not higher than 10% in any variant and there occurred hardly any changes in the coloration of assimilatory organs 2 years after outplanting) and growth dynamics of rooted cuttings were very good. This is the reason why we suppose that the favourable effect of the genetic quality of clone stock will be expressed over a longer period of growth in specific conditions similarly like in other experiments of ours.

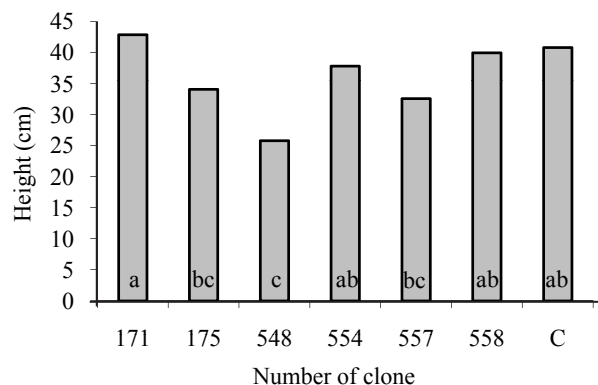


Fig. 5. Average shoot height of vegetative progenies of spruce (2nd generation clones) in Černohorská rašelina locality 2 years after outplanting – different letters in columns indicate statistically significant differences (5% significance level), C – control

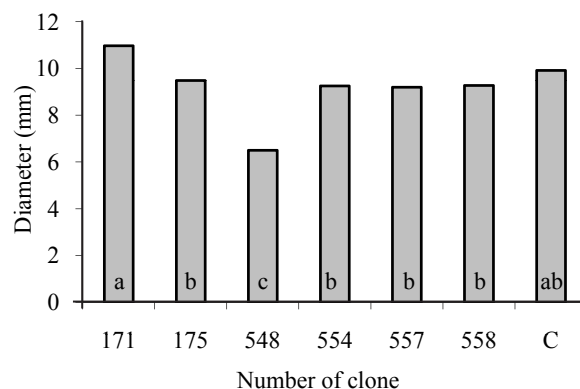


Fig. 6. Average root collar diameter of vegetative progenies of spruce (2nd generation clones) in Černohorská rašelina locality 2 years after outplanting – different letters in columns indicate statistically significant differences (5% significance level), C – control

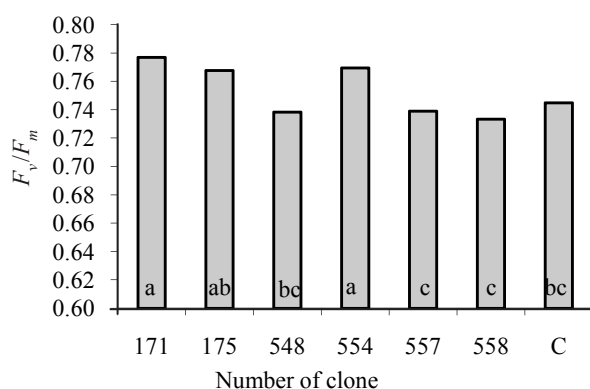


Fig. 7. Maximal quantum yield of chlorophyll fluorescence F_v/F_m of needles of spruce samples from Černohorská rašelina RP – different letters in columns indicate statistically significant differences (5% significance level), C – control

Evaluation of the physiological state of spruce plants in the 2nd generation clone plantation

The physiological state of selected clone progenies was evaluated in the 2nd generation clone plantation on Černohorská rašelina RP. Chlorophyll fluorescence was measured in the spring season and the intensity of water losses was assessed in laboratory conditions in one-year shoots from the previous year.

The evaluation of chlorophyll fluorescence shows the very good state and function of photosynthetic apparatus in rooted cuttings of all studied clones. The best values were measured in trees of clone 171 again. The results document very good adaptation of rooted cuttings to conditions of an extreme mountain locality. They also indicate the better state

of photosynthetic apparatus in comparison with control generative plants of the spruce mountain population (Fig. 7).

The evaluation of water content in shoots after 15 and 180 minutes of controlled desiccation in laboratory conditions (Figs. 8 and 9) suggested the worse ability of trees of clone 548 to resist drought. On the contrary, the best-growing clone 171 was able to maintain a high water content during desiccation. The results of evaluation of the physiological state of the 2nd generation rooted cuttings correspond to data on the growth of particular clones acquired in repeated *in situ* measurements.

DISCUSSION

Ortet and clone plantations were established in the last years mainly for the purposes of silvicultural research, i.e. successful artificial forest regeneration in extreme mountain conditions and formation of stable forest ecosystems. It is not a classical breeding programme that would allow using standard breeding methods of data processing. The objective was to acquire new knowledge essential for forest regeneration in extreme mountain locations.

The results of field surveys showed the same relations in height and diameter growth among the studied clones in generative mother plantations and clone plantations of the 1st and 2nd generation. The higher growth dynamics of clones obtained from the best-quality trees with the best health status is a well-known fact (ROULUND 1977; ELERSEK, JERMAN 1988; IŞIK et al. 1995; SONESSON, ALMQVIST 2002; LEUGNER et al. 2008) and the clone selection

Table 3. Analysis of variance for shoot height on Černohorská rašelina RP

Sums of squares	Degrees of freedom	Mean squares	F_{exp}
Variants (clones) Sa = 14,889.2	6	245,319	11,159
Error Sr = 127,641.1	574	220,071	
Total Sc = 142,530.3	580		

Conclusion of test: effect is statistically significant at the $\alpha = 0.05$ level

Table 4. Analysis of variance for the values of chlorophyll fluorescence F_v/F_m on Černohorská rašelina RP

Sums of squares	Degrees of freedom	Mean squares	F_{exp}
Variants (clones) Sa = 0.045	6	0.000713	15.438
Error Sr = 0.087	178	0.000471	
Total Sc = 0.132	184		

Conclusion of test: effect is statistically significant at the $\alpha = 0.05$ level

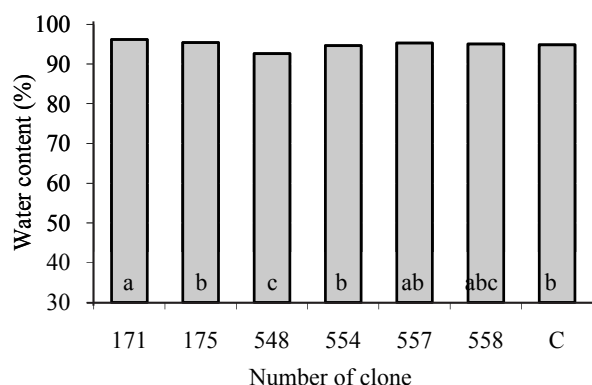


Fig. 8. Water content in annual shoots after 15 minutes of desiccation in laboratory conditions (in % of the initial water content) – different letters in columns indicate statistically significant differences (5% significance level)

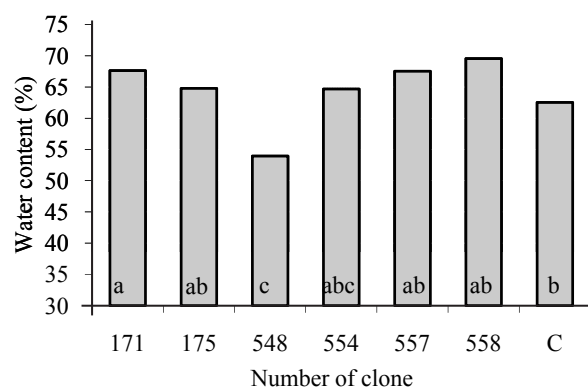


Fig. 9. Water content in annual shoots after 180 minutes of desiccation in laboratory conditions (in % of the initial water content) – different letters in columns indicate statistically significant differences (5% significance level)

in Norway spruce is used in forest operations to increase the production of vegetatively propagated planting stock.

The growth of Norway spruce mountain populations is rather different compared to populations from lower locations. Besides, the primary objective in extreme mountain conditions is not to ensure production but first of all to provide for the stability of forest ecosystems. Mountain populations of Norway spruce have lower growth rate compared to populations from lower locations (KOTRLA 1998; OLEKSYN et al. 1998; UHLÍŘOVÁ 1999; MODRZYŃSKI, ERIKSSON 2002) and different growth rhythm (LANG 1989; WESTIN et al. 1999; HANNERZ, WESTIN 2000; WESTIN et al. 2000b; MODRZYŃSKI, ERIKSSON 2002). Earlier termination of elongation growth and bud formation are marked characteristics (HOLZER et al. 1987; MOLMANN et al. 2006). Such growth dynamics is fixed genetically, and spruce seedlings maintain it at least in the first year of growth even though they are grown in completely different conditions (greenhouse, growth chamber) (HOLZER 1984; QUAMARUDDIN et al. 1995). Adaptation to the adverse environment at the cost of growth is considered to be one of the main causes (OLEKSYN et al. 1998).

In extreme mountain conditions the aim of planting stock selection is not higher growth rate but it is the best adaptation to adverse environmental factors. MODRZYŃSKI and ERIKSSON (2002) reported higher resistance to drought in spruce populations originating from high altitudes above sea level compared to spruce from lower locations; their higher frost hardiness is also known (HAWKINS, SHEWAN 2000; WESTIN et al. 2000a). Therefore progenies of trees best surviving and growing in these specific extreme conditions should be used for the reforestation of extreme localities.

The results of morphological surveys in our trials document good growth dynamics of the selected 2nd generation clones. Although the differences in growth dynamics were not statistically significant in all cases, these findings are very interesting, confirming a hypothesis that the selection of clones for extreme climatic conditions can be done through natural selection in mother plantations in exposed mountain localities (SCHACHLER et al. 1986).

In our trials the study of the 2nd generation clone plantations showed high variability of growth not only among the clones within one progeny but also within some clones. Three years after outplanting the influence of transplant shock was still visible in these extreme conditions. The influence of differences in microsites within one locality was also considerable. The observed large intraclonal differences are consistent e.g. with data of JOHNSEN and SKRØPPA (1992), who observed high variability of growth within some clones of Norway spruce while other clones were homogeneous. WONISCH et al. (1999) reported that in the particular localities the conditions of small-area sites, i.e. soil conditions, in combination with large-area influences such as altitude contributed to the stress of trees. Based on detailed evaluation of a number of biochemical and physiological characteristics they found out that small-area soil influences, e.g. insufficient supply of water, could contribute to the overall stress of spruces in a crucial way. High sensitivity of young spruces to microsite conditions was reported by JONSSON (1999). Other authors also described a significant clone × site interaction in Norway spruce (IŞIK et al. 1995). KARLSSON and HÖGBERG (1998) and KARLSSON (2000) stated that the height growth of clones by site interaction often changed with the age of clone plantation. The selection of clones

propagated by cuttings according to their height in a nursery influenced the height of clones 6 years after outplanting to a small extent only (HÖGBERG 2003). IŞIK et al. (1995) also concluded that the height of cuttings in a nursery was not a reliable indicator of future development after outplanting. It is recommended to select clones older than 8 years for growth (GEMMEL et al. 1991).

A comparison of selected clones with the control planting stock of the Norway spruce population Krkonoše 3 years after outplanting indicated relatively good growth and physiological quality of generatively propagated plants, which is consistent with data reported by KRIEGEL (2003), who also compared the growth and health status of vegetatively and generatively propagated planting stock of Norway spruce from the 7th and 8th forest altitudinal zone in the Krkonoše Mts. Genetic quality gained by vegetative propagation of high-quality spruce plants is not mostly expressed immediately after outplanting, which was documented e.g. by SONESSON and ALMQVIST (2002), who evaluated 5,000 spruce clones in Sweden and ascribed the large height increment of spruce clones compared to generative plants 6 years after outplanting, besides good genetic characteristics, to better characteristics of planting stock when rooted cuttings had thicker stems and were generally more robust than seedlings. Rooted cuttings on Černoohorská rašelina RP had very good health status and growth dynamics. It is assumed that the favourable influence of genetic quality will be expressed after a longer period of growth in specific conditions similarly like in other experiments of ours (JURÁSEK et al. 2005).

Different dynamics of physiological processes is described in rooted cuttings compared to seedlings, e.g. later onset and lower intensity of dormancy and cold hardiness and earlier flushing in spring (FENNESSY et al. 2000). The evaluation of the 2nd generation rooted cuttings in Černoohorská rašelina research locality did not reveal any larger differences in the intensity of water losses between rooted cuttings and generatively propagated material. Certain differences observed among the clones corresponded to the growth rate of these clones. The measurement of chlorophyll fluorescence may provide detailed information on the photochemistry of photosystem II, which is sensitive to adverse environmental factors such as strong light, low temperature, overheating or drought (MAXWELL, JOHNSON 2000; KITAO 2004; LICHTENTHALER et al. 2005). The values of maximal quantum yield of fluorescence measured in our clone plantation document the better state of photosynthetic apparatus in selected clones compared to control plants.

CONCLUSION

The study of the growth and vitality of selected clones in ortet and clone plantations brought about the following information:

- Identical relations of growth among the studied clones were observed on research plots with ortet and clone plantations in different site conditions. In all localities the growth of clone No. 171, which represents dynamically growing clones in original generative mother plantations, was markedly the best. On the contrary, the clone that was selected as a representative of the lowest-quality clones in the generative ortet plantation was the worst again in all types of sites. Relatively good growth in the extreme mountain locality Černoohorská rašelina was also observed 2 years after outplanting in the control (generative) planting stock of the spruce mountain population.
- The above-mentioned differences in morphological traits of clone plantations correspond to physiological characteristics studied in the 2nd generation clone plantation. The maximal quantum yield of photosystem II photochemistry (F_v/F_m) was measured in the best-growing clone 171. This clone also had the lowest water losses during controlled desiccation. On the other hand, the worst-growing clone 548 had the least favourable values of these parameters.
- The results of measurements of chlorophyll fluorescence and water losses during controlled desiccation indicated the better instantaneous physiological state of studied clones compared to the control plants of generative origin. They confirmed the better adaptation of selected clones of local provenance to the specific conditions of mountain locality.
- The results illustrated very good growth dynamics of selected clones in extreme climatic conditions provided that cuttings were taken from vital parent trees growing in exposed mountain localities.

The growth of the 2nd generation clone plantation will require subsequent measurements in a longer time series in order to eliminate the potential influence of transplant shock and of the clone growth by site conditions interaction. But the results confirm a possibility of using the spruce clone stock and *in situ* selection for the selection of potentially more stress-tolerant clones. As a frame of newly established forest stands this planting stock could contribute to the stabilization of forest ecosystems in extreme mountain conditions.

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