Benefits of genetic testing for introduction of Douglas fir to Lithuania

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To facilitate establishment of a breeding program as a new step of Douglas Fir introduction to Lithuania, this study was made with he following objectives: (1) to assess differences in height, growth and quality among three subspecies of Douglas fir ("green, "blue", and "gray") grown as grafted clones in a seed orchard, and (2) to study the quality of open-pollinated families of Douglas fir originating from three provenances from the north-west of the USA.

The seed orchard study showed that the stem diameter of the "green" subspecies of Douglas fir was the largest (though the differences were small). There was no marked difference among the subspecies in stem quality and tolerance to the fungi *Rhabocline pseudotsugae* causing the needle fall. Trees of the "green" Douglas fir subspecies with even bark were less damaged by needle fall fungi and grew taller than trees with patterned bark. Given the positive association between wood yield and tolerance to needle fall fungi, selections for the new seed orchard shall mainly be made among the trees of the "green" subspecies.

A study of North American Douglas fir provenances assessed at age 3 showed that the provenances originating within approximately the same geographical area performed similarly, which means that a cost-efficient approach when establishing the future provenance tests shall not include too much entries from the same geographical area. A more northerly provenance performed slightly better than the other provenances; this points to frost tolerance and a suitable growth rhythm, which then may be the major traits to base the future selections on.

Key words: breeding plan, exotic species, *Pseudotsuga menziesii,* provenance, *Rhabdocline pseudotsugae*

INTRODUCTION

The first seeds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) were introduced to Europe 170 years ago [1, 2]. Because of its high wood yield produced in a relatively short time, Douglas fir has been target exotic tree species for introduction to Lithuania over the past 100 years [3]. At the beginning, like all new tree species introduced to Lithuania, Douglas fir was planted as an ornamental tree in parks and later on was used as a commercial species for timber production. Groups of Douglas fir trees originating from this first introduction may be found in the Bitėnai forest close to Endrijavas, Girija forest close to Viržulionys park, Bebruja forest close to Radviliškis. The area of Douglas fir plantations increased after the Second World War.

Studies on the growth of Douglas fir in Lithuania were made by Kairiūkštis [4]. Optimum and marginal environments for a healthy development of Douglas fir are when the mean annual air temperature is about +7.5 and +6 °C, respectively. The mean annual air temperature in Lithuania varies at about +6 °C, which, though marginal, may allow to produce Douglas Fir stands of good commercial quality. As in Norway spruce, there is a high risk for spring or autumn frost damage in young Douglas fir plantations established on open land. Thus, introduction of new provenances shall be based on the compatibility of their growth rhythm (start and end of active growth) with a given climate.

The following three subspecies of Douglas fir were introduced in Lithuania: "green" *Pseudotsuga menziesii* var. *viridis* Franco, "blue" *Pseudotsuga menziesii* var. *glauca* Franco, and "grey" *Pseudotsuga men-*

ziesii var. *caesia* Franco. Among those, the "green" Douglas fir performed best in Lithuania. It is a relatively fast growing subspecies able to produce wood of high commercial value and suitable for enriching recreational forests around cities. Our data show that a 100-year-old stand of this subspecies can yield 1000 m^3 of wood per ha; wood basic density can on the average reach 0.55 g/cm^3 (this value for Scots pine is 0.51 g/cm^3), and the mean proportion of latewood within an annual ring varies by about 55% [5]. The major problem with "green" Douglas fir is frost damage. As a consequence, the trees are often forked and curvy. Trees damaged by frost may be more sensitive to pest insect and fungi damage, such as the recently observed wide-spread infection by *Rhabdocline pseudotsugae* (Syd.) fungi, which infects needles and causes needle fall [6]. Thus, owing to such environmental stresses as frost followed by pest insect or fungi damage, establishment of commercial forest stands was not successful. A reason for this may be a failure to benefit from genetic testing: (1) the seed sources were chosen without testing them to identify the most suitable provenances for certain climatic zones of Lithuania, (2) no breeding plan was developed to select and test superior individuals within the most suitable geographical range.

Lead by D. Pirags, a Douglas fir breeding program was established in Latvia [2]. This program allowed to achieve a mean annual increment of Douglas fir stands of 11 m^2 per ha, and at the same age Douglas fir stands produced by 25–30% more wood than did Norway spruce stands of the 1st bonitet. The parent–progeny correlation for height ranged at about 0.62–69, showing that phenotype-based selection may give a good result. One of their conclusions was that the provenances that already grew for one generation in Europe performed better than direct introductions from North America. Therefore, when selecting Douglas fir provenances to be tested in Lithuania, it is important to include the provenances (land races) already introduced to Latvia, Belarus, Poland and Russia. For instance, at age 59, Douglas fir stands grown in Svetlogorsk forest in Kaliningrad region reach the mean height of 30 m, mean diameter of 53 cm and produce 894 m^3/ wood per ha (the corresponding parameters at age 76 were: 34.5 m, 50.5 cm and 1137 m^3/ha).

To facilitate establishment of a breeding program as a new step of the species introduction, this study was made with he following objectives: (1) to assess differences in height growth and quality among three subspecies of Douglas fir grown as grafted clones in a seed orchard in Lithuania, and (2) to study the quality of open-pollinated Douglas fir families originating from three provenances from the north-western USA.

MATERIALS AND METHODS

The first study was made in a seed orchard of Douglas fir. This seed orchard was established in 1966 over the area of 5.5 ha in Compartment 46 of Alytus forest district in Lithuania. The spacing was 6.5 × × 3 m. The clones originated from Douglas fir stands of Lithuania and Kaliningrad region of Russia. The clones represent the following three subspecies of Douglas fir: "green" *Pseudotsuga menziesii* var. *viridis* Franco, "blue" *Pseudotsuga menziesii* var. *glauca* Franco, and "gray" *Pseudotsuga menziesii* var. *caesia* Franco. The orchard was established by grafting the scions into roots stocks, which had been already planted at the orchard site. However, the survival of the grafted scions was very low – only 3%, which means that most of the trees in the orchard were ungrafted root stocks. In 2001 (at age 35), the height, diameter, stem straightness and forking of the orchard trees were measured. In addition, to determine the subspecies of the orchard trees, needle color, cone size and shape of cone scales [7], [8] (Fig. 1) and crown shape [9] were assessed. At the time of measurement only 59% (1647 trees) of the initially planted trees were alive.

Genetic gain (G) from selection of trees thicker than one standard deviation from the mean and ticker than two standard deviations from the mean was estimated according to the following formula:

$$G = S\ h^2,$$

where S is the selection differential and h^2 is additive heritability for tree diameter (the estimate were obtained from other studies on Douglas fir; the values ranged from 0.46 to 0.56 at a similar age as in our study).

The second study was made in a nursery trial of open-pollinated Douglas fir families ("green" subspecies) from three USA populations. The open-pollinated seeds were collected (1) from Sant Paul seed orchard clones which originate from Vernona provenance in USA (46° 23', 123° 56', 450 m a.s.l.), (2) in a stand in Raymond district in State of Washington, USA (46° 30', 123° 34', 520 m a.s.l.) and (3) in a stand in Solduk district in State of Washington, USA (48° 00', 123° 56', 600 m a.s.l.). In 1994, the nursery trial was established with 10 × 10 cm spacing. The height of the seedlings was measured at the age of 1, 2 and 3 years.

RESULTS AND DISCUSSION

The seed orchard study

A large number of trees in the seed orchard were of low stem quality (mainly forked) and of poor

Table. **Mean values of tree growth, stem quality, cone and needle traits of three Douglas fir subspecies assessed in Alytus seed orchard at age 35. Coefficient of variation is given in brackets**

Subspecies	Tree number	Cone		Diameter, cm	Height, m	Crown width, m	Crown length, m	Needle color
		length, cm	scale no.					
Green	29	6.9 (15)	51 (7)	34 (21)	15 (8)	5 (15)	12 (17)	Light green
Blue	31	5.9 (10)	48 (7)	29 (20)	14 (9)	5 (19)	11 (17)	Light blue
Gray	25	4.8 (11)	39 (14)	31 (18)	15 (7)	5 (16)	12 (17)	Gray blue

growth as well as often damaged by *Rhabocline pseudotsugae* (Syd.) – a fungi causing needle fall. At age 35, the tallest tree was 17 m high and the thickest tree was 48 cm thick. The interpretation of the result was made by comparing the growth and quality traits among the subspecies (Table), which were identified according to the needle, cone and crown properties (Fig. 1).

As regards the quantitative traits (height and diameter), the subspecies did not differ much from each other (Table). The stem diameter in the "green" subspecies of Douglas fir was by 17% larger than in "blue" and by 10% larger than in the "gray" subspecies. When identifying conifer subspecies, the quantitative traits usually are more affected by the environment and, therefore, less informative than the qualitative traits. For instance, the "blue" subspecies of Douglas fir has a short crown and a less sharp branching angle. There was no marked difference in the proportion of forked trees among the subspecies (about 30%) as well as in the tolerance to the fungus *Rhabocline pseudotsugae* Syd causing the needle fall. However, there were differences in the sensitivity to needle fall fungi among trees with a specific bark pattern within the "green" Douglas fir subspecies. The trees with even bark were less damaged by needle fall fungi and grew taller than trees with patterned bark (in small scales). However, a negative property of these even-barked trees was somewhat curved stems (usually 2 waves). Therefore, when making selections for a new seed orchard, the even-barked trees with straight stems shall be favored.

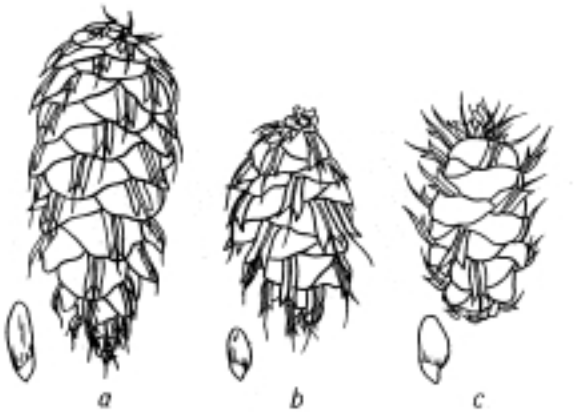

Fig. 1. Cones and seeds of the three subspecies of Douglas fir: (a) "green" *Pseudotsuga menziesii* var. *viridis* Franco, (b) "gray" *Pseudotsuga menziesii* var. *caesia* Franco, and (c) "blue" *Pseudotsuga menziesii* var. *glauca* Franco

On the individual tree level, crown size strongly positively correlated with stem diameter (correlation coefficient r = 0.72 ***) and the tolerance to needle fall decease strongly positively correlated with wood yield (r = 0.78 ***).

Genetic gain from the new selections may depend on the selection intensity. The diameter of the trees that are by more than one standard deviation ticker than the total mean is by 41.5% larger whereas in the trees that are by more than two stand deviations thicker than the trial mean the diameter is by 58.5% larger (Fig. 2). Consequently, genetic gain in case of lower selection intensity would be 19.1% and in case of higher selection intensity 26.9%. Given a positive association between wood yield and tolerance to needle fall fungi, there were 130 trees that were tall and least damaged by the fungi in the seed orchard. If the requirements for stem straightness would be included, the number of such trees would drop to 64; the latter may be used as female parents for establishing a new seeding orchard of Douglas fir. To rise the gene diversity in the seed crops of this new orchard, additional selection can be made in other seed orchards or in the forest to increase the number of families to 100. Then, a possibility for further improvement may be maintained via selective thinning of the worst families later on.

The seed orchard studied may be excluded from the list of seed collection sources and given a status of gene conservation stand instead.

The conclusion is that the trees of "green" subspecies with an even bark pattern performed best in the orchard and exhibited the highest resistance to needle fall fungi, appropriate wood yield and good stem quality. Therefore, selection for the new seed orchard should mainly be made among the trees of the "green" subspecies.

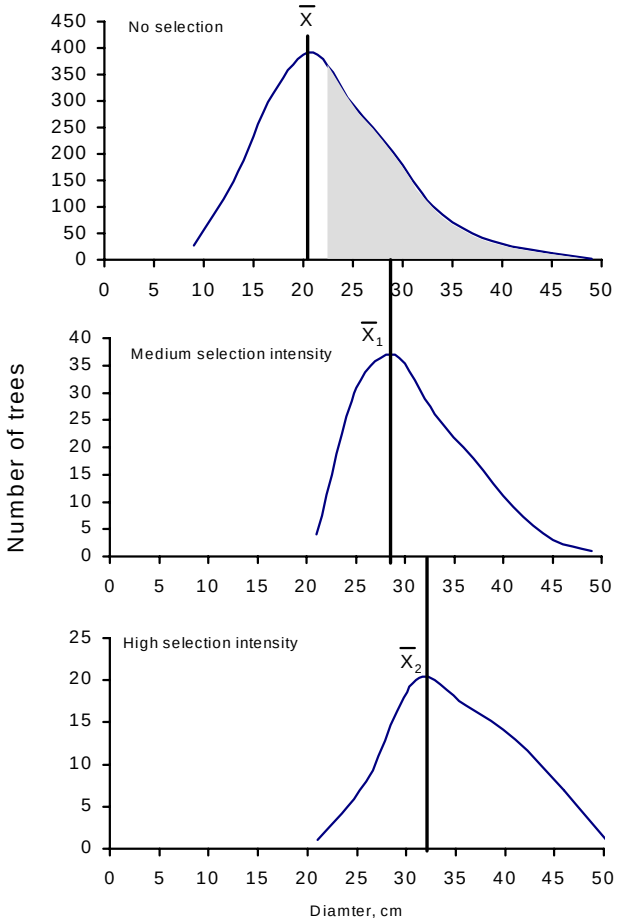

Fig. 2. Frequency plots showing the mean diameter of trees selected with medium (plot in the middle) and high intensity (lower plot). In case of medium selection intensity, trees with a larger diameter than one standard deviation from the total mean are selected; in case of high selection intensity trees with a larger diameter than two standard deviations from the total mean are selected. Vertical lines show the total mean

Nursery trial of North American Douglas fir provenances

At age 3, the ranking among the provenances in seedling height was the following: Solduc (mean height 100 cm), Vernona (mean height 95 cm) and Raymund (mean height 83 cm) (Fig. 3). However, the differences were not large. As the provenances originate from approximately the same geographical area, notable differences can hardly be expected. Nevertheless, it is worth noting that the best performing Solduc provenance represents an area which is by 2 degrees of latitude further north and elevated about 200 m higher than the other provenances. An interpretation may be that the Solduc provenance represents a more northerly environment than the other provenances and is thus more adapted to frost-related stresses, which may harm Douglas fir seedlings in the climate of Lithuania. In order to repair the frost-damaged tissues, seedlings may grow more slowly and bear such defects as forking and double leaders. All this results in a reduced seedling height. In this context, a new study on Douglas fir provenances representing a number of climatic zones would be of value.

The age-age family mean correlation coefficients for height at age 1 to 3 and age 1 to 2 were 0.79 and 0.90, respectively. The correlation coefficients among the height of the leader shoots were lower: age 1 to 2 – 0.54, age 2 to 3 – 0.23 and age 1 to 3 – 0.80. This indicates that seedlings at age 3 are far too young for selection of the candidates. Our experience shows that the optimum age for selection would be when the trees are about four meters tall.

The variation in seedling height among families within a provenance was greater than among the provenances (Fig. 3). This indicates a high intrapopulation variation in height, which used to be a regularity when assessments concerned the traits of adaptive value [12, 13]. Given this high intrapopulation variation, the provenance trails with family structure shall contain a sufficient number of families for each provenance.

The strategy for introduction of Douglas fir to Lithuania may consist of the following steps: (1) a large-scale provenance test without family structure, representing the most interesting areas in North America and the second-generation introductions from Europe; selection towards a suitable growth rhythm (timing of active growth) and frost tolerance as well as height growth, (2) after identification of the most promising geographical region, a new small-scale provenance test with family structure aiming at selection of the best provenances and the best families within these provenances as the founders of a breeding program.

In conclusion, this study shows that (1) provenances of Douglas fir originating within approximately the same geographical area perform similarly,

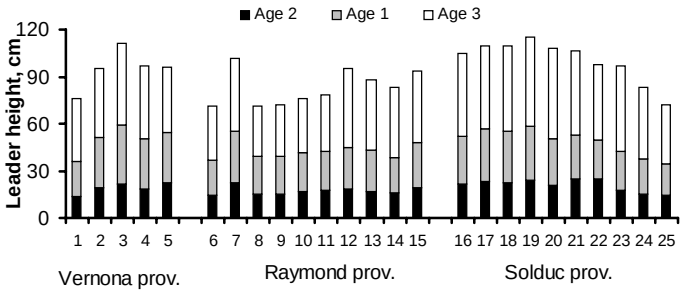

Fig. 3. Mean leader height of Douglas fir families ("green" subspecies) originating from three provenances of the north-western USA (assessed at age 1, 23 and 3 in a nursery trial)

which means that a cost-efficient approach when establishing the future provenance tests shall not include too much entries from the same geographical area; (2) the intrapopulation variation in height growth is large, therefore, to make correct selections, new provenance trials with family structure shall contain a sufficient number of families from each provenance; (3) a more northerly provenance performed slightly better than the other provenances, pointing to frost tolerance and suitable growth rhythm, which then may be the major traits to base the future selections on.

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DUGLASO POCŪGĖS INTRODUKCIJOS PERSPEKTYVA LIETUVOJE TAIKANT GENOTIPINĘ ATRANKĄ

S a n t r a u k a

Siekiant pabrėžti selekcijos priemonių būtinumą sėkmingai pocūgės introdukcijai Lietuvoje, buvo atlikti du tyrimai, kurių tikslai buvo: 1) įvertinti augimo ir kokybės skirtumus tarp trijų pocūgės porūšių (pilkosios, melsvosios ir žaliosios), augančių Alytaus sėklinėje plantacijoje ir 2) įvertinti trijų Šiaurės Amerikos žalsvosios pocūgės kilmių augimo skirtumus jaunystės stadijoje.

Tyrimai sėklinėje plantacijoje parodė, kad žalsvojo pocūgės porūšio medžiai buvo storiausi, tačiau tiek augimo, tiek kokybės požymių skirtumai tarp porūšių buvo nedideli. Nepavyko nustatyti, kuris iš porūšių labiau toleruoja pocūgių škotiškąją spygliakritę (*Rhabocline pseudotsugae*), tačiau pastebėta, kad žalsvojo porūšio lygiažieviai medžiai yra mažiau pakenkti spygliakritės nei kiti šio porūšio medžiai. Esant teigiamam ryšiui tarp produktyvumo ir tolerancijos spygliakritei, rekomenduojame sudaryti naują pocūgės sėklinę plantaciją, atrenkant žalsvojo porūšio pocūgės medžius be škotiškosios spygliakritės pakenkimų.

Trijų Šiaurės Amerikos žalsvosios pocūgės kilmių trejų metų augimo įvertinimas parodė, kad kilmės, kilusios iš daugmaž to paties regiono, auga panašiai. Todėl ekonomiškiau ateityje netirti per daug kilmių iš to paties regiono. Iš šiauresnio klimato atkelta kilmė augo šiek tiek geriau nei kitos bandomos kilmės. Tai leidžia daryti prielaidą, kad šalčių išvengimas ir tinkamas augimo ritmas (augimo pradžia ir pabaiga) gali būti vieni iš esminių požymių, į kuriuos reikėtų atsižvelgti atliekant labiausiai tinkamų Lietuvai kilmių atranką.

Raktažodžiai: kilmė, *Pseudotsuga menziesii, Rhabdocline pseudotsugae,* selekcija, svetimos rūšys