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Efficiency of methods to support natural regeneration in Scots pine genetic reserves

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Artificial regeneration of forests affects their genetic composition. Under natural regeneration, the evolutionary processes are less disturbed and the initial genetic composition of the populations is efficiently preserved. The objectives of this study were to assess natural regeneration in three genetic reserves of Scots pine in Lithuania and to determine the main factors affecting the success of regeneration. The results have shown that regeneration fellings of variable intensity are the most suitable method in poor sites with a low grass and shrub cover. On richer sites, specific means to eliminate the suppressing vegetation and to support natural regeneration are necessary. The felling shall be synchronized with a year of abundant seed yield. To ensure a sufficient genetic diversity of the new generation, the number of seed trees and their even distribution over the site are important factors to consider. For this purpose, the list of measures in the National Regulations on Forest Genetic Reserves are appropriate for a successful regeneration of Scots pine genetic reserves.

Key words: gene conservation, genetic recourses, natural regeneration

INTRODUCTION

The climatic change causes such natural calamities as storms, droughts, temperature fluctuations, invasion of pests and diseases, which all markedly affect forest genetic resources. An urgent revision of the present-day gene conservation programmes based on a strict "non-touch" approach is needed, where development and adjustment of existing measures to support natural regeneration and create a stable multi-aged stand structure are a high priority. In each eco-climatic zone, natural regeneration may have specific aspects which may require modifications of the well-established forest regeneration practices.

One of the most natural gene conservation methods is establishment of genetic reserves, their protection and promotion of natural regeneration under generation shift. A genetic reserve is a long-term conservation means and is defined as autochthonous forest stand(s), where the target tree species dominates and represents the genetic composition evolved in a local adaptive environment or contains a certain valuable property. Forest genetic reserve is established over a definite forest area (greater than 3 ha) and may comprise one or several forest stands. Genetic reserves may also be used for tree breeding. Silvicultural means in forest genetic reserves must secure a natural regeneration of the target tree species and form a multi-age structure. This preserves the stability of the new generation and the genetic structure of the parental generation [1, 2]. Favourable conditions for regeneration of forest stands are provided: soil scarification, opening of more light for understory and elimination of suppressors of naturally regenerating trees of the target tree species. By applying the intergraded gene conservation, natural conditions and natural selection processes in forest stands are supported [3]. There is a number of regeneration felling methods used in Lithuania [4]. In practice, a simplified type of felling over two intensity steps is commonly used.

Presently, forest genetic reserves make up 0.7% of the total forest area in Lithuania. There are 67 genetic reserves of Scots pine with the area of 2374.6 ha, which is 0.43% of the total area of Scots pine stands in the country. What is the state of gene conservation stands in the other countries with similar eco-climatic conditions as in Lithuania? According to the *in situ* gene conservation methods used in Austria, Poland, Slovakia and Scandinavia, gene conservation reserves should make up 1–2% of the species area [5]. In Sweden, a systematic provenance transfer was started in 1950 and the gene conservation programme was started in 1980. The main requirements: strict rules regarding clonal forestry and genetically modified trees; in tree breeding programmes, gene diversity is considered of similar importance as the genetic gain, and the long-term breeding programme is based on the multiple population principle and balanced within family selection; genetic resources are conserved in natural and artificial populations by including means to conserve rare alleles; the gene reserves are allocated by a systematic grid which not necessarily includes phenotypically superior stands [6]. In Norway, the gene conservation programme is mainly focused on assessment of genetic diversity, studies of epigenetic effects and establishment of the *in situ* and *ex situ* gene conservation units [7]. In Germany, dynamic and static gene conservation methods are used. The dynamic methods are the following: (1) conser-

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vation *in situ* and the natural regeneration or artificial support with local seed to form the multi-aged structure of the stands, (2) conservation *ex situ* of the endangered and therefore evacuated populations. The static methods are clonal archives, seed orchards and gene banks [8]. DNA fingerprinting of the natural populations is planned. In Switzerland and Austria, genetic reserves are the main means of gene conservation with an average area of a reserve 10–20 ha [9]. In Austria, genetic recourses are mainly conserved via genetic reserves and forest stands of a comparably small area. The genetic reserves are usually larger than 30 ha to capture the genetic variation and create favorable conditions for its evolution. *Ex situ* means are viewed as complementary measures [10]. In Poland, forest genetic resources are conserved in state national parks, forest reserves and other small-scale gene conservation units. 1.85% of the total forest area in the country is used for *in situ* gene conservation [11]. In Slovakia, the biodiversity of forest ecosystems is conserved in nature reserves, whereas forest genetic resources – in forest genetic reserves. 15% of the total forest area in the country is used for *in situ* gene conservation. It is foreseen to enlarge the conservation areas up to 2% of the total forest area in the country [12]. In Canada, forest genetic resources are conserved *in situ* (genetic reserves) and *ex situ* (archives and gene banks) [13]. The gene conservation guidelines prepared within the competence of the EUFORGEN encourage dynamic gene conservation together with long-term breeding programmes, where genetic reserves remain the most efficient *in situ* gene conservation measure [14]. This review indicates that genetic reserves are and will remain among the main measures for conserving forest genetic resources, and there is a need for recurrent revisions of their management plans to consider the threats caused by climatic fluctuations.

The objective of our study was to assess the efficiency of alterative methods to naturally regenerate the genetic reserves of Scots pine on poor and rich soils and to discuss not only the silvicultural aspects, but also the ability to sample a sufficient adaptive diversity in the new generation.

MATERIALS AND METHODS

The following three Scots pine genetic reserves were studied:

- Braziukai, area 74.1 ha (in Braziukai forest district of the Kazlų Rūda forest enterprise). Felling over several occasions in 1999 to 2004.
- Prienai No. 1, area 16.6 ha (compartment 91, in the Prienai state forest enterprise, the site type normally irrigated on medium fertility sandy-loam soils (Ncl). Felling over several occasions in 1999 to 2004.
- Prienai No. 2, area 4.5 ha (compartment 86, in the Prienai state forest enterprise, the site type normally irrigated on poor sandy soils (Nbl). Felling over several occasions in 1999 to 2004.

After regeneration felling in the Braziukai genetic reserve, 70–80 seed trees per ha were left and the site was ploughed in furrows (1.5–2 m apart from each other) and scarified in squares (5 to 7 thousands of squares per ha). In the Prienai genetic reserves, the site was ploughed in furrows at each 1.5–2 m and in addition, squares were scarified where furrows were irregular. The natural regeneration success was assessed by counting the naturally regenerating seedlings of Scots pine in the sample plots which were laid out as follows [15]: 4 × 5 m large sample plots were allocated at each 50 m along the longest diagonal of the felling area in each of the forest genetic reserves. The following assessments were made in the sample plots: the number of naturally regenerating seedlings of Scots pine and the number of seedlings located at least 0.7 m apart from each other (possible survivors to form a young understory). In all three reserves, 54 such sample plots were established and assessed. The grass abundance was assessed in scores: 1 – abundant cover (90–100% of the scarified area is covered with grass), 2 – medium abundance (11–89% of the scarified area is covered with grass) and 3 – weak grass cover (less than 10% of the scarified area is covered with grass). Specific data on the genetic reserves are given in Tables 1–3 and the management criteria in [16, 17]. The list of genetic reserves in the country may be found in [18]. The law on national genetic resources is given in [19].

RESULTS AND DISCUSSION

In the Braziukai genetic reserve, the mean number of naturally regenerating seedlings per sample plot was 60.9 (or 30.4 thousand per ha) and 79.7 (or 39.8 thousands per ha) in the reserve parts scarified in squares and ploughed in furrows (Table 1). It is a fairly good regeneration statistics for Scots pine. The mean plot number of seedlings, which where spaced at least 0.7 m apart from each other (indicating the uniformity of regeneration), was 36.2 seedlings (18.1 thousands per ha) and 37.9 seedlings (19.9 thousands per ha) in the part of the reserve scarified in squares and furrows, respectively (Table 1). These numbers are sufficiently large to form a new understory. Thus, scarification in furrows was more efficient than scarification in squares (the latter creates a more scarified surface), though both provided a sufficient number of seedlings for natural regeneration. Scarification in squares may be distributed less irregularly than in furrows, what allows a more natural way of regeneration.

Table 1. **Mean number of seedlings per sample plot and mean grass cover score in the Braziukai genetic reserve (sample plot size 20 m²)**

Descriptive statistics	Grass cover, score	Site prepared in squares (5–7 thous. squares /ha)		Site prepared in furrows 1.5–2 m apart from each other	
		Total	No. of seedlings at least 0.7 m apart from each other	Total	No. of seedlings at least 0.7 m apart from each other
Mean	2.88	60.9	36.2	79.7	37.9
Standard deviation	0.39	35.1	8.9	52.3	7.7

Apparently 70–80 seed trees on this poor site were enough to provide a sufficient number of seedlings and create an optimum light regime for Scots pine seedlings to be competitive with grasses. As regards gene diversity, 50 unrelated genotypes are regarded as a sufficient number to carry out the common alleles important for adaptation [20–22]. Since in natural stands there are clusters of relatives, this number of 50 can be increased more than twice to some 70 –150 tree per ha. Since genetic reserves may also serve as seed collection stands, a sufficient diversity level is also critical [6, 23–25]. Genotypes with a higher

fitness relatively stronger contribute to the genetic composition of the future generation [28]. In Finland, Sweden, Estonia and Poland, natural regeneration of Scots pine is a common forest regeneration method, leaving 100–150 seed trees per ha depending on the site type and age (crown size). The seed trees are usually removed when the young uderstory of Scots pine reaches the height of 0.5 to 0.7 m [26]. In addition to seed tree number and soil scarification, availability of seeds (seed crop) and their dispersal time and patterns are also important factors for a successful regeneration. Air humidity has a significant effect on the opening of cone scales, and wind affects seed dispersal patterns. In spring, seeds are usually dispersed within two months. Approximately 30% of the seeds are dispersed around the tree's crown and the most of the seeds are falling within the 20–30 meter cycle around the tree [27]. Only ca. 3% of the seeds reaches the distances of 100 meter away from the seed tree [27]. Seed germination and initial growth of sprouts is manly affected by the temperature and humidity. In thinned stands, the soil surface is warming up relatively more. Scots pine seeds start to germinate at a temperature of +4 °C. Soil moisture shall also be optimal: if too dry – germination is weak, if too moist – the young seedling growth and vigor are weak. The optimum humidity for germination of Scots pine seeds is 35%, and the start of seed germination is very sensitive to the shortage of humidity [27].

In the Prienai genetic reserve No. 1 (compartment 91), after fellings in 1998, 2002 and 2003, there were 126, 75 and 6 seed trees, respectively. The site was prepared in furrows at each 1.5–2.0 m, but in plots where the remaining stumps inhibited furrowing the site was prepared in squares. The mean number of naturally regenerating seedlings per sample plot was 23.6 (Table 2). The mean number of seedlings spaced at least 0.7 m apart from each other (indicating the uniformity of regeneration) was 11.8 thousands per ha. However, there was a marked difference between the number of regenerating seedlings in different parts of the reserve, and it depended on the abundance of the grass cover. In the parts with an abundant grass cover, there were merely 1–2 seedlings per sample plot. The moist harm is caused by tall grasses, such as ferns, and there are no Scots pine seedlings in such plots. In plots with a medium grass abundance, there were 8.8 thousands of seedlings per ha. If appropriate support measures are applied, such number is sufficient to form a new stand.

The support measures would include elimination of tall grass, lower shrubs and fast-growing soft-wood deciduous trees, because the sites were wetter (Lbl) with a more abundant grass cover. In plots with a low grass abundance, there were 20 thousands of seedlings per ha. This is a very good regeneration record. To summarize data on this reserve, in over 53% of the reserve area natural regeneration is good, over 27% satisfactory, and over 20% insufficient or very weak. In the weakly regenerating part, it is necessary to use artificial regeneration with the local seed-born seedlings.

In the smallest Prienai genetic reserve No. 2 (compartments 86, area only 4.5 ha, poor soils), after fellings in 2000 and 2004, there were 80 and 10 seed trees, respectively. The site was prepared in squares (7000 squares per ha). The mean number of naturally regenerating seedlings per sample plot was 36.9 (18.4 thousands per ha) (Table 3). The mean number of seedlings,

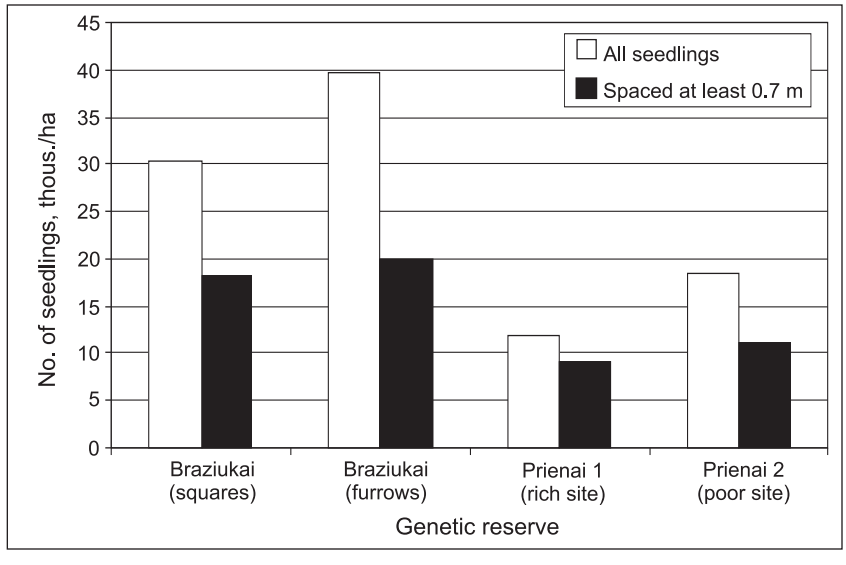

Figure. Comparison of number of regenerating seedlings per ha in Braziukai (soil treatment in furrows and squares) and in two Prienai reserves

Table 2. **Mean number of seedlings in the sample plot given by seedling height (less and more than 50 cm) and grass cover score in Prienai genetic reserve No. 1 (91 compartments) (sample plot size 20 m²). Site type Ncl (rich)**

Descriptive statistics	Seedling number		Seedling number by grass cover				
	<50 cm	>50 cm	>90% of the scarified area covered with grass	50% of scarified area covered with grass		<10% of scarified area covered with grass	
			<50 cm	< 50 cm	>50 cm	<50 cm	>50 cm
Mean	22.9	0.7	0.5	16.6	1.0	32.5	0.7
Standard deviation	20.9	1.2		11.3	0.6	18.8	1.7
Variation coefficient	79.0	63.8		68.2	34.6	48.3	86.6

which where spaced at least 0.7 m apart from each other (indicating a uniformity of regeneration) was 11.2 thousands per ha. This is a sufficient regeneration density to form a new young undergrowth. Only in over 10% of the reserve area the natural regeneration was weak. In this reserve, ca. 50 seed trees per ha were left as the seed source and shading for grass. In this reserve, measurements of the space gaps between the seed trees showed that if the distance between the edges of the crowns of seed trees is less than 10 m (or the stocking level is no more than 0.4 out of 1 where 1 means complete closure), natural regeneration was weak. Grass cover had a major effect on natural regeneration success (Table 3, compare the last two columns). The variation in seedling number between the sample plots with an abundant grass cover was much less (indicating a uniformly weak regeneration over such plots).

Table 3. **Mean number of seedlings per the sample plot given by grass cover score in Prienai genetic reserve No. 2 (86 compartments) (sample plot size 20 m^2). Site type Nbl (poor)**

Descriptive statistics	Mean seedling number, total	Mean seedling number by grass cover	
		50% of scarified area covered with grass	<10% of scarified area covered with grass
Mean	36.9	22.5	40.1
Standard deviation	9.4	10.6	5.7
Variation coefficient	25.4	47.1	14.1

In all three reserves, the natural regeneration success was mainly dependent on the abundance of grass cover and on site fertility. In the Braziukai genetic reserve, there was a relatively low grass coverage (score 2.88 of the highest 4 scores) and the highest number of naturally regenerating seedlings (Tables 1, 2 and 3). In the Prienai reserve No. 2, on poor soils, the number of regenerating seedlings was 1.5 times greater than in the Prienai reserve No. 1 on relatively richer soils, where also a more aggressive grass cover prevailed (Table 4).

Table 4. **Comparison of seedling number per ha after regeneration cuttings in Prienai genetic reserves Nos. 1 and 2**

Reserve	Felling area size, ha	Site type	Time of felling	Seedling number according to grass cover density			
				Low	Medium	Dense	Mean
No. 2 (comp. 86)	4.5	Poor (Nbl)	1999–2004	29.0	11.2	–	18.4
No. 1 (comp. 91)	16.6	Rich (Ncl)	1998–2004	16.6	8.8	0.2	11.8

Regardless of the regeneration success after fellings, we recommend collecting seeds from the felled representative trees (selected over all parts of the area) and to use part of the seeds to produce seedlings to support the natural regeneration. Felling and soil scarification shall be synchronized with seed years. Supplementary soil scarification to support natural regeneration may be made at least within a 3–4-year period (which is the periodicity of seed years) over which most of the genotypes may have the possibility to bear seeds and disperse them.

CONCLUSIONS

1. Regeneration felling over several occasions with a variable felling intensity gives good results only on normally irrigated and poor soils (Nbl) with a weak grass, dwarf shrub and lower shrub cover. For this purpose, *vaccinium* (Nabl) and *vaccinium-myrtilosum* (Nbl) site types are most appropriate.

2. Since the number of naturally regenerating seedlings was higher on sites scarified in furrows than on the sites scarified in squares (with squares, a smaller area is scarified), scarification in furrows is recommendable.

3. The best time to scarify soil is immediately after the first thinning when the seed trees are left. The measure to support natural regeneration should be synchronized with the years of abundant seed yield.

4. After the first felling, it is important to leave an optimum number of seed trees – 70 to 100, evenly distributed (because the number of trees affects the regeneration success). Since Scots pine seedlings do not tolerate overshadowing, the best regeneration results were obtained when the stocking level after the first felling was no more than 0.4 (1 meaning crown closure). Regeneration of Scots pine is weak and tree height is 3 times greater (owing to the lack of light) when the distance between the tree crown projections is less than 10 m.

5. To secure natural regeneration in genetic reserves, a proper timing of the following means is needed: appropriate felling methods, sufficient number of seed trees, means to support natural regeneration, control of suppressing ground vegetation.

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References

1. Gabrilavičius R, Miškininkystė 1995; 35: 6–18.
2. Gabrilavičius R. Žemės ūkio mokslai 1998; 3: 56–60.
3. Geburek T, Stephan B, Scholz F Forstw. Cbl 1989; 108: 204–11.
4. Labanauskas B 1969. Reports of the Lithunian Forest Reseach Institute. XII; 118–22 (in Russian).
5. Merlo M, Päivinen R. EFI News 1998; 2: 5–25.
6. The Swedish Forest Gene Conservation Programme. National Board of Forestry 1997: 7.
7. Skrøppa T. Skogsternes Genetiske Mangfold 1996: 28.
8. Konzept zur Erhaltung Forstlicher Genresourcen in der Bundesrepublik Deutschland. Forst und Holz; 15: 379–404.
9. Bonfils P, Müller-Stark G. Forstliche Generezervate in der Schweiz 1993: 31.
10. Geburek Th, Mueller F. National forest genetic resources programme of Austria. Federal Forest Research Centre. Vienna 1996: 38.
11. Program zachowania leśnych zasobów genowych i hodowli selekcyjnej drzew leśnych w Polsce na lata 1991–2010. Warszawa 1993: 62.
12. Longauer R, Zachowanie biodiversity leśnych ekosystémow. Zvolen 1995: 22.

13. Nieman TC, Mosseler A, Murray G. Forest Genetic Resource Conservation and Management in Canada. Petawawa National Forestry Institute 1995: 103.
14. Conservation of genetic resources of Noble Hard-words. Roma, IPG RI, EUFORGEN 1996: 53.
15. Miško atkūrimo ir įveisimo nuostatai. Miško sodmenų, želdinių ir žėlinių apskaitos ir vertinimo metodika 2001: 71.
16. Gabrilavičius R, Danusevičius J, Gradeckas A, Riepšas E. Lietuvos miško genetinių išteklių išsaugojimo, miško genetinio pagerinimo ir atkūrimo koncepcija. Mokslinė ataskaita. Kaunas-Girionys 1996: 30.
17. Forest genetic resources. Catalogue of Lithuanian Plant Genetic Resources. Dotnuva-Akademija 1997: 223–76.
18. Augalų (miško) genetinių draustinių nuostatai. Augalų nacionalinių genetinių išteklių įstatymas ir poįstatyminiai aktai. Vilnius 2004: 75–8.
19. Lietuvos respublikos augalų nacionalinių genetinių išteklių įstatymas (2001). Augalų nacionalinių genetinių išteklių įstatymas ir poįstatyminiai aktai. Vilnius 2004: 5–11.
20. Donell O. Proceedings of the Nordic group of tree breeding. Edinburg, Scotland 1993: 80–94.
21. Danusevičius J, Gabrilavičius R. Botanica Lithuanica 1999; 2: 125–34.
22. Pliūra A. Botanica Lithuanica 1999; 2: 105–24.
23. Krusche D, Geburek T. Mitteilung der BFH fur Forst-und Holzwirtschaft 1990; 164: 67–81.
24. Riepšas E. Metodinės rekomendacijos. Vilnius 1990: 55–58 (in Lithuanian).
25. Muller F, Schultze U. Erhaltung genetischer Resourcen im Wald 1998: 120–35.
26. Zasady hodowli lasu 2002: 45–83.
27. Suchockas V. Baltic Forestry 2001; 1(12): 79–83.
28. Сольбриг О, Сольбриг Д. Популяционная биология и эволюция. Москва 1982: 488.

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PAPRASTOSIOS PUŠIES GENETINIŲ DRAUSTINIŲ EFEKTYVŪS NATŪRALAUS ATKŪRIMO METODAI

Santrauka

Dirbtinai atkuriant miško populiacijas gerokai pakeičiama jų genetinė struktūra. Atsikuriant natūraliai mažiau pažeidžiami gamtiniai evoliucijos procesai ir labiau išsaugoma pirmykštė populiacijos genetinė sudėtis. Tyrimų tikslas – nustatyti miško genetinių draustinių regeneraciją bei įvertinti tam įtakos turinčius veiksnius. Analizuojant pušies regeneraciją trijuose miško genetiniuose draustiniuose išaiškinta, kad atkuriamieji kirtimai labiausiai tinka augavietėse, kuriose negausiai želia žolė ir puskrūmiai. Derlingesnėse augavietėse būtina aktyviai kovoti su pušaites stelbiančia augmenija bei taikyti žėlimą skatinančias priemones, kirtimus derinti su sėkliniais metais. Svarbus optimalus priedanginių-sėklinių genotipų skaičius po pirmojo kirtimo, kad nauja karta turėtų pakankamą palikuonių genetinę įvairovę. Taikant miško genetinių draustinių nuostatuose nurodytas atkūrimo priemones ir prisilaikant regeneracijos reglamentavimo, pušies genetiniai draustiniai visiškai atkuriami.