

Combining production of improved seeds with genetic testing in seedling seed orchards

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Abstract

In Poland usually a seedling seed orchards are established as a objects combining production of improved seeds with genetic testing. The aim of this paper is description of the typical approach applied on SSO and discussing the advantages and disadvantages that kind of management. As example was chosen Scots pine seedlings seed orchard in forest district Olesnica, planted in 1993 year. The trees were planted 5 m x 5 m in random, single tree plot distribution in four block replication. Area of the plantation was 5.06 ha, the number of families were 65 and seedlings 1680. After 12 year of growth for all trees diameter at breast height (DBH) and height were measured. Also for each trees quality features were assessed: stem straightness, crown width, branch diameter. The ANOVA and estimation of variance components for the measured and assessed traits were performed also family and single tree index values were calculated. There are considerable differences among families. Range between the worst and the best families comes from 15% up to the 45% depending on the traits. Basing on family and tree index value genetic thinning was planned. The description on thinning is presented on fig. 1 and 2. As a result of thinning the effective family size decreased from 64.96 to 57.16. After thinning the mean traits values increased about 1%. The presented way of SSO management is applied in wide scale into the forestry practice in Poland. The conception of using of SSO as testing plots isn't new. It was being raised already in many publications but so far it only in Poland found wider applying. The special software - connected with Database of Forest Reproductive Material were created to help with calculations and planning of the thinning. Of course using this method requires the compromise between the testing and production requirements. Presented example supports management of seedling seed orchards also as the testing plots. This approach would result into continuous genetic progress of SSO before they reach biological phase of cone harvest.

Keywords:

Seedling seed orchards, genetic testing, index selection

Introduction

The typical method of producing genetically improved seed in operational quantities is to using the seed orchard approaches. There are many definitions for seed orchard. One is formulated by Eeilberg and Soegaard (1975): "A seed orchard is a plantation of selected clones or progenies which is isolated or managed to avoid or reduce pollination from outside sources, and managed to produce frequent, abundant, and easily harvested crops of seed". Seed orchards are not always solely for genetic improvement of specific characteristics but can be used for conservation of genetic resources and also testing of forest reproductive material. There are many kinds of seed orchards, but they typical fit into one of two groups. Vegetative established by (grafts, cuttings, tissue culture or other methods). The other group is called a seedling seed orchard. These are established by planting seedlings followed by later roughing that will remove the poorest trees, generally leaving the best trees of the best families for seed production.

In the past there has been much discussion about the best types of seed orchards to use. Discussion was so animated during the early 1960s that a special issue of *Silvae Genetica* was published (Toda, 1964). The special issue contained articles on the for and against of the two types of orchards. Since then; many articles have been published that champion one of the two types of orchards. Scots pine seedling seed orchard are widespread in Poland. The simple fact is that Seedling Seed Orchard (SSO) area is 699 ha, about 35% of the all orchard (Matras 2007). It is influenced by many factors and the reasons not to be discussed in that paper. Some of the most important is that we in Poland pay big attention to conserve forest genetic biodiversity. SSO are similar to forest seed stands especially when families are coming from one region. Another reason is technical, it consist in incompatibility between scion and stock, in that case if severe problems are encountered with the health of vegetative propagated orchards, then seedling orchards are better alternative. The last main important reason is connection between seed orchard program and genetic testing and breeding. In Poland usually a seedling seed orchards are established as a objects combining production of improved seeds with genetic testing.

The aim of this paper is description of the typical approach applied on SSO in Poland and discussing the advantages and disadvantages that kind of management.

Methods of SSO establishment in Poland.

The first SSO was established in 1967. From the beginning until 1989 the spacing for all species was 3 m x 3m. In 1990 y. several SSO was panted using spacing 4 x 4 m. From

1990 until nowadays SSO are planting in initial spacing 5 m x 5 m. At the beginning systematic thinning are planed and proportionally to the number of trees in family seedlings are randomly distributed to consider this. Area of SSO typically are divided for several blocks where all genotypes are represented. The experimental design is single tree plot. Trees are distributed in that way to assure obtain the maximal possible distance between the same genotype. The planting materials are open pollinated half sib progenies of plus trees. In single SSO the families originated from one seed region. The minimal number of families is 40 for Scots pine and Norway spruce and 30 for other species. The number of trees per family is usually bigger than 20. Very important is choosing the appropriate localization to reduce pollen contamination. When SSOs reaches the stage where it becomes to dense (generally after 8-10 years after planting), the first genetic thinning are planned after prior measurements. Until that time no crown forming were applied. For the all trees the DBH and the height are measured as well as quality traits (stem straightness, crown wide, branch diameter) are visually scored. The method is described in State Forest technical bulletin (Zarządzenie 7a).

Seed production on SSO in Poland.

All seed orchards in Poland are belong to the State Forest. And that fact influenced methods of rather extensive managements. There are no official information concerning seed production but it can be estimated from cones collection data. Information are available on the scale on whole country as the report "LPIO-5" the output from information system of the Polish State Forest (personal communication with U. Zabrocka, specialist from General Directorate of State Forest GDLP). Concerning Scots pine cones collection during last 5 years only 1% of collected cones comes from SSO, 9% comes from seed orchards, 80% from so called economic seed stand and 10 % from selected seed stands. The statistic is different for another species. For example 40% of European larch seeds comes from seed orchard.

Example application of family testing using SSO (real example).

The Scots pine (*Pinus sylvestris* L.) seedlings seed orchard is located in forest district Olesnica. Open-pollinated progenies (half-sibs) were planted in 1993 year, which in this paper are referred to as families. While the mothers are plus trees, fathers are not known but it is assumed that they are from nearby stands. Each mother tree is assigned a number to identify the family. Identification numbers of families are related to numbers used in national register of plus trees. The trees were planted 5 m x 5 m in random, single tree plot distribution in four

block replication. Area of the plantation is 5.06 ha, the number of families were 65, and seedlings 1680. Family no 3566 was a least numerous represented by 23 trees but most families were represented by 26 trees. Effective family was is 64.96.

Methods.

After 12 year of growth for all trees diameter at breast height (DBH) and height were measured. Also for each trees quality features were assessed: stem straightness, crown width, branch diameter.

The ANOVA and estimation of variance components for the measured and assessed traits were performed according to the following model:

$$Y_{ij} = \mu + F_i + E_{ij}$$

μ - total mean

F_i - random effect of family i

E_{ij} - random effect of tree j in family i (random error)

Variance components for the family and the residuals were estimated using S-plus statistical software. Heritabilities for families and for single trees were calculated and indexes.

σ_E^2 - Variance component for residual

σ_F^2 - Variance component for family

$V_{Ph} = \sigma_E^2 + \sigma_F^2$ - phenotypic variance

$V_F = \frac{\sigma_E^2}{n} + \sigma_F^2$ - family variance

$h_F^2 = \sigma_F^2 / (\frac{\sigma_E^2}{n} + \sigma_F^2) = (A - B) / A$ - family heritability

$h_s^2 = 4\sigma_F^2 / (\sigma_E^2 + \sigma_F^2) = 4\sigma_F^2 / V_{Ph}$ - single tree heritability

$h_w^2 = h_s^2 \left(\frac{1 - 0.25}{1 - h_s^2 \cdot 0.25} \right)$ - within family heritability (Falconer, Mackay 1996)

$$W_f = h_{F1}^2 d_{F1} E_1 + \dots + h_{Fk}^2 d_{Fk} E_k \quad - \quad \text{family index value (Giertych and Mąka 1994)}$$

where:

h_{F1}^2 - family heritability for trait number 1

d_{F1} - selection differential for family and trait number 1 for family

$$d_F = (\bar{x}_F - \bar{X}) / \sqrt{V_F}$$

- \bar{x}_F - mean value of trait x for family (arithmetic average)
- \bar{X} - total mean for trait x
- V_F - family variance
- E_1 - economical value for trait no. 1 (arbitrarily chosen as presented in Table 1)

Index value for a tree was calculated using a combined selection of the genetic value of its family and the genetic value of the tree within its family where taken into account:

$$W_T = W_f + W_t \quad - \quad \text{Genotypic value of the tree}$$

were:

$$W_t = h_{w1}^2 d_{w1} E_1 + \dots + h_{wk}^2 d_{wk} E_k \quad - \quad \text{genetic index value of a tree within its family}$$

$$d_{wk} \quad - \quad \text{selection differential for trait } k$$

$$d_w = (x - \bar{x}_R) / \sqrt{V_w} \quad - \quad \text{selection differential}$$

$$V_w = \sigma^2_E - \sigma^2_{E/n} \quad - \quad \text{within family variance (Falconer, Mackay 1996)}$$

$$X \quad - \quad \text{trait value}$$

Group coancestry (Θ) for the half-sib population was calculated as, Fedorkov et al. (2005) :

$$\Theta = \frac{0.5 \sum n_i + 0.125 \sum n_i (n_i - 1)}{(\sum n_i)^2}$$

Status number (N_s) is an effective number defined by Lindgren et al. (1996) as half the inverse of the group coancestry:

$$N_s = \frac{0.5}{\Theta}$$

Effective family number N_e is equivalent to Robertson's (1961) definition of effective population size which is widely applied to populations with a family structure (Wei 1995).

$$N_e = n^2 / \sum n_i^2$$

where: n denotes the number of trees on the area and n_i the number of trees in particular families.

Results

There are considerable differences among families. Range between the worst and the best families comes from 15% up to the 45% depending on the traits. The best families in terms of DBH and the height are at the same time a little bit worse with quality features, especially for a crown width and the branch diameter. Seed orchard was established in homogenous area and therefore 10% of the total variability can be explained by the family structure. Heritabilities of analysed traits are big (tab. 1). Biggest heritability value have DBH (0.63), and the smallest heritability have branch diameter (0.31). Simple selection index was calculated and it is presented on fig. 1. Basing on family and single tree index values cutting was planned removing 7 worst families in terms of index value. At the same time all trees in the seven best families marked on of fig 1 were kept on the orchard. On fig. 2 it is illustrating how the cuts were planned on the part of block 1. Trees selected to cut were marked with the red colour, trees marked with green colour were protected even if they planned to be removed in systematic thinning. In order to assure better conditions for the development of crowns and the production of cones additionally the schematic thinning was planned. As a result of thinning the effective size of families on the plantation decreased from 64.96 to 57.16 and status number from 232.6 to 199. After thinning the traits values increased only about 1%. Average height of trees increased from 6.15 m to the 6.19 m it is 0.6% of the mean before thinning, and DBH increased for 1.3 % from 14.2 cm to 14.4 cm.

Table 1. Variance and heritability of measured traits.

Trait	E weights	Variance explained by family [%]	F	Heritability (standard error)	
				Family	Individual
DBH	2,5	8,8	2,63**	0,63 (±0.10)	0,24 (±0.11)
Height	2,0	7,0	2,01**	0,50 (±0.08)	0,15 (±0.05)
Stem straightness	1,0	7,6	2,22**	0,55 (±0.20)	0,18 (±0.05)
Crown size	1,0	7,4	2,13**	0,54 (±0.22)	0,17 (±0.05)
Branch diameter	1,0	5,3	1,45 ^{ns}	0,31 (±0.15)	0,07 (±0.01)

- significant on the level, **($p=0.01$), *($p=0.05$), ^{ns} – not significant

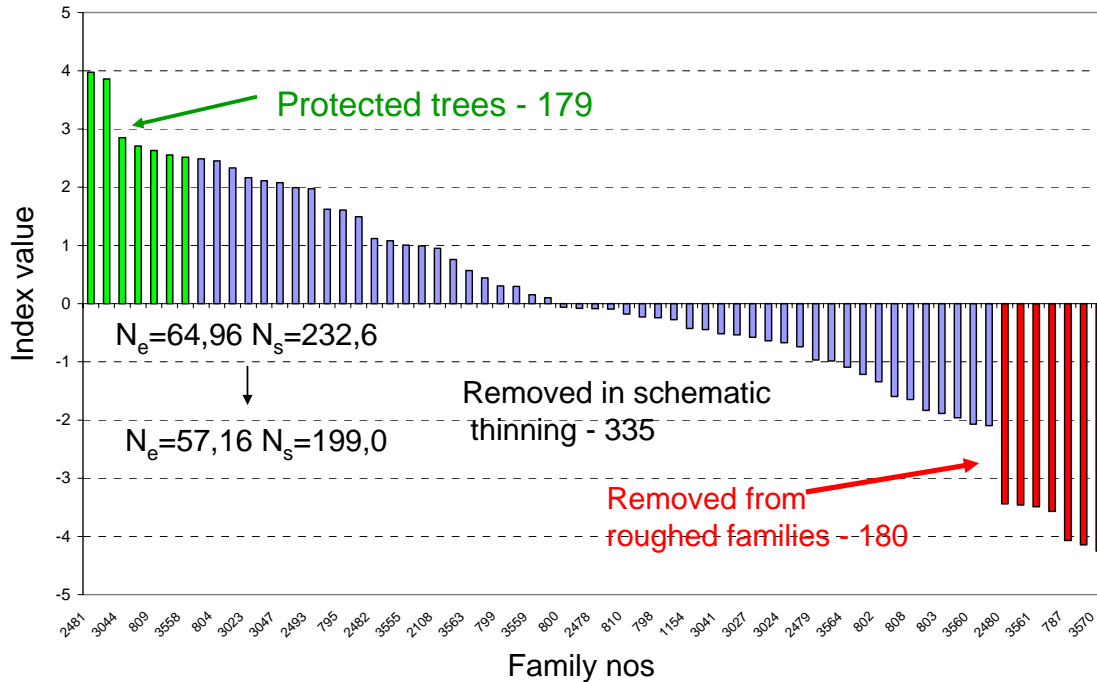


Fig 1. Estimated index values of families and thinning description.

	K01	K02	K03	K04	K05	K06	K07	K08	K09	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20
R01	793 1 X 0,78	799 3 0,23	2478 1 X 0,50	802 3 0,29	794 1 X -1,6	3026 3 1,32	2111 1 X 1,81	807 3 1,04	798 1 X -0,4	2493 3 1,06	3564 1 X -0,1	3570 3 X -1,4	2481 1 !! 0,14	3044 3 !! -0,0	2493 1 X -0,4	3024 3 -0,3	810 1 X -1,7	2482 3 -2,1	3559 1 X 0,06	2481 3 !! 1,03
R02	3046 4 0,22	798 2 0,57	3561 5 X 0,44	790 2 0,84	3047 4 0,77	800 2 0,50	2495 5 0,25	806 2 !! 1,37	3032 4 0,56	802 2 1,01	2478 5 0,87	3559 2 0,43	3043 4 X -1,2	796 2 0,11	3573 5 -1,7	787 2 X -0,5	792 4 -1,5	2479 2 -1,2	789 5 1,49	809 2 !! 1,24
R03	792 1 X -0,7	804 3 0,39	807 1 X 0,27	3560 3 -0,3	3565 1 X 1,36	801 3 X -1,2	795 1 X 0,84	796 3 -0,1	3566 1 X 0,15		790 1 X -1,2	3561 3 X 0,17	799 1 X 0,82	3026 3 -0,6	3557 1 X 0,47	3025 3 0,29	3032 1 X 0,34	1154 3 -0,4	2109 1 X -0,5	796 3 -0,1
R04	3032 5 0,33	3028 2 0,62	788 4 !! 0,61	809 2 !! 0,83	787 5 X -1,0	799 2 0,10	2481 4 !! 1,39	3570 2 X 1,02	2108 5 -1,0	3558 2 !! -1,3	810 4 -0,3	804 2 1,97	788 5 !! 0,60	795 2 0,37	3555 4 -0,0	803 2 -0,1	3556 5 -0,5	3567 2 -0,4	790 4 -0,1	804 2 -0,1
R05	3556 1 X 2,01	800 3 -0,3	3025 1 X 0,10	803 3 -0,1	789 1 X 1,54	2110 3 0,56	3564 1 X 0,02	3567 3 -0,0	791 1 !! 0,77	803 3 -0,4	2482 1 X 0,82	1154 3 0,25	3554 1 X -0,6	806 3 !! 0,41	3564 1 X -0,8	808 3 -0,1	3041 1 X -0,2	3570 3 X -0,3	3560 1 X -0,2	806 3 !! 0,16
R05	794 4 0,03	3573 2 -0,6	808 5 -0,1	802 2 0,91	1154 4 -0,1	3026 2 -0,8	3044 5 !! 1,02	793 2 -1,0	3556 4 0,89	2109 2 -0,7	3568 5 -0,9	794 2 1,08	809 4 !! 0,89	3565 2 0,03	3046 5 -0,8	3563 2 -0,1	807 4 2,24	801 2 X -4,1	2480 5 X 0,96	3558 2 !! 1,53
R05	2480 1 X 1,86	3563 3 0,42	3568 1 X 0,08	3562 3 2,31	3027 1 X 0,22	790 3 -1,2	800 1 X -0,8	3043 3 X -1,3	3571 1 X 1,12	795 3 0,94	800 1 X -0,3	789 3 -1,9	3570 1 X -0,4	3571 3 0,05	796 1 X 0,26	3028 3 0,61	2482 1 X 1,75	2108 3 1,52	3044 1 !! 0,27	3554 3 -0,3

Fig. 2. Tree distribution on the part of the block 1. The row and column numbers are written left and top. Each cell representing one tree. On the top is the family number, below is the systematic thinning number (from 1 - trees to be removed to 5 – destination trees). On the bottom of each cell is written tree index value. Trees marked on red color are planned to be removed.

Discussion

The presented way of SSO management is applied in wide scale into the forestry practice in Poland. All SSO area are established in this way to assure evaluation breeding values of families prior to planning genetic thinning. Such an approach requires the

compromise between the function of testing and seeds production. Compromises as regards testing is lack of replication in different locations. However it is possible to justify the lack of repeating with the fact that seeds from SSO are planned to using only in relatively small seed region. Another one issue it that conditions of the grow are different on SSO in comparing to typical testing experiments. The most important difference is a wide initial spacing. It is causing that the breeding value is calculated with some error. Compromises also concern production of seeds. At the beginning before making the first thinning, production of seeds is on the second plan. Most of the SSO in the first few years after plantation to do not produce seeds because tree are to young. Later when trees are growing the spacing is increased and in the end it is planned as 10 m by 10 m. So far on SSO in Poland is not applying crowns forming. That way, it is not disturbing in the assessment of the development of the crowns and the branches. However if such treatments will be planed in the future it seriously conflict with testing. In the presented example index value is calculated without taking into consideration correlations between traits. However the simplicity of the used method is causing it, that seed orchard managers easily understand how the index values are calculated. Additionally single tree index value is calculated as the combined index, it is the most effective way of selection (Falconer, Mackay 1996) but it is only useful to select trees within families. The consequences of using only combined index selection at high selection intensity could have undesirable effects on inbreeding and genetic diversity. During the planning genetic thinning on SSO many factors is taking into consideration. First one, is the minimum population size (N_e). If N_e is bigger than 40 for pine and spruce and bigger than 30 for other species, genetic thinning are possible. Trees are marked to cut after analyzing of all condition which are appearing on SSO (special distribution, health condition etc.). Families could be tested in field trials also and results could be used when calculating criteria for thinning but so far only some families are represented in existing field experiments. We do not use the procedure elaborated by Lindgren and Matheson (1986) with theoretically maximizing the genetic gain and the genetic variability. In the future it would be worthwhile using it after some modification concerning the spatial distribution of trees.

The conception of using of SSO as testing plots isn't new. It was being raised already in many publications but so far it only in Poland found wider applying. To help with calculations and planning of the thinning, special software connected with Database of Forest Reproductive Material were created. Of course using this method requires the compromise between the testing and production requirements.

Conclusions

This example supports management of seedling seed orchards also as the testing plots. This approach would result into continuous genetic progress of SSO before they reach biological phase of cone harvest.

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