Seed orchards

Proceedings from a conference at Umeå, Sweden, September 26-28, 2007

Editor: Dag Lindgren

The photo is from a Scots pine seed orchard, which has reached the commercial seed productive phase, close to Umeå airport shot by Davorin Kajba 2007.

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These proceedings can currently (2008-01-30) be read and downloaded in .pdf format starting from the link: http://www-genfys.slu.se/staff/dagl/Umea07/ (Dag Lindgren web). They are unlikely to vanish before mid-2009, and the file may be updated and corrected, so downloads are recommended from where in first hand. The proceedings file is posted in an open archive on: http://pub-epsilon.slu.se/151/ . Reporters made summaries of some of the presentations, including some which are not in the proceedings, which are currently on the Treebreedex web http://treebreedex.mediasfrance.org/pages/body/homePage.jsp
and these proceedings may be there also.

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Preface

Seed orchards constitute the cradle for most cultivated forests. Often seed orchards are needed or preferred just to get a reliable and reproducible seed supply, but as forest tree breeding has left its infancy, the genetic gain becomes often of major importance. Seed orchards are by far quantitatively the most important interface between forestry on one side and tree breeding and supporting research on the other. By seed orchards we create resources future generations will need: seed orchards are one tool in the fight against global warming and for sustainability; and better seed orchards means a better future World. Seed orchards can also have an important role for gene conservation. Seed orchards have through the past decades not been regarded as a new research frontier, but as a mature science and a matter for stump-jumpers and not scientists. However, knowledge and experiences of seed orchards and their role and management have accumulated during the last decades. Many developments have not got much attention, because they seldom reach fancy journals and are a concern only for a few specialists and managers. The time has come for a conference to synthesize and debate this new knowledge and amalgamate it into the old.

An opportunity arose, as seed orchards was an issue for Treebreedex, Activity 6. Treebreedex is a consortium of 28 organizations involved in tree breeding and forest genetics in Europe supported by the European Union 2006-2010. Participation in the conference was however open to anybody.

At the conference 36 Oral presentations and 17 posters were presented on different aspects of seed orchards. There were 90 registered participants from 27 countries. All oral presentation were given the same time lot and they were presented in alphabetic order according to the presenter. There were no concurrent sessions. Besides the lectures it was discussions about possible forest genetic consequences of recent fires in Greece and possible use of DNA techniques associated to seed orchards based on a conference immediately preceding the seed orchard conference. The public defense of a PhD thesis (Prescher F 2007 Seed Orchards – Genetic Considerations on Function, Management and Seed Procurement. Acta Universitatis Agriculturae Sueciae 2007:75) took place so participants could attend. A half-day excursion to seed orchards around Umeå was done.

The manuscripts published in these proceedings were submitted as word files and included in the proceedings as received, except pagination and sometimes minor formatting and conversion to PDF files. The content of the contributions is the responsibility of the authors. The manuscripts have not been reviewed except for some technical considerations. Of the presentations, 36 are documented as papers in these proceedings.

With advances in digital technology, proceedings are often published online because of obvious advantages: It is hassle free. It is a marginal cost for the publisher and almost free for the customer. It is easily accessible for anyone. The downside of online publishing is that sites hosting the material will sooner or later vanish or change URL! It is unpredictable how long the proceedings will be public available, so it is a good idea to download it. Anyone may place it available on another web.

Sponsoring is acknowledged from Treebreedex and The Swedish Forest Tree Breeding Association. Staff from my own department (like Jan-Erik Nilsson and Anders Fries) has done a great job assisting me in organizing the conference! Much help is acknowledged from Skogforsk (in particular Bengt Andersson who organized the excursion). Swedish University of Agricultural Sciences and its Faculty of Forest Sciences and Umeå Plant Science Centre are acknowledged for hosting the conference. Organizational help and discussion about the arrangements have been received from several participants. The main cost and main effort with a conference is the time and dedication of the participants and contributors and those funding their time and effort, and the most important acknowledgement is to their contributions.

An example of a possible way to make a citation:
Hannerz M and Ericsson T 2008. Planter's guide - a decision support system for the choice of reforestation material *In* Lindgren D (editor) Proceedings of a Seed Orchard Conference, Umeå, Sweden, 26-28 September 2007. p 88-94.

Organizing a conference is a lot of work, but it is also a lot of fun. I truly enjoyed the seed orchard conference. I hope the other participants liked it as much as I did. I see it as a great privilege to be allowed to arrange a conference about such an important, but still neglected subject, as seed orchards.

Umeå 10th February, 2008

Dag Lindgren,

Conference chair, proceedings editor

Dag Lindgren

Participants

Abrahamsson, Sara

Ackzell, Lennart

Alan, Murat

Alizoti, Evi

Almqvist, Curt

Andersson, Bengt

Andersson, Jörgen

Berlin, Mats

Bilir, Nebi

Byram, Tom

Chalupka, Wladyslav

Chládek, Jan

Codesido, Verónica

Danusevicius, Darius

De Cuyper, Bart

de Vries, Sven MG

Douglas, Gerry

Dutkowski, Greg

El-Kassaby, Yousry

Ericsson, Tore

Fjellström, Joakim

Foffova, Elena

Fries, Anders

Frýdl, Josef

Fussi, Barbara

Gailis, Arnis

Garcia Gil, Rosario

Gaspar, Maria J

Gomory, Dusan

Gong, Peichen

Granlöf, Jonatan

Haapanen, Matti

Hall, David

Hallander, Jon

Hallingbäck, Henrik

Hannerz, Mats

Hansen, Ole

Högberg, Karl-Anders

Isik, Fikret

Jansons, Āris

Jansson, Gunnar

Johnsen, Øystein

Johnskås, Ragnar

Kajba, Davorin

Kang, Kyu-Suk

Karlman, Lars

Karlsson, Bo

Kjær, Erik D.

Kleinschmit, Jörg

Klintenäs, Maria

Kowalczyk, Jan

Kroon, Johan

Li, Yue

Lindgren, Dag

Markiewicz, Piotr

Martinsson, Owe

Matras, Jan

McKeand, Steve

McRae, Tony

Meland Edvardsen, Øyvind

Moriguchi, Yoshinari

Nielsen, Ulrik Bräuner

Nikkanen, Teijo

Nilsson, Jan-Erik

Normark, Erik

Novotný, Petr

Parnuta, Gheorghe

Persson, Torgny

Pliura, Alfas

Prescher, Finnvid

Pöykkö, Sirkku

Reid, David

Rosenberg, Olle

Rosvall, Ola

Ruotsalainen, Seppo

Shen, Xihuan

Sigurgeirsson, Adalsteinn

Steffenrem, Arne

Stener, Lars-Göran

Tigabu, Mulualem

Tolkamp, Wim

Waldmann, Patrik

Walfridsson, Erik

Vander Mijnsbrugge, Kristine

Wang, Xiao-Ru

Varghese, Mohan

Wei, Run-Peng

Wennström, Ulfstand

Westin, Johan

Ylioja, Tiina

Conference photo with most of the participants at 2007-09-27 (Dag Lindgren 65 Year Anniversary)

DNA and seed orchards

Darius Danusevicius, Yousry El-Kassaby, Maria Gaspar, Øystein Johnsen and Xiao-Ru Wang

Witness reports about what was learnt of relevance to seed orchards in the foreseeable future from the GENECAR meeting immediately preceding the seed orchard conference: "Application of DNA based tools for genetic research, molecular breeding, management and monitoring of genetic resources". Participants registered for both meetings were suggested to make statements, five volunteered, and their statements appear in the following:

Darius Danusevicius: Development of DNA markers for the DNA sequences associated with phenology traits would especially be desirable for reproduction genetics with the ultimate aim to test the hypothesis of "future pre-recorded in the past"- is there a pattern in environmental variation during gamethogenesis, fertilization and embryogenesis to affect the genetic and epigenetic variation of the future generations? We also may use epigenetic technologies to manipulate trees: e.g. silencing by RNRi. Better understanding of genomic imprinting and paramutation in trees: reciprocal crossings among individuals with contrasting flowering traits with known specific-allele markers to identify presence of absence of a particular allele and phenotypic expression of the trait.

Yousry El-Kassaby: The precise estimation of clonal gametic contribution to seedlots has never been a challenge for the maternal component point of view; however, the paternal side is a daunting task. The problem is not only restricted to the "within" orchard paternal contribution but also is to when and how much the "outside" pollen migration takes place. Recently the combined use of DNA fingerprinting technology and pedigree reconstruction, not only made the parental contribution determination possible, but also it allowed us to estimate it to the individual clone level and by phonological classes. The more we work and develop the necessary tools, the better our gene resource management gets.

Maria Gaspar: DNA based tools, like SSR markers, are now being used in different aspects of seed orchard management. These tools can assist the resolution of some existent dysfunctions in the clonal seed orchard, through clone identification, evaluation of pollen contamination, selfing rates and percentage of pollen contribution. Moreover, they can be very useful to obtain better estimations of genetic parameters used in tree-breeding programs. In populations where plus trees are selected, pedigrees are usually unknown, and it is assumed that all plus trees are genetically unrelated. Deviations from this assumption may lead to greater inbreeding and loss of genetic gain. Thus, genetic markers can be used for pedigree reconstruction, evaluation of the coancestry coefficient, providing more accurate estimations, increasing the predictable improvement of economical important traits

Øystein Johnsen: To become more efficient**,** we should implement fingerprint technology in tree breeding along the guidelines presented as “Breeding without breeding”. Marker-based breeding can be brought to applications within the next five years, we heard. A new EU-project (NOVELTREE) will reveal if this is realistic for more tree species. We need to explain why phenotypes do respond differently to identical environmental cues. Is variation in gene regulation (leading to quantitative difference in gene products) causally more important than sequence difference in structure genes (giving qualitative difference within gene products)? Search for DNA variation in promoter regions, in genes coding for transcription factors, as well as in regions coding for microRNAs, will tell us if we can use DNA variation as a major predictive tool in future breeding.

Xiao-Ru Wang: Genomic research and investigations into the molecular mechanisms controlling important traits, such as growth cessation, bud set, flower initiation, and wood quality, are progressing fast in *Populus*. In conifers, the on-going large scale genome scan and candidate gene-based association studies will gradually provide valuable information on the genetic architecture underlying adaptive and productive traits. At current stage, the most relevant applications of molecular techniques in tree breeding are in the areas of evaluation of genetic resources and the function of breeding programs, seed lots classification, and forest and nursery healthy examinations. The use of high-resolution DNA markers for pedigree reconstruction in open pollinated progenies of seed orchard, as BWB suggested by El-Kassaby, is an example of how molecular techniques can facilitate an innovative breeding strategy for outcrossing conifers.

Seed Orchard Planning and Management in Turkey

Murat ALAN, Hikmet OZTURK and Sadi SIKLAR
Forest Tree Seeds and Tree Breeding Research Directorate 06560 Gazi/Ankara/TURKEY
E-mail: alanmur@yahoo.com

Tree breeding studies have been started at 1964 in Turkey. Two sample seed orchards were established at the training area of Faculty of Forestry of Istanbul University in 1964 by using 10 clones. Because of low clone number, production was not aimed in those seed orchards. Later, it was aimed to establish seed orchards by the purpose of seed production. For this reason, seed transfer regions were determined for the economically important tree species. Plantation areas and seed requirements were assessed for each species. Selection of seed stands and plus trees from seed stands were followed by clonal seed orchard establishment with grafted seedlings. First clonal seed orchard was established at 1976 by *P. brutia*. Breeding studies had been continued in this manner until 1993. Seeds orchards established in that time generally contained 30 clones and were not tested genetically.

The National Tree Breeding and Seed Production Program (NTBSP) for Turkey were implemented at 1994. *Pinus brutia, Pinus nigra, Pinus sylvestris, Cedrus libani and Fagus orientalis* were determined as target species. It was aimed to meet seed demand of 150.000 ha/year plantation for those species in the program. By considering seedling number per hectare and seed amount needed for a seedling, seed requirements were determined for each species. Seed sources (seed stands and seed orchards) were planned according to seed demand of species. Breeding studies have been accelerated by the progeny trials by this program. In addition, seed orchards were established by higher number of clones (41-152 clones).

By the year 2006 there are 174 seed orchards occupying 1200 ha in Turkey. Ninety two percent of seed orchards have been established by *Pinus brutia, Pinus nigra, Pinus sylvestris* and *Cedrus libani*. All of the seed demands of plantations are supplied from seed orchards for the first 3 species. Since seed production by *C. libani* takes longer time, seed production in seed orchards is not sufficient to provide seed requirement of plantations. All seed orchards are phenotypic. However, two seed orchards were established by *P. brutia* in two breeding zones according to the first results (4th year) of progeny tests. These two seed orchards will be converted to genotypic seed orchards at the end of progeny tests. Any results of progeny tests in other breeding zones have not been obtained yet.

Table 1. Seed orchards in Turkey

Species	Minumum clone	Maximum clone	Seed Ochard Number	Total Area (ha)
Pinus brutia	10	146	67	472,2
Pinus nigra	10	122	52	431,1
Pinus sylvestris	10	152	20	111,3
Cedrus libani	15	55	12	66,0
Pinus pinea	30	30	4	47,2
Picea orientalis	30	50	9	30,5
Pinus halepensis	10	20	2	8,2
Liquidambar orientalis	30	30	1	2,2
Sorbus torminalis	59	59		-[a]
Pinus nigra var. pyramidata	20	21	2	4,3[b]
Pinus nigra var. *pendula*	8	8	1	1,2[b]
Pinus nigra var. *seneriana*	10	10	1	1,8[b]
Pinus brutia var pyramidalis	18	18	1	1,2[b]
Pinus sylvestris var. *compacta*	8	26	2	4,8[b]
		Total	**174**	**1182**

[a] Scions were grafted, it will be established in next spring, [b] seed orchard for *exsitu* conservation.

Research that would guide seed orchard management was limited before NTBSP in Turkey. So implementations about management had been limited by protection and renewal. In that time, seed orchards are surrounded by a fence against animal attacks. Field between seedlings is harrowed by disc harrow each year. Seed orchards were surrounded by trees to isolate from pollen contamination. Although there is no serious problem related to insects, pest management is also done. The researches considering seed orchard management have been begun after NTBSP. Research projects including studies about flower counting, pruning, hormone application and molecular genetics have been started and some of them have been finished. Knowledge that could be gained by acceleration of this kind of studies will improve applications of seed orchard management in future.

Synchronization and Fertility Variation Among *Pinus nigra* Arn. Clones in a Clonal Seed Orchard

P.G. Alizoti[1], K. Kilimis, P. Gallios

[1]Aristotle University of Thessaloniki, School of Forestry and Natural Environment
Laboratory of Forest Genetics and Tree Improvement, 54124 Thessaloniki, Greece
E-mail: alizotp@for.auth.gr

Clonal seed orchards are the main source of forest reproductive material for the most economically important conifer species. The establishment of seed orchards aims mainly towards the production of seed crops of higher genetic value compared to those obtained from seed stands.

The flowering synchronization among clones in a clonal seed orchard has been proved to be a crucial factor for the genetic composition of the seed crop, as it affects the exchange of genes among clones. Lack of synchronization among female flower receptivity and pollen shedding can violate the basic assumption for an idealized seed orchard, which is the panmictic equilibrium (El-Kassaby and Askew, 1991; Matziris, 1994; Kang and Lindgren, 1999; Kang and Mullin, 2006). The above knowledge is fundamental for the effective management of a seed orchard.

Aims of the present contribution were to determine the clonal variation in the development of different phenological stages of female and male flowers in a *Pinus nigra* Arn. clonal seed orchard; to investigate the synchronization among female receptivity and pollen shedding; to record the variation of female and male strobili number among clones; and to evaluate the response of clones to the contrasting environmental conditions recorded among the two years of study.

The study was carried out in a black pine clonal seed orchard that comprises sixty clones originating from Northern Greece. The study was carried out in two successive years with contrasting environmental conditions (one year with normal temperature and precipitation conditions, and one dry year with exceptionally high temperatures). The different phonological stages (scale 1-5 for male and scale 1-4 for female strobili) were recorded every second day in eight male and eight female strobili for each one of the two ramets per clone that were included in the study. Fertility was assessed during the second year of the study, by counting the total number of female and male strobili per ramet and clone.

Figure 1. Black pine clonal seed orchard (Clonal male fertility variation).

Figure 2. Receptive female strobili (phenological stage 3).

The data were analyzed separately for each individual year and combined for both years, following a mixed linear model analysis (Proc Mixed, Proc Varcomp; Sas, 1996).

From the results obtained it was shown that significant differences in male and female fertility exist among the black pine clones. Also, significant variation among clones for the male strobili dimensions was found.

It was also revealed that high genetic variation exists among clones for both the female and male flowering developmental phases. The genetic component of both male and female flowering earliness is very strong in all cases.

The year effect was statistically significant in all cases. No significant clone by year interaction was detected for the female onset, termination and duration of flowering for the stages recorded, while a statistically significant interaction was found for the pollen shedding duration.

The high heritability values found indicate that selection for flowering synchronization could be effective and result in rapid genetic gains.

The assumptions for an ideal seed orchard, as far as synchronization and panmictic equilibrium are concerned, were not met. Especially during the dry and exceptionally hot year, a well expressed asynchrony among the black pine clones was recorded, with apparent consequences on the genetic composition of the seed crop. The above finding could indicate the effect of the predicted climate change (with prolonged periods of drought and high temperature in the Mediterranean region) on the phenology of flowering and the genetic composition of the seed crop obtained from black pine seed orchards.

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Practical use of $GA_{4/7}$ to stimulate flower production in *Picea abies* seed orchards in Sweden

Curt Almqvist
Skogforsk
Uppsala Science Park
SE-751 83 Uppsala
Sweden

curt.almqvist@skogforsk.se

Abstract

In 2005 we conducted the first practical attempt to stimulate flower production in *Picea abies* seed orchards in Sweden by applying $GA_{4/7}$. Four seed orchards were treated: two old orchards at the end of their production phase, and two young orchards at the start of their production phase. Only one treatment of $GA_{4/7}$ was applied to each orchard, and treatment was done at one single occasion in each orchard. Every second row of trees was left untreated and used as a control. Male and female flowering was scored during the flowering season in 2006 from a random sample of trees in all four orchards. In autumn 2006, the number of cones produced per tree was counted in three of the four orchards, and the seed quality was assessed in two of them. Our results show that the $GA_{4/7}$ treatment had a significant positive effect on male flowering in the two young orchards, but not in the two old orchards. $GA_{4/7}$ treatment also had a significant positive effect on female flowering in three of the orchards, but in one of the old orchards it had no significant effect on female flowering. A positive significant $GA_{4/7}$ effect was found in the number of cones produced in only one of the young orchards. Seed quality was not affected by the $GA_{4/7}$ treatment. Although the $GA_{4/7}$ treatment did not always have a clear positive effect, economic calculations based on cone count data showed that $GA_{4/7}$ treatment is still a cheap and highly profitable way of increasing the seed production in *P. abies* seed orchards.

Introduction

Flower stimulation using $GA_{4/7}$ has shown positive effects in a large number of conifer species (see e.g. Owens & Blake 1985). Also in *Picea abies* positive results has been reported (Dunberg 1980, Högberg & Eriksson 1994, Fogal et al. 1996). Consequently, flower stimulation, using $GA4_{/7}$ treatment, has been used routinely for a long time in order to facilitate crossings in the Swedish breeding programmes. However, seed orchard managers have hesitated to use $GA_{4/7}$ in seed orchards, especially in *P. abies,* due to its irregular and unpredictable flowering patterns.

In 2005 we conducted the first practical, commercial-scale attempt to stimulate flower production by $GA_{4/7}$ treatment in *P. abies* seed orchards. The objectives of the study presented here were to measure the effects of the $GA_{4/7}$ treatment on flower, pollen and cone production.

Material and methods

Location of seed orchards

Four *P. abies* seed orchards were treated with $GA_{4/7}$: two old orchards near the end of their production phase and two young orchards at the beginning of their production phase. The locations of the orchards are shown in figure 1, and descriptions of the orchards are given in table 1.

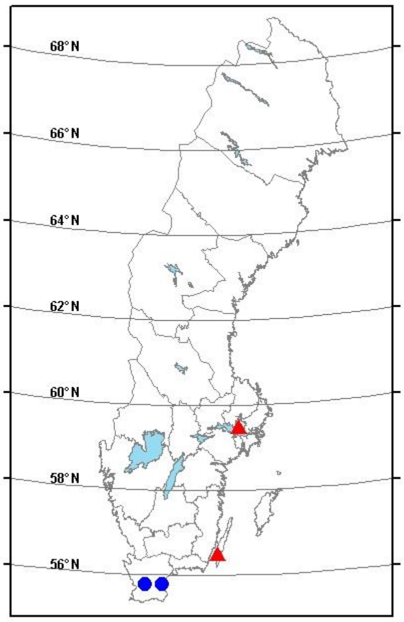

Figure 1. Locations of the seed orchards. Blue circles show the locations of the two old orchards: 52 Maglehem and 68 Slogstorp. Red triangles show the locations of the two young orchards: 501 Bredinge on the island of Öland and 504 Ålbrunna, outside Stockholm.

$GA_{4/7}$ treatment

A single $GA_{4/7}$ treatment was applied to each orchard, and the dates of application are shown in table 1. Every second row was left as untreated control to enable statistical assessment of the treatment. Each graft was given a $GA_{4/7}$ dose corresponding to its diameter at breast height, table 2. In all orchards, a standard method of $GA_{4/7}$ application was used, i.e. a hole was drilled into the xylem in the trunk and a $GA_{4/7}$ solution was applied using a micropipette.

However, in 504 Ålbrunna we also evaluated a different injection method, using equipment from ArborSystems that was developed for injecting insecticides into trees, figure 2.

Table 1. Description of the seed orchards studied, $GA_{4/7}$ treatment dates in 2005, and the numbers of grafts and samples included in this study.

	504 Ålbrunna	501 Bredinge	52 Maglehem	68 Slogstorp
Established	1984	1983	1958	1965
Seed orchard managers	Bergvik Skog AB	Södra Odlarna AB	Svenska skogsplantor AB	Svenska skogsplantor AB
Size (ha)	25	26	5	19
Number of clones	137	100	36	45
Spacing (m)	7 × 4	7 × 3.5	5 × 5	7 × 3.5
$GA_{4/7}$ treatment dates in 2005	July 6–8	June 30 – July 2	July 7	July 4–5
No. grafts, flower score	1600	243	363	400
No. grafts, cone count	210	99	100	-
No. samples, seed quality	9	4	2	-
DBH (mm) of grafts in cone count. Min – mean – max	73 – 171 – 278	126 – 185 – 298	111 – 290 – 445	-

Figure 2. The Wedgle™ Direct-Inject™ tree injection unit from ArborSystems (Omaha, USA) tested in 504 Ålbrunna.

Table 2. Doses of $GA_{4/7}$ and the number of application points on each graft used in the $GA_{4/7}$ treatment of the seed orchards.

Graft diameter at breast height (cm)	$GA_{4/7}$ dose (mg/graft)	Number of application points (drilled holes using the standard method or injection points using the injection method)
< 7	15	1
7–15	30	2
15–20	45	3
20–25	60	4
> 25	100	5

Flower scoring

Female and male flowering were scored during flowering in 2006 by examining a random sample of trees in all four orchards, as listed in table 1. Flowering was scored in one of ten classes; from 0 (no flowers), to 9 (corresponding to the most abundant flowering observed in each orchard).

Cone count and seed quality assessment

In the autumn of 2006, the number of cones produced per tree was counted in three of the orchards, table 1. From each graft all cones, including vital cones and those affected by insects and fungi, were collected to estimate the production potential of the trees. Seed quality was assessed for the different treatments in the two young orchards 504 Ålbrunna and 501 Bredinge. Each sample consisted of 30 cones. In Ålbrunna three samples of each treatment were analysed, and in Bredinge two samples of each treatment were analysed. At 52 Maglehem, the samples for the control and the $GA_{4/7}$ treatment were unintentionally mixed up, so for this orchard only data from two samples consisting of cones from both control grafts and $GA_{4/7}$ treated grafts could be analysed. Seed quality traits analysed were 1000-grain weight and the number of filled seeds per cone.

Statistical analyses

The Proc GLM module of the SAS program (SAS 1999) was used for statistical analysis. Data were analysed separately for each seed orchard, and the traits included in the analysis were the numbers of female and male flowers per graft, and the number of cones per graft. We analysed the data using the following linear model:

$$y_{ijk} = \mu + b_i + c_j + g \cdot x_{ijk} + e_{ijk}$$

where:

y_{ijk} = dependent variable, *i.e.* the number of female flowers per graft

μ = overall mean

b_i = fixed effect of the block

c_j = fixed effect of the treatment

g = regression coefficient

x_{ijk} = diameter at breast height (only used in the analyses of cone count data)

e_{ijk} = residual (N(0, σ_e^2))

The significance of any differences between the control and treatment least square means was tested within the GLM procedure.

Economic calculations

Based on the cone count and seed quality results from 504 Ålbrunna, along with costings for the $GA_{4/7}$ solution and $GA_{4/7}$ application, we calculated the cost of production for one additional kilogram of filled seeds. The $GA_{4/7}$ cost was €14 per gram, the labour cost was

€228 per day and it was possible to treat 600 grafts per day. We assumed that 80 percent of the originally planted grafts were alive and that 75 percent of the cones contained filled seeds. Rate of exchange used: 1 € = 9.20 SEK.

Results and discussion

Flower and pollen scoring

We found a significant positive effect of the $GA_{4/7}$ treatment on male flowering in the young orchards, but not in the old orchards (table 3), and scores for female flowering showed a significant positive effect of the $GA_{4/7}$ treatment in three of the orchards. In one of the old orchards, the treatment had no significant effect on female flowering, table 4. The standard $GA_{4/7}$ treatment method yielded higher, but not significantly higher, flowering scores than the injection method for both male and female flowering at 504 Ålbrunna. A possible reason for this difference between the treatment methods is that the $GA_{4/7}$ is introduced into the xylem of the tree with the standard method, and into the phloem with the injection method. Further comparative studies between these two methods are needed to confirm whether or not there is a real difference in the results obtained using these application methods.

The higher average flower scores in the $GA_{4/7}$-treated trees, compared to the controls, were due to larger proportions of control grafts in the lowest scoring classes, and larger proportions of $GA_{4/7}$-treated grafts in the highest scoring classes, figure 3. The finding that more $GA_{4/7}$-treated grafts flowered than the controls implies that more $GA_{4/7}$-treated clones participated in the flowering, suggesting in turn that the treatment enhanced the genetic diversity in the seed crop. The finding that more grafts flowered abundantly after $GA_{4/7}$ treatment also implies that the treatment enhanced the genetic diversity of the seed crop.

Table 3. Results of male flower scoring.

Seed orchard	Flower score, controls	Flower score, $GA_{4/7}$-treated grafts	$GA_{4/7}$ treatment effect (percent)	Pr > |t|	
504 Ålbrunna, standard method	4.08	4.55	11.6	0.003	**
504 Ålbrunna, Injection method	4.08	4.50	10.4	0.008	**
501Bredinge	3.55	3.95	11.2	0.041	*
52 Maglehem	4.78	4.87	1.9	0.596	n.s
68 Slogstorp	4.32	4.34	0.6	0.909	n.s.

Table 4. Results of female flower scoring.

Seed orchard	Flower score, controls	Flower score, $GA_{4/7}$-treated grafts	$GA_{4/7}$ treatment effect (percent)	Pr > |t|
504 Ålbrunna, standard method	3.18	4.52	42.1	<.0001 ***
504 Ålbrunna, Injection method	3.18	4.30	35.4	<.0001 ***
501 Bredinge	3.46	4.20	21.3	<.0001 ***
52 Maglehem	4.54	4.59	1.2	0.740 n.s.
68 Slogstorp	2.84	3.31	16.7	0.048 *

The absence of any significant effect of $GA_{4/7}$ treatment in the old orchards may be explained by the doses given, since they may have been too small in relation to the graft size. The highest doses of $GA_{4/7}$ (100 mg/graft) were used for grafts with diameters larger than 25 cm, and at 52 Maglehem and 68 Slogstorp the grafts had an average diameter of 29 cm and a maximum diameter of 44 cm.

Cone count

In the young orchard 504 Ålbrunna the standard $GA_{4/7}$ treatment resulted in a significant ($P<0.049$) 69 percent increase in the number of cones compared to the controls, while the injection method resulted in a non-statistically significant 33 percent increase, table 5. In the other young orchard, 501 Bredinge, there was no positive effect of the $GA_{4/7}$ treatment on cone production. However, in this orchard both the treated and control grafts' production levels were extremely high; on average 877 cones were produced per graft, with a maximum cone production for one graft of 2828. In years when high natural flower production occurs, the grafts probably cannot bear any further cones, so the increased flowering indicated by the flowering scores may not result in a larger cone crop.

Table 5. Results of cone counts.

Seed orchard	Cones per control graft	Cones per $GA_{4/7}$-treated graft	$GA_{4/7}$ treatment effect (percent)	Pr > |t|
504 Ålbrunna, standard method	169.7	286.9	69.0	0.049 *
504 Ålbrunna, Injection method	169.7	225.5	32.9	0.352 n.s.
501 Bredinge	876.8	830.0	-5.3	0.749 n.s.
52 Maglehem	479.4	494.7	3.2	0.850 n.s
68 Slogstorp	-	-	-	-

Seed quality

The seed quality data show, as expected from other investigations (e.g. Eriksson et al. 1998), that the $GA_{4/7}$ treatment resulted in slightly lighter seeds. The differences between the treated and non-treated grafts were not statistically significant in this respect (table 6), but this may have been due to the small sizes of our small samples.

Table 6. Seed quality results.

Seed orchard	1000-grain weight, controls (g)	1000-grain weight, $GA_{4/7}$-treated grafts (g)	No. of filled seeds per cone, controls	No. of filled seeds per cone, $GA_{4/7}$-treated grafts
504 Ålbrunna, standard method	8.1	7.8	105	106
504 Ålbrunna, Injection	8.1	6.9	105	135
501 Bredinge	9.5	8.9	147	140
52 Maglehem	9.0 [1)]	-	150 [1)]	-
68 Slogstorp	-	-	-	-

[1)] Average for both control and $GA_{4/7}$ treated grafts.

Economic calculations

Results from the economic calculations based on data from 504 Ålbrunna showed that the cost of producing an additional kilogram of seeds was €14 (table 7), much lower than the market price of high quality orchard seed in Sweden (ca. €1100). Even if only one in every seven treatments with $GA_{4/7}$ was successful, the cost of producing an extra kilogram of seed would be less than €100; less than 10 percent of the market price of the seed. So, $GA_{4/7}$ treatment appears to be a cheap and highly profitable way to increase seed production in *P. abies* seed orchards.

Table 7. Economic calculation results.

Cost of $GA_{4/7}$ treatment (€/ha)	311
Extra production of filled seed (Kg/ha)	22
Cost of the extra production of filled seed (€/Kg)	14
Success rate	Cost of the extra production of filled seed (€/Kg)
Every time	14
1 out of 3	42
1 out of 5	70
1 out of 7	99

Conclusions

The treatment of *P. abies* with $GA_{4/7}$ increases both the genetic diversity and size of the seed crop in seed orchards. Although a positive effect of $GA_{4/7}$ treatment is not always obtained, economic calculations based on our cone count data indicate that $GA_{4/7}$ treatment is a cheap and highly profitable way to increase the seed production in *P. abies* seed orchards, even if only one treatment in seven is successful.

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Seed orchards and seed collection stands of Scots pine in Turkey

Nebi Bilir & M. Denizhan Ulusan

Faculty of Forestry, Suleyman Demirel University, 32260 - Isparta/Turkey
(nebilir@orman.sdu.edu.tr or nebilir@hotmail.com)

Abstract

Scots pine (*Pinus sylvestris L.*) has 22 seed orchards covering 116 ha and 36 seed collection stands (seed stands) covering 4813 ha. The seed orchards had on average 5.6 ha and 21 years, 41 clones and 36 ramets per clone. The effective number of clones was on average 37.3, which is 91% of census number indicating that the clones are represented by similar number of ramets. The stands had 134 ha, 1660 m. altitude, 105 years, 26 m. height, 35 cm diameter on average, were briefly documented in this study. Seed orchards in Turkey usually draw their clones from a single seed collection stand. Although it was changed for years based on annual plantation program, at beginning of the century more than 90 % of seed demand for forest plantation in Turkey was covered from the seed stands for some years, and the impact of seed orchard crop is raising.

There were large differences for number of clone and ramets per clone and total number of ramets among the orchards. Large differences for the documented characters were also found among the seed stands. Results of the study were discussed based on seed source management and breeding programs.

Key words: *Pinus sylvestris*, seed sources, effective number of clone, breeding program.

Introduction

Scots pine

Scots pine ***(Pinus sylvestris* L.)** is one of the economically and ecologically most important forest tree species for both Europe and Asia. It has also an interesting growing as an introduced exotic species such as Korea, China, Mexico and New Zealand, where provenance and cultivation trials have been established (Boratyński, 1991). Scots pine occupies between 37°-70° N latitude and 7°-137° E longitude in the world (Anonymous, 2001).

Scots pine is one of the five native pines (*Pinus brutia*, *P. nigra*, *P. sylvestris*, *P. pinea*, *P. halepensis*) in Turkey. It is classified as one of the economically important species for Turkish forestry and the "National Tree Breeding and Seed Production Programme" which is a part of the Turkish–Finnish project, implemented in cooperation between the Turkish Ministry of Forestry and Enso Forest Development Oy Ltd (Koski and Antola, 1993). While Scots pine grows between 0-2700 meters (mainly 1000-2500 m.) above sea level in Turkey, it also grows up to 3125 meters. Within its natural area it grows in different ecological conditions. Such a wide range of ecological conditions could favor the formation of a variety of ecotypes. For instance, Turkey has *Pinus sylvestris* ssp. *hamata* var. *hamata* and *P. sylvestris* ssp. *hamata* var. *compacta* (Anonymous, 2001; Genc, 2004). Pure stands of the species occupy roughly 750 000 ha in mostly Black sea coastal mountains at northern part of Turkey (38°41'-41°48' N latitude, 28°00'-43°05' E longitude) where the climate is humid, of which roughly 475 000 ha (65%) are considered to be productive forests. Scots pine had only 3 % of the artificial and 1 % of the natural regeneration area during the last 20 years (Koski and Antola, 1993). Annual yield is 1 976 688 m^3 in the species. Annual increment is 10.43 m^3 varied between 1.33 and 26.81 in Turkey (Anonymous, 2001).

Afforestation is one of the important ways to turn into productive forest of unproductive forest and to increase its current distribution area. For instance, its distribution was increased from 738 192 ha (Anonymous, 2001) to 757 426 (Caliskan, 1997) between 1980 and 1997. But, suitable area for afforestation of the species is about 500 000 ha (Anonymous, 2001). Seed collection stand and orchards are more important seed sources for the plantation. The natural range of the species was divided geographically into four main and seven sub breeding zones based on climatically and ecological conditions in the "National Tree Breeding and Seed Production Programme" (Koski and Antola, 1993) (*Figure 1*). Collected seed both seed orchard and seed stands are mostly tested such as germination percentage by Forest Tree Seeds and Tree Breeding Directorate. And some of the collected seed are exported or sold to local private companies. For instance, 15 kg Scots pine seeds were exported during last five years (Anonymous, 2004).

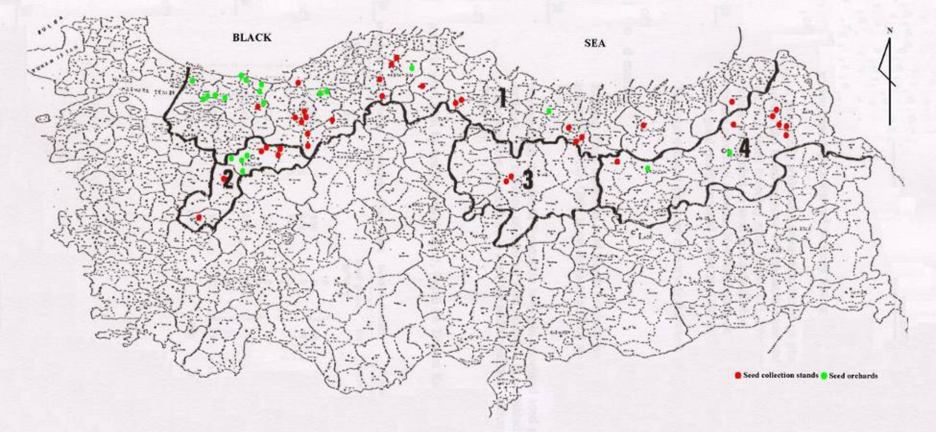

Figure 1. Breeding zones, present seed orchards and seed collection stands of Scots pine.

Seed orchards

The first trial seed orchard with Scots pine (10 clones and 96 ramets) was established in Turkey in April of 1964. Turkey has currently 171 seed orchards in 1165.4 ha of eight forest tree species (*Pinus brutia*, *P. nigra*, *P. sylvestris*, *P. halepensis*, *P. pinea*, *Cedrus libani*, *Picea orientalis*, *Liquidambar orientalis*). Tree breeding in Turkey on an operational scale was initiated with plus tree selection and establishment of seed orchards in 1972, Establishment of seed orchards have been continued with 6952 plus trees selected phenotypically (e.g. growth rate, stem and branch forms) mostly in seed collection stands of 11 species (www.ortohum.gov.tr, 2007). It was characteristics of the present Turkish seed orchards that they have been established with about 30 plus tree clones each, selected from a single seed stand at the same breeding zone. While phenotypic selection was used in establishment of early seed orchards, phenotypic selection and progeny test will be combined in establishment of new seed orchards based on National Tree Breeding and Seed Production Programme. Both establishment of seed orchards and selection of seed collection stands are administered by "Forest Tree Seeds and Tree Breeding Directorate" of Ministry of Environment and Forestry.

Seed collection stands

There are 338 seed collection stands (seed stands) in 46 086 ha of 27 forest tree species in Turkey. They are selected phenotypically based on geography (natural distribution area) and target characters of the species in breeding program. For instance, the most important characters of Scots pine which should be improved by means of breeding are height growth. In addition the quality character that effect the usefulness of stem should be improved. Besides, forest resistance is essential for high elevation areas near the forest tree line such as Kars-Sarikamis (more than 2200 m elevation) (Koski and Antola, 1993). The seed collection stands are also used as source of vegetative materials/ancestor of long-term breeding populations to establish seed orchards.

Seed orchards and seed collection stands of Scots pine in Turkey were documented based on establishment (number of clone and ramets, effective number of clones) and selection characters (altitude, area, age, height and diameter). Results of the study were discussed for National Tree Breeding and Seed Production Programme.

Material and Methods

Seed orchards

General information of seed orchards was taken from Forest Tree Seeds and Tree Breeding Research Directorate. In this study following criterions of the orchards were used based on Turkish forestry.

Number of clones and ramets per clone

Number of clones and ramets per clone have important roles on gene diversity in seed orchard crops and economy of the seed orchard (i.e., selection of clones, growth of graft, establishment, thinning). If many clones were used, genetic diversity would be high; establishment cost also would be high. When a small number of clones are used, some rare allele in a base population may be lost in a seed orchard due to sampling effect (Bilir *et al.*, 2004). For gene diversity of orchard crops, the number of clone may be more important than to use equal number of ramet among clones (Kang *et al.*, 2001). Classes of number of clones were determined according to the minimum clone numbers (30) (Bilir *et al.*, 2004).

Effective number of clones

The effective number of clones (N_c) was estimated based on ramet numbers of clones (n) (Kang *et al.*, 2001) as:

$$N_c = \frac{n^2{}_{total}}{\sum_{i=1}^{N} n_i^2} = \frac{1}{\sum_{i=1}^{N}\left(\frac{n_i}{n_{total}}\right)^2} = \frac{1}{\sum_{i=1}^{N} r_i^2}$$

where n_i is the number of ramets for i^{th} clone; N is the census number of clones and r_i is the proportion of the i^{th} clone in the seed orchard. If the clones are unrelated and not inbred the effective number of clones can be considered as equivalent to the status number of clones (Kang *et al*, 2001).

Seed collection stands

Seed stands data were taken from website of "Forest Tree Seeds and Tree Breeding Research Directorate (www.ortohum.gov.tr, 2007). Following characters of the stands were documented in the study.

Age

Age is important for reproductive characters such as cone and pollen production of individual/stand. Age classes were determined according to the rotation age (80-100 years) of the species (Genc, 2004).

Altitude

The character is important for both seed production used in plantation and gene conservation. Altitude classes were determined according to the low (≤1200 m), middle (1200-1600) and high (1600≤) zones (Koski and Antola, 1993).

Area

It is important for selection/breeding program cost, thinning, and protection. Its classes were determined according to the minimum area number, which is about 100 ha (Koski and Antola, 1993).

Height and diameter

Height has an important role in seed cost (e.g. seed collection). Height and diameter are important phenotypical characters during selection of seed stand and clone.

Results and Discussion

Seed orchards

Scots pine has 22 seed orchards in 116 ha (*Table 1*, *Figure 1*), two of which is *Pinus sylvestris* var. *compacta* in 4.8 ha. One of the orchards is shown in *Figure 2*. Current seed orchards covered 9 % of seed demand for some years (e.g. 2002) (Cengiz, 2003). But, it was related to annual plantation program. For instance, plantation area was 7542 ha in 2002; 1227 ha in 2003; 3897 ha in 2004; 3149 ha in 2005; 2000 ha in 2006 (Personal communication with M. Alan). So, seed orchard crop was fully covered for 2003, but was not sufficient for 2002. It could be also changed for good/poor seed year or age of the orchards. 30 % of the orchards including 404 clones (49% of total clones) at 39.6 ha (34% of total area) is lower than fifteen years (*Table 1*). Male strobili appear sporadically in the first three years after grafting regardless of origin. Until 12 to 15 years graft produce more female strobili than male. At the age of 15 to 20 years, the production of male strobili was about 5 times higher than female (Chalupka, 1991). Bilir *et al*. (2007) estimated average seed production as 2.3 kg per hectare in three Turkish seed orchards of the species for one year data. New seed orchards are established as emphasized in National Tree Breeding and Seed Production Programme.

Table 1. Number of clones (N), total number of ramets ($\sum n$), effective number of clone (N_c), the relative effective number of clones (N_r), coefficient of variation (*CV*) among clones in ramet numbers and general knowledge.

Breeding zone	N	$\sum n$	N_c	N_r (= N_c /N)	CV	Area (ha)	Spacing (m)	Age (year)
1	10	94	9.9	0.99	8.5	0.2	5x5	43
3	25	1222	23.2	0.93	27.3	6.0	7x7	25
1	28	1035	25.3	0.91	32.0	5.1	7x7	25
2	29	1100	26.7	0.92	29.2	5.4	7x7	24
1	29	1193	26.8	0.93	28.1	6.2	7x7	21
1	30	1034	22.9	0.76	55.6	5.1	7x7	19
4	30	1050	27.4	0.92	30.2	5.5	7x7	17
1	30	1154	27.4	0.92	31.0	5.8	7x7	30
2	30	1200	27.9	0.93	27.0	7.2	7x7	23
4	30	1274	27.9	0.93	27.1	6.5	7x7	20
2	30	1406	27.0	0.95	33.1	6.9	7x7	15
1	30	1807	28.4	0.95	23.0	6.7	6x6	10
1	30	1987	28.4	0.96	23.6	7.2	6x6	12
1	31	917	28.2	0.91	31.3	5.1	7x7	18
1	31	1258	28.5	0.92	29.2	4.0	5x5	31
4	36	2060	32.5	0.66	32.7	5.7	5x5	3
1	43	728	34	0.79	51.3	3.6	7x7	30
1	43	1220	39.7	0.93	28.5	6.0	7x7	30
2	126	453	107.7	0.90	41.2	3.2	7x7	7
2	152	2021	145.3	0.86	31.3	9,9	7x7	9
average	41	1211	37.3	0.91	31.0	5.6	-	21
range	10-152	94-2060	9.9-145.3	0.76-0.99	8.5-55.6	0.2-9.9	-	3-43

There were large differences among the orchards based on number of clones (N), total number of ramets ($\sum n$) (*Table 1*). There were fifteen times differences between the lowest (N=10) and the highest (152) seed orchard for the number of clones. Besides, average of number of ramets per clone was 36 and varied between 4 and 67. Most of the orchards (70 %)

had more than 30 clones and more than 30 ramets per clone (*Table 1*). Bilir *et al*. (2006-a) suggested less clones (e.g., 5-10 clones) based on fertility and gene diversity in establishment of seed orchards both economy and balance among clones. Bilir *et al*. (2004) reported that even though the production of strobili is assumed to be proportional to the successful number of gametes, the number of 30 clones could hardly deliver entire gene diversity of the base population into the seed orchard. When used many clones in establishment of seed orchard, fertility variation among and within clone can be high (e.g., CV) and then balance of fertility by seed orchard manager can be hard (Bilir *et al*., 2006-a). Besides, effective number of clone can be low. So, fewer clones (e.g., 5-10 clones) should be used at establishment of seed orchard. It could be reduced to 20 or fewer clones after genetic rouging (Lindgren and Prescher, 2005). While early Turkish seed orchards were established with about 30 plus tree clones selected phenotypically from a single seed stand, new seed orchards have been established with 100-200 clones based on progeny test and the breeding program. Besides, number of seed stands or base populations used establishment of seed orchard should be increased for high gene diversity in seed orchard crop and transmit of gene diversity by afforestation to next generations and resistance of afforestation to biotic and a biotic factors.

Figure 2. Scots pine seed orchard established at Mengen, Bolu, with spacing 7x 7 m., 1034 grafts from 30 clones selected from a seed collection stand in 1988.

Effective number of clones

There were small differences between effective number of clones and number of clones, more than 90% of relative effective number of clones (*Table 1*). Using of equal or similar number of ramets per clone is important for effective number of clone. For instance, effective numbers of clone were 22.9 (N_r = 0.76, CV=55.6) and 28.4 (N_r = 0.96 CV=23.6) which have the same clone number, respectively. Effective number of clone (N_c) was also estimated based on number of clones (N) and coefficient of variation (*CV*) for the number of ramets among clones as $N_c= N/(CV^2+1)$ (Kang *et al*., 2001).

Effective number of clones (N_c) becomes the status number (N_s) of the orchard crop if parents are unrelated, non-inbred and parental fertility is proportional to the ramet number and all parents are included (Lindgren and Mullin, 1998; Nikkanen and Ruotsalainen, 2000; Kang *et al*., 2001). Gene diversity (*GD*) can be related with the effective number of clones ($GD=1-0.5/N_c$) by Lindgren and Kang (1997) or with status number (N_s) as: $GD = 1- 1/ (2*N_s)$, and also with the relative effective number of clones (N_r) and clone numbers (N) as: ($GD=1-0.5/N*N_r$). Maximum gene diversity of seed orchards crop for a given clone number is attained when all parents contribute equally to the gamete gene pool (Kang, 2001). In this situation coefficient of variation (*CV*) among clones for ramet numbers play important role in gene diversity of seed orchards crop.

Table 2. Number, area (ha), number of clones and number of ramets for seed orchards and number of plus trees selected and proposed for Scots pine and estimated seed demand in the four Turkish breeding zones.

	Breeding zones			
	1	2	3	4
Number & (%)	11 & (55 %)	5 & (25 %)	1 & (5%)	3 & (15 %)
Total area & (%)	55 & (49 %)	32.6 & (29 %)	6.0 & (6%)	17.7 & (16 %)
Number of clones & (%)	335 & (41 %)	367 & (44 %)	25 & (3%)	96 & (12 %)
Number of ramets & (%)	12440 & (51 %)	6180 & (26 %)	1222 & (5 %)	4384 & (18 %)
Current selected plus trees[1]	1124	380	475	440
Proposal number of selected plus trees[1]	1500	500	500	500
Seed demand (kg) & (%)[2]	985 & (68%)	24 & (2%)	164 & (11%)	270 & (19%)

[1] Koski and Antola, 1993 ; [2] Anonymous, 1992

There were large differences among seed orchards for the coefficient of variation (*CV*) of ramet numbers among clones (*Table 1*). But, it could be balanced by genetic thinning together with reproductive characters. Bilir et al. (2006-b) reported higher variation within clones than among the clones for strobili production in three Turkish Scots pine seed orchards. Most of the orchards were established in first breeding zone as seen from *Table 2* and *Figure 1*. It is normal that because about 41 % of the natural distribution is in first breeding zone.

Seed collection stands

Scots pine has 36 seed collection stands, (*Figure 1*) in 4813 ha, selected phenotypically such as stem and crown forms, small branch (*Figure 3*). The general characters of the stands of the species were given in *Table 3*.

Figure 3. Seed collection stand (left) where a plus tree was selected (right) (1300 m).

More than 90% of the seed demand for plantation in the species is covered from these stands for some years as related to annual plantation program. They are also base population of vegetative material to establish seed orchards. Although seed collection stand is a way of cheap breeding such as short-term breeding, seed orchard has also some advantage such as harvesting cost and quality in seed crop. For instance, while average of one thousand seed weight is 4 g, it increases up to 6-7 g in seed orchard crop (Urgenc, 1982). Also, seed harvest is possible on ground in seed orchard. While it was found 9.2 g in natural populations (Boydak, 1977) and 9.9 g in eleven seed collection stands (Turna, 2003) and 9.3 g in three seed collection stands (Ucler, 1991) in Turkey, it was estimated 11 g in three Turkish Scots pine seed orchards (Bilir *et al.*, 2007). Besides, seed orchard crop (Bilir, 2007 unpublished data) had bigger size than seed stand seed (Turna, 2003). The differences were also valid for subspecies and variety of the species. For instance, 1000 seed weight was 2-4 g in *Pinus sylvestris* ssp. *lapponica*, 4-7 g in *P. sylvestris* ssp. *sylvestris* (Molotkov and Patlaj, 1991).

Table 3. Averages and ranges of the seed stands.

	area (ha)	altitude (m)	age (year)	Height (m)	diameter (cm)
Average	134	1660	105	26	35
Range	45-303	900-2350	78-164	16-36	17-45

The seed stands are 0.7% of total area of Scots pine. It should be at least 3-5% for gene conservation and seed harvest. Seed stand is also a way of the cheap breeding. So, new seed stands should be selected. 47 % of the current seed stands are over than rotation age of the species (100 years). The species starts seed production at natural stands 30 years (Anonymous, 1986). Urgenc (1982) suggested that seed stand in Scots pine could be selected up to 90 or 135 years. Stands of high quality and of high productivity at an age of 60-80 years are best suited for the selection of plus trees or up to 100 years depending on the felling age (Philgas, 1991). Besides, size and quality of seeds harvested from more than 200 years seed stand could be low (Genc, 2004). While 100 ha were proposed as minimum seed collection stand area (Koski and Antola, 1993), 35 % of the stands had less than 100 ha. Half of the stands were between 1200 and 1600 meters, while 44 % of them were more than 1600 m. The seed stands for altitude are normal. Then, while new seed stands are selected, age and areas should be considered. There were large differences among the stands for the characters such as seven times differences between the lowest and largest area of the stands.

More than 50 % of the seed stands are in first breeding zone based on number and area, it is well accordance with natural distribution area of the species in first zone (*Table 4*).

Table 4. Natural distribution and seed collection stands (ha) based on breeding zones.

	Breeding zones			
	1	2	3	4
Number & (%)	20 & (56%)	7 & (19.5%)	2 & (5.5%)	7 & (19%)
Total area & (%)	2772 & (58%)	600 & (12%)	377 & (8%)	1064.5 & (22%)
Core area & (%)	1890 & (62%)	260 & (9%)	313 & (11%)	582.5 & (19%)
Natural area & (%)*	299 205 & (40.5%)	5170 & (0.7%)	278 038 & (37.6%)	155 191 & (21%)

*; Anonymous, 1980

While natural distribution area of the species is high in third zone, number and area of the selected seed stand is very low (*Table 4*). It can be because of the similarity of distribution of main stands (*Figure 1*) and also seed demand in the zone (*Table 2*). While annual seed demand of Scots pine is reported about 715 kg (Cengiz, 2003), it is reported in another study as 1 440 kg (Anonymous, 1992). The differences can be because of the good seed year or annual plantation program.

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Do We Need Flower Stimulation In Seed Orchards?

Władysław Chałupka
Polish Academy of Sciences, Institute of Dendrology,
Parkowa 5, 62-035 Kórnik, Poland
wrchal@man.poznan.pl

Abstract

After long-term studies, an application of growth regulators, mostly gibberellins, became an efficient method of flower stimulation practically used in seed orchards. However, results of experiments indicate also some other possibilities of flower regulation, i.e. control of the strobili sex, changes in the strobili distribution within the crown, and differential effectiveness of growth regulators depending on the natural ability of clones to flowering. These effects are shortly discussed in the paper with regard to question of proposed selective stimulation of strobili bearing.

Introduction

Studies on physiology of hormonal flower induction in seed orchards of forest trees started on the turn of the 1950s and they were developing extensively until the 1990s (for review see, e.g. Pharis and Kuo 1977, Zimmermann et al. 1985, Owens and Blake 1985, Bonnet-Masimbert 1987, Chałupka 1991a and 2007). After several years of investigations, many practical recommendations were formulated for seed orchard managers how to apply growth regulators more efficiently and on a large practical scale (e.g. Philipson 1990, Almquist 2007).

However, from the past research activity in that field we are able to draw some additional conclusions which could be also useful in practical application of hormones to stimulate flowering in seed orchards.

Harmonization of inter-clonal variability in flowering

One of the main principles of seed orchards management is the promotion of genetic diversity in progeny. For that reason, when planning the lay-out of seed orchard, the same or very similar number of grafts of each clone is initially planted, assuming that this will result in an equal or at least similar contribution of clones to the genetic composition of seed orchard progeny. However, from many literature data it is known, that finally more or less half of the clones in the seed orchards produces nearly all male or female strobili (and seed crop as a consequence) (e.g. Jonsson 1976, Wesoły 1984, Nikkanen and Ruotsalainen 2000) (Fig. 1).

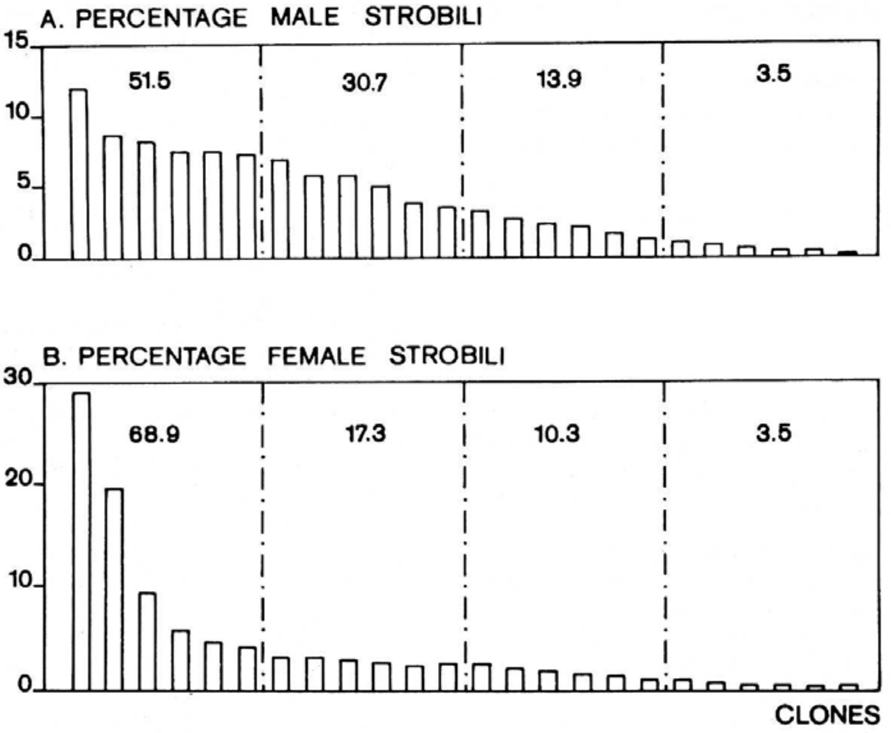


Fig. 1. Mean percentage of male and female strobili produced by 24 clones in a Polish Scots pine seed orchard (after Wesoły 1984).

This means that significant disparity exists between the level of genetic diversity in seed orchard progeny expected from the proportions of planted clones, and the level of genetic diversity resulted from observed participation of clones in genetic diversity of seed crop.

It was revealed in some investigations that the efficiency of flower inducing treatments could be higher in poor flowering clones than in good flowering ones (Fig. 2) . Such increase in the number of strobili in poor flowering clones creates a possibility to equalize to some extent a strobili production by clones and support the participation of poor flowering clones in progeny gene pool.

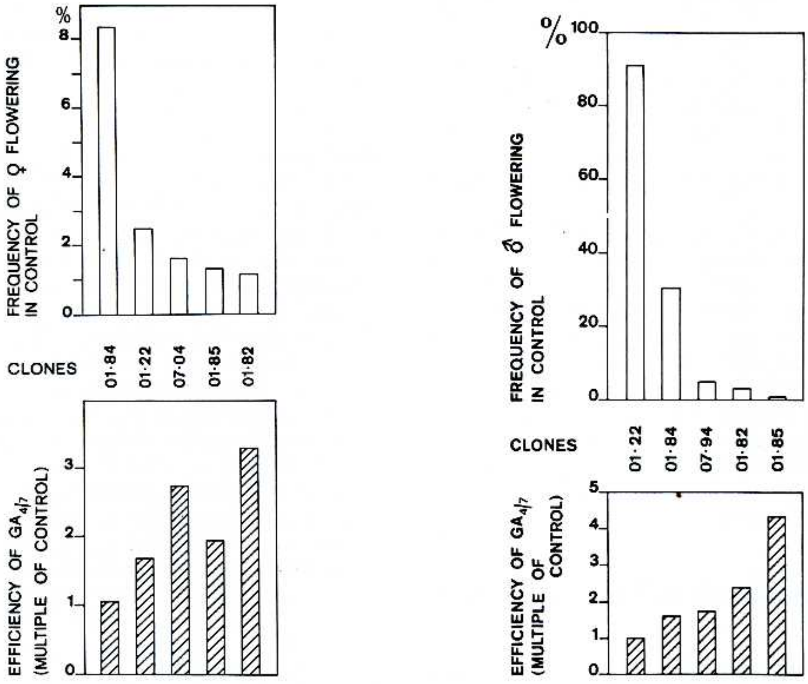


Fig. 2. Efficiency of GA4/7 treatment in Scots pine clones with different female and male flowering ability (after Chalupka 1991b).

Changing in the distribution of strobili in crown

Natural differences exist in the distribution of female and male strobili in the crown of grafts (Fig. 3).

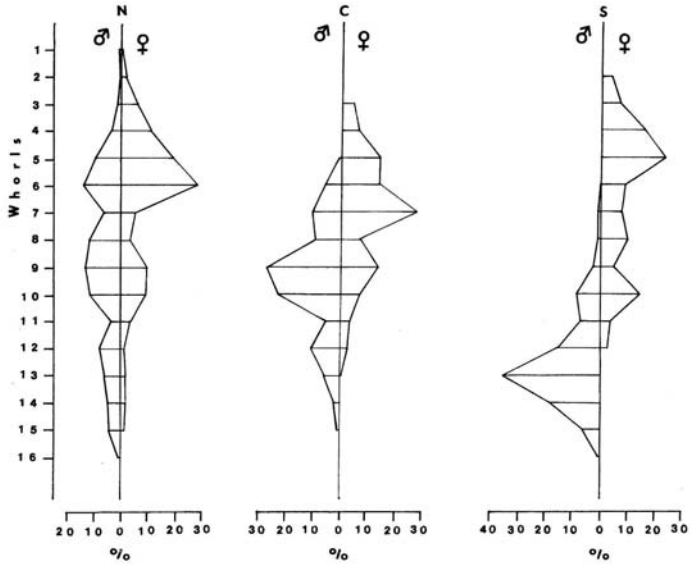


Fig. 3. Distribution of male and female strobili in the crown whorls of 16-year-old grafts of Scots pine in northern (N), central (C) and southern (S) Finland (based on data of Bhumibhamon 1978).

It has been established that gibberellins were effective in promoting female flowering in the lower part of graft crowns in *Larix* sp. (Bonnet-Masimbert 1982), and *Picea glauca* (Marquard and Hanower 1984 b). Also in *Pinus sylvestris* (Chałupka 1980) the efficiency of the gibberellin treatment was much higher in the middle and lower branches that in upper ones (Fig. 4).

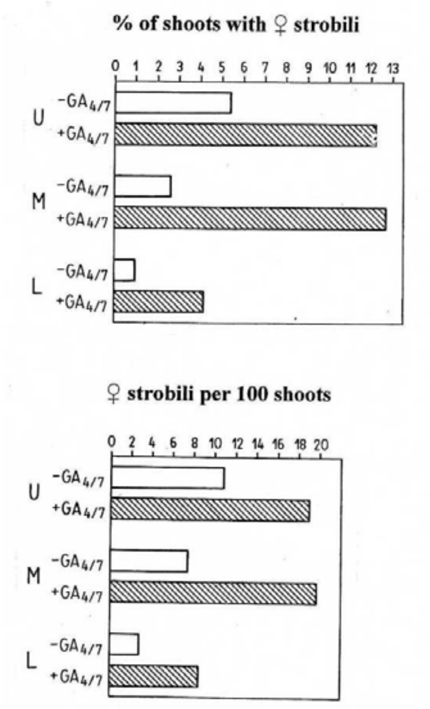
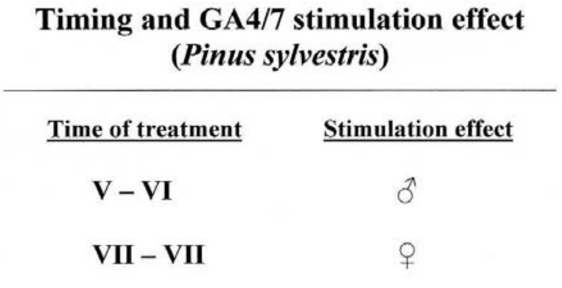


Fig. 4. Effect of GA4/7 on the percentage of shoots flowering female and number of female strobili in upeer (U), middle (M) and lower (L) branches of Scots pine grafts (after Chałupka 1980).

Timing

There are some disproportions between clones and/or grafts in the ratio of female to male strobili produced. Some timing experiments revealed that the sex of strobili initiated depends on time of gibberellins application.

Table 1. Summary of results on timing experiments with Scots pine grafts (after Lukkanen 1980, Chałupka 1984 and Almqvist 2003).



Timing and GA4/7 stimulation effect
(*Pinus sylvestris*)

Time of treatment	Stimulation effect
V – VI	♂
VII – VII	♀

It has been established that application of gibberellins at early stage of the growing season promoted the initiation of male strobili, while application in later stages increased female strobili production (Luukanen 1980, Chałupka 1980 and 1984, Almqvist 2003). The summarized results of those experiments are presented in Table 1.

Conclusions

Results demonstrated above, i.e. higher efficiency of gibberellin treatment in poor flowering clones and in lower part of crowns, and effect of timing of treatment application on sex of strobili induced, allow us to modify strobili production in seed orchards by treating only selected clones with gibberellins application at proper time. Therefore, the possibility exists to harmonize participation of clones in genetic diversity of seed crop.

Answering the title question it is obvious that the flower stimulation in seed orchards is needed, however it could be selective one. A more or less equal contribution of all clones to genetic diversity of seed crop should be a target of such selective stimulation. However, further detailed studies on morphogenesis of generative organs are necessary to develop hormonal treatment more effective.

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Using SYNCHRO.SAS, a program to facilitate phenological data processing, in a radiata pine seed orchard in northern Spain.

Codesido V[1]. and Fernández-López J.
[1] Verónica Codesido Sampedro
CINAM-Lourizán
Ap.127, 36080, Pontevedra, Spain
Tel.0034986805068 or mobil: 003462653172
Fax: 0034986856420
Email: vcodesido.cifal@siam-cma.org

ABSTRACT

Flowering process in a seed orchard is of great importance, since it affects the gene exchange between the clones and consequently the genetic composition of the seed produced. The knowledge of flowering phenology is of great importance and fundamental need for the successful operation of any seed orchard. Several different techniques have been developed for quantifying the degree of reproductive synchronization between all mating pairs of clones. A programme intended for SAS-pc (SYNCHRO.SAS) has recently been created to facilitate phenological data processing and to compute several phenological synchronization indices for each male-female combination and to enable construction of the male and female phenograms as well as other simple graphics that may help in the interpretation of phenological synchronization parameters. Reproductive phenology was studied in a radiata pine seed orchard, located in northern Spain. Timing of flowering was determined on the basis of data recorded by visual observations made three times a week in 2000, 2001 and 2002 flowering period. In general, the flowering periods of the different clones overlapped. The male flowering clones that best synchronised with the females appeared to be those that started flowering earlier. The phenological overlap index varied greatly among clones, whether male or female. SYNCHRO.SAS was a very useful tool for calculate the overlap indices and obtain phenological graphs.

INTRODUCTION

Radiata pine (*Pinus radiata* D.Don) is one of the most exotic planted forest in the world and was introduced in Galicia (northern Spain) at the middle of the 19th century.

Actually it is the third most important forestry specie in Galicia occupying the 12% of the Galician forest area (Anonymous, M.M.A., 2001), being one of the most important commercial specie for local wood industries that are an important economic sector in this area.

The main use of radiata pine in Galicia is for furniture, sawn-timber and poles. Therefore, a radiata pine tree improvement program was initiated in 1992 in Galicia by the CINAM (Centro de Investigación e Información Ambiental de Lourizán) with plus tree mass selection in Galician plantations and the use of this material for seed production in a clonal seed orchard (Sergude, A Coruña, Codesido and Merlo, 2001). The selection criteria for plus trees were superior height growth, an excellent straight stem, flat branch angle and they must to be freedom for disease and defect.

We recollected grafts to install a clonal seed orchard and seeds to install progeny tests from the plus trees selected. The seed orchard was installed far away from radiata pine plantations to avoid pollen contamination.

Flowering process in a seed orchard is of great importance, since it affects the gene exchange between the clones and consequently the genetic composition of the seed produced. The knowledge of flowering phenology is of great importance and fundamental need for the successful operation of any seed orchard.

Several different techniques have been developed for quantifying the degree of reproductive synchronization between all mating pairs of clones (Askew, 1988; Askew and Blush, 1990; Xie *et al*., 1994; Gömöry *et al*., 2000, 2003). A programme intended for SAS-Pc 6.12 (SYNCHRO.SAS) has recently been created to facilitate phenological data processing and to compute several phenological synchronization indices for each male-female combination and to enable construction of the male and female phenograms as well as other simple graphics that may help in the interpretation of phenological synchronization parameters (Zas *et al*., 2003)

The objectives of this study were:

- ■ Determine the phenological variation in female and male flowering in a radiata pine seed orchard
- ■ Describe the timing of female receptivity and pollen shedding
- ■ Comprobe the utility of the SYNCHRO.SAS program in calculate phenological data

MATERIAL AND METHODS

The radiata pine seed orchard was established in Sergude between 1997-1998, and consists of 68 clones coming from selected plus trees in Galicia. The orchard is divided in 10 randomized complete blocks. Each block contains one ramet of each of 68 clones. The spacing is 1.5 x 3m.

In 2000, 2001 and 2002, flower bud development was monitored in 25 randomly selected clones in the orchard. Between January and late March, the reproductive phenology of three male and three female marked branches on each tree was observed three times a week to determine the phenological stage of each strobili in each branch at a given date, until all pollen was released and seed cones were no longer receptive (Codesido *et al.*, 2005). The number of ramets per clone varied between 5 and 8. Observations of flowering were made on the same trees each year, but not always on the same branches. We distinguished four female stages and four male stages. The female stages were described by Matziris (1994) as follows: stage 1, the female bud is increasing in size, becomes cylindrical, but is still completely covered by the bud scales (0% female receptivity); stage 2, the apex of the enlarged cylindrical bud is opened and the first ovuliferous scales appear. At this stage the ovules are not receptive, but pollen grains may get inside the bud scales and if they survive they may to be able to take part in fertilization (20% female receptivity); stage 3, the scales of the female conelet are gradually separated and almost form right angles with the axis of the conelet. This is the stage of maximum receptivity (100%), and stage 4, the ovuliferous scales increase in size and thickness so that the strobili are no longer receptive (0% female receptivity).

The male stages were described as follows (Codesido and Merlo, 2001): stage 1, the round brown strobili are covered by the bud scales (0% pollen shedding); stage 2, the male strobili burst through the bud scales and elongate (0% pollen shedding); stage 3, the yellow strobili start shedding their pollen (100% pollen shedding) and stage 4, end of pollen shedding. The male strobili wither and fall down (0% pollen shedding). When there was more than one female flower per branch, we considered the beginning of female receptivity in all of the branch when some of the flowers reached the receptive stage. In the same way, receptivity was not considered to be concluded until all the female strobili of the branch had reached the end of the receptive stage (Askew and Blush, 1990).

Phenological scores must be assessed on each branch in each ramet on each day of the study. The data were processed using the SYNCHRO.SAS programme, introducing the percentage of female receptivity or the percentage of pollen shedding associated with each phenological score and the date of the start and the end of assessment to obtain: the predicted beginning, end and duration of female receptivity and pollen shedding for each clone and for each ramet; the overall synchronicity graph, which represented the overall mean female receptivity and mean pollen shedding in all clones over time (e.g. Matziris, 1994); the male and female phenograms presented as bands over time, which represented the relative female receptivity (or pollen shedding) of each clone at a given date (see e.g. Askew and Blush, 1990).

RESULTS

The variation in the flowering patterns from year to year can be observed in the graph of overall phenology synchronicity (Figure 1). Maximum female receptivity was earlier than the peak of pollen shedding by 7 days in 2000 and by 2 days in 2001. Both peaks coincided in 2002. Note that in 2000, 15% of receptive female strobili remained in the seed orchard after pollen shedding stopped, so that 15% of the strobili were not pollinated in this year. This did not occur in the following two years. In 2000 the receptive period extended from February 3rd to March 18th and the pollen shedding period from February 11th to March 9th. The maximum number of receptive strobili (65%) occurred on February 20th, one week before the maximum pollen release (98%). In 2001 the receptive period extended from January 15th to March 1st and from January 25th to February 25th for pollen shedding. On February 5th, 90% of all of the flowers monitored were receptive and only two days later, 55% of male strobili were shedding pollen. In 2002 the pattern was very similar to that observed in 2000, with the receptive period occurring between February 2nd and March 17th, but on February 13th the percentage of receptive strobili was 90%. For male strobili, the period of pollen shedding occurred between February 11th to March 17th with a maximum of 70% on February 25th. Pollen shedding always began some days after the commencement of seed-cone receptivity (8 days in 2000, 11 days in 2001 and 11 days in 2002).

The large variation among clones in the initiation, cessation and duration of female receptivity and pollen shedding was evident in the phenograms (Figure 2). The differences between the clones that were earliest and latest in starting the receptive period were between 8 and 19 days for the beginning of the female flowering. The same clone (clone 41) was earliest in starting the receptive period in all three years, and clone 09 was always the latest. On the right of each phenogram appeared the overage clonal overlap index, POij index, which is a quantitative measure of the proportional symmetry of the female and male phenograms, is the ratio of the common area to the maximum area between the female and male phenograms summed across all registered days and for each pair of clones. The variation in the index calculated for female flowering ranged between 0.13 and 0.54 in 2000, and for male flowering, the index ranged between 0.17 and 0.47. In 2001 PO values varied between 0.19 and 0.37 for female and from 0.21 to 0.41 for male. In 2002, they varied between 0.23 and 0.51 for female and between 0.05 and 0.58 for male.

In general, the clone 42 was the best overlapping male flowering clone and the best female one was the 18. In 2000 and 2001 every female clone was able to be pollinated by every male clones and every male clone was able to pollinate every female clones. Nevertheless, in 2002 five pair of mating clones were not possible. There was no pollen available in clone 38 when clones 11, 12, 20, 39 and 41 were receptive.

The distributions of the phenological overlap index (Figure 3) varied greatly between years with averages of 0.34 in 2000, 0.29 in 2001 and 0.42 in 2002. The synchronization of male and female phenology for each pair of mating clones varied between 0.01 and 0.74 in 2000; between 0.07 and 0.52 in 2001 and between 0 and 0.82 in 2002. In 2000, 43% of the pairs of clones had POij values that were higher than the seed orchard mean, in 2001 it was 45% and in 2002 it was 55%. In general, most clones were synchronized, the overlap indices generally being higher than 0.40.

CONCLUSIONS

1. Flowering phenology was characterized by a steady increase in the number of receptive trees and the absence of pollen donors at the beginning of the receptive period. After a few days of female receptive period in the seed orchard, there was a steady increase in pollen production with a peak in pollen shedding.
2. The percentage of strobili shedding pollen was greater than the percentage of female receptive strobili in 2000 but not in 2001 and in 2002, possibly because of the rainfall patterns. Conditions were drier in 2000 than in 2001 and 2002.
3. During the three years under study, we observed a gradual shift in flowering phenology, probably due to the juvenility of the seed orchard, with synchronization between female receptivity and pollen shedding periods improving with time.
4. Stabilization between all pair of clones appeared to be reached, each clone could pollinate each other at some moment of the pollen shedding process and could also be pollinated by any clone in some moment of its receptivity phase.

5. The indices varied greatly among any one male or female parent and also among years. The overall seed orchard PO index value was 0.34 in 2000, 0.29 in 2001 and 0.42 in 2002.
6. In 2002 the orchard reached desirable PO values, except for clone ID38 which was severally affected by *Rhyacyonya buoliana* the summer before and all its ramets appeared to be unhealthy. This was the only clone in 2002 that could not pollinate some of the other clones. If we eliminate this clone from the orchard we could obtain good flowering synchronization, with an orchard mean PO value about 0.6.
7. The best overlapping male flowering clones appeared to be those clones that started flowering earlier. Nevertheless, the highest overlap indices for female flowering were observed for intermediate flowering clones
8. The reproductive synchronization of the seed orchard was not perfect, but improved each year.
9. The PO values were useful for following the development of young seed orchards until reaching reproductive stability.
10. SYNCHRO.SAS was very useful to help us to calculate the overlap indices and to draw phenological graphs

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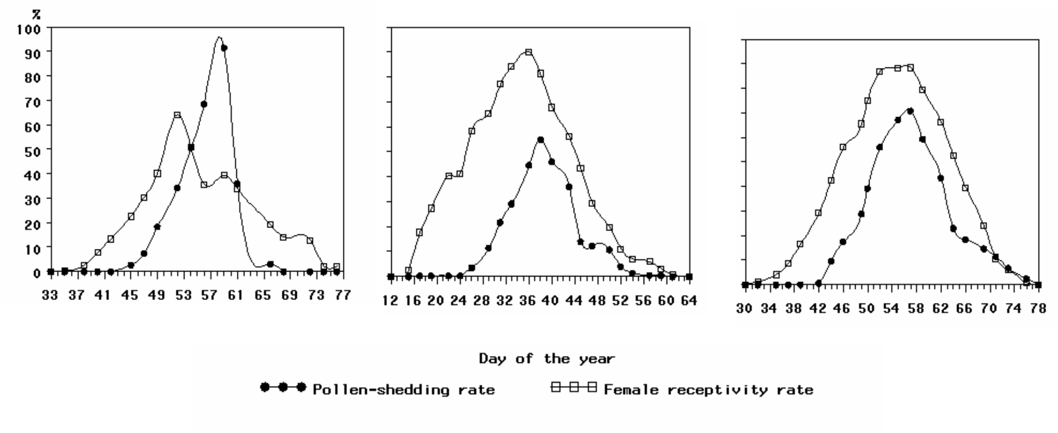
FIURES

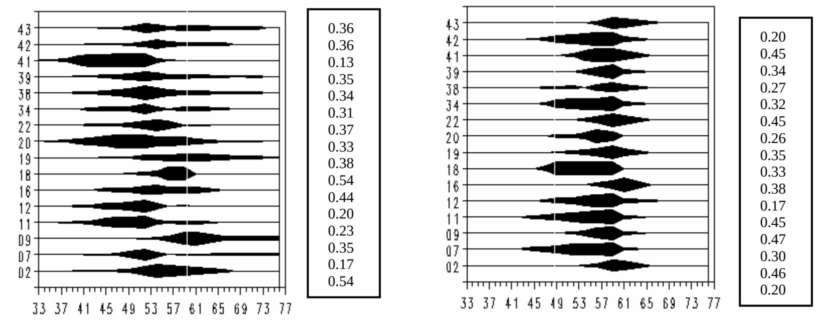
Figure 1. Graphs of overall phenological overlap. The percentage of receptive strobili and shedding pollen strobili are shown.



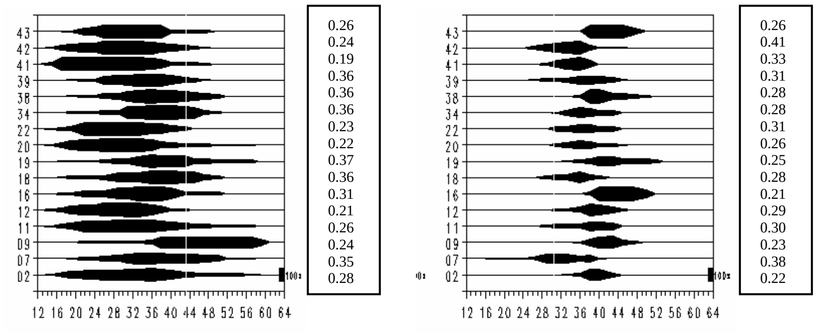
a)2000 b)2001 c)2002

Figure 2. Flowering phenograms. PO_{ij} mean clonal index values are shown in boxes on the right hand side.

a)2000. Female Male



b)2001



c)2002

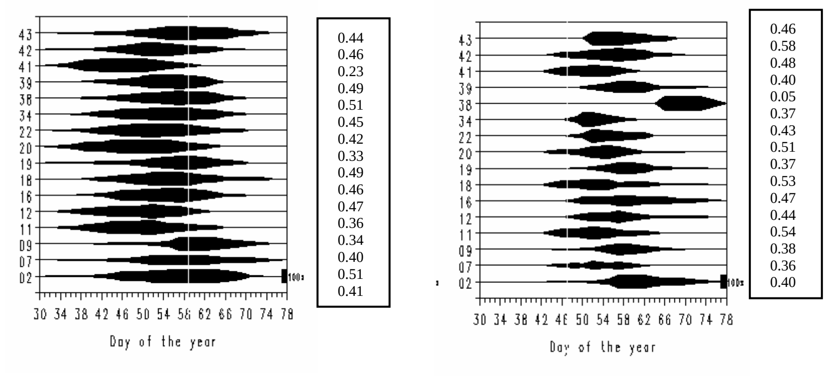
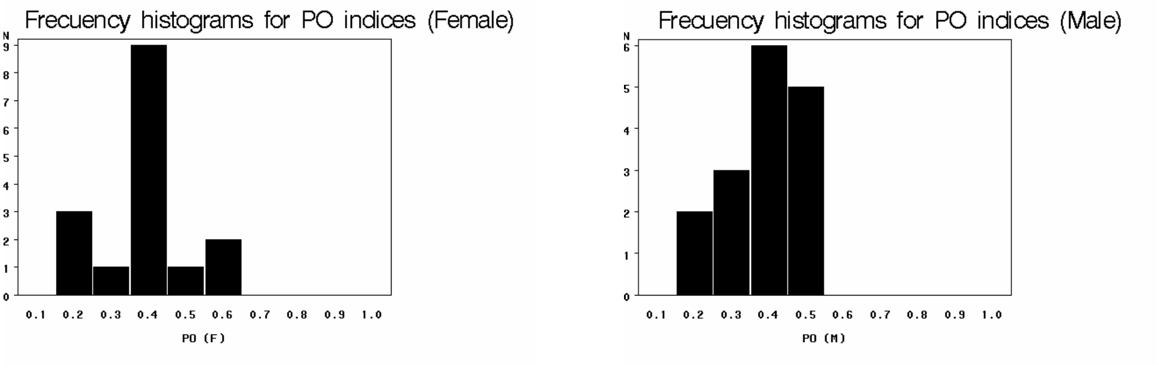
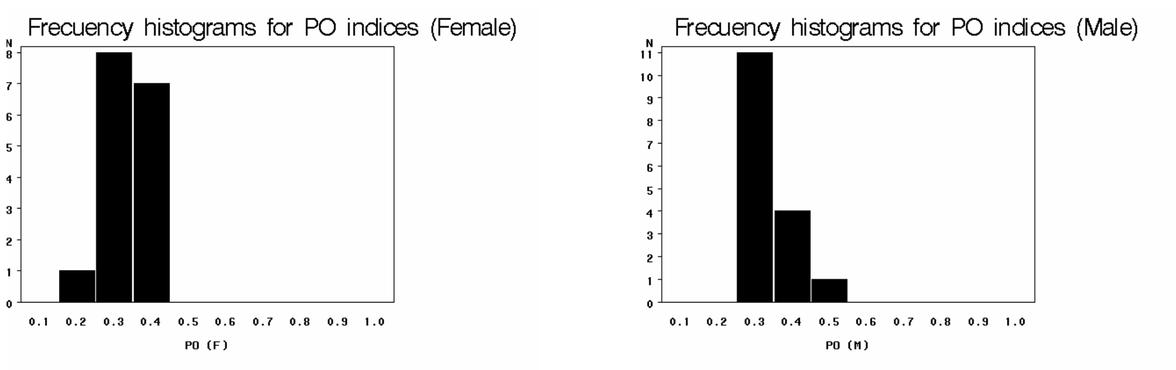


Figure 3. Frequency histograms for PO indices.

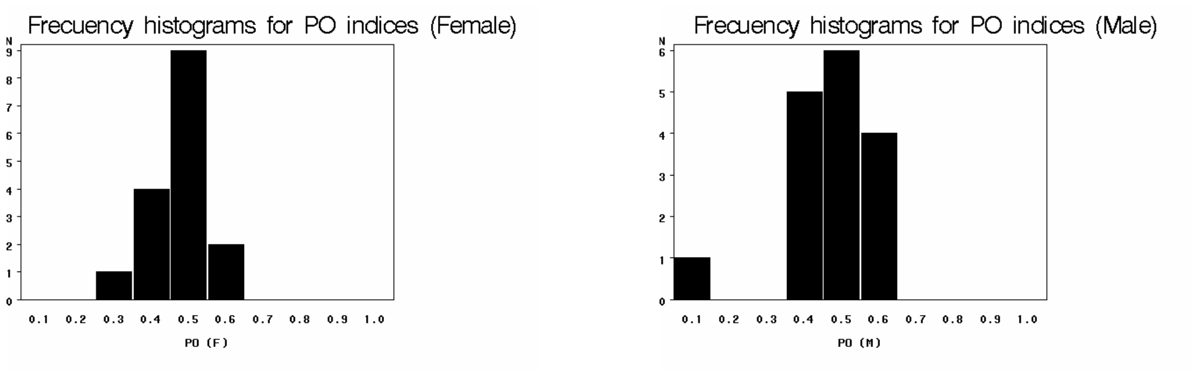
a)2000.



b)2001



c)2002



A New Generation of Clonal Seed Orchards of Wild Cherry. Selection of Clones and Spatial Design

Bart De Cuyper

Research Institute for Nature and Forest, Gaverstraat 4, 9500 Geraardsbergen, Belgium
E- mail bart.decuyper@inbo.be

Abstract

Forest policy in Flanders (Belgium) strongly promotes the use of indigenous hardwoods, among which wild cherry (*Prunus avium* L.), for re- and afforestation and for stand conversion,. This line of policy generates a strong demand for high quality forest reproductive material, which cannot be met by the currently available basic material. The selection and breeding programme attempts to remedy the discrepancy between supply and demand by creation of a new generation of clonal seed orchards characterised by (i) a high yield and (ii) a high genetic quality and diversity of the offspring. This goal was achieved by selection of 52 genotypes based on half-sib progeny trials. Yield and genetic quality of the offspring was enhanced to a further extend by adjusting the spatial design of the seed orchard to the phenologically and gametophyticaly cross-compatibility of the selected genotypes. Background pollination is reduced to a minimum by establishment of the seed orchard at a minimum distance of 400 m from other wild cherry populations and sweet cherry plantations

Introduction

The use of wild cherry (*Prunus avium* L.) for re- and afforestation is motivated by the acknowledgement of its high silvicultural, ecological and economical importance. Wild cherry is a tolerant tree species allowing the establishment of mixed forest stands and is a relatively fast growing hardwood producing very high quality timber. Furthermore, the species is often mentioned as a potential alternative to poplar for the afforestation of abandoned and set-aside farmland. Through the implementation of the EU Council Directive 1999/105/EC in the Flemish Forest Decree, the certification of forest reproductive material of wild cherry became compulsory and, thus, the genetic quality of the reproductive material had to be warranted. The present annual demand for reproductive material, amounting to 30 kg of pure seed on average, cannot be met by the available indigenous basic material. Firstly, reproductive material of the category "source-identified" is not allowed for forestry purposes, i.e. wood production. On the other hand, only two Flemish seed stands and one seed orchard are presently recorded in the Belgian Catalogue of Forest Basic Material.

The area of the seed stands is very restricted, *viz* "Vrebos" (0.28 ha) and "Rattenberg" (0.37 ha). Consequently, the yield is very low, being about 10 kg of pure seeds in good crop years. Moreover, the harvest is not cost-effective as the cost price is about four times the selling price. Observation of the fruit morphology of the cherries harvested in "Vrebos" strongly indicates an introgression from sweet cherry cultivars. Moreover, this seed stand is characterised by a predominant regeneration through root suckering resulting in a marked clonal structure: the 402 individuals only represent 64 different genotypes with a fairly low genetic distance (~ 0.23). Therefore, the seed stand "Vrebos" is likely to be removed from the above-mentioned catalogue. Finally, the potential for the selection of additional seed stands is limited, due to the occurrence of wild cherry as individual trees or small clusters scattered throughout mixed forest stands.

The sole seed orchard "Mommedeel", established in 1988 and covering 0.82 ha, suffers from recent severe dieback of the constituents due to the unsuitable site conditions and to the abortion of the grafts.

The selection and breeding programme attempts to remedy the discrepancy between supply and demand by creation of a new generation of clonal seed orchards characterised by (i) a high yield and (ii) a high genetic quality and diversity of the offspring. Additionally, seed orchards offer the possibility for intensive management and the advantage of less labour-intensive harvest.

The adopted research strategy pursues a fourfold aim:

i. Assessment of the genetic diversity of the basic collection with a view to detecting identical or closely related accessions.
ii. Identification of the very best clones within the basic collection by assessment of adaptive traits in half-sib progeny trials
iii. Designing the layout of the seed orchard, i.e. determination of the optimal spatial arrangement of the clones within the orchard.
iv. Establishment of minimum isolation standards with regard to surrounding natural populations of wild cherry and cultivated sweet cherries.

Material and methods

From the early 80's till the late 90's, 168 phenotypically superior plus trees of wild cherry were selected in 27 wild cherry populations covering its entire distribution area in Belgium. Vegetative copies of these plus trees, obtained by grafting or budding, were planted in seven experimental comparative plots.

The genetic diversity of this basic collection was assessed by six AFLP markers and 11 highly informative microsatellite markers with DP values ranging from 0.76 to 0.95 (Clark *et al.* 2003, Downey *et al.* 2000, Vaughan *et al.* 2004). Estimation of the genetic distance D between individuals was based on the proportion of shared alleles: $D = 1 - P$ with $P = \Sigma_u S/2u$ where the number of shared alleles S is summed over all loci u.

In 1995, seeds were harvested in seven multiclonal plantations established with vegetative replica of the selected plus trees. This resulted in the establishment of 13 half-sib progeny trials covering a total surface of 15.3 ha. Adaptive traits such as vigour (height growth), morphology (stem straightness, branching habit, apical dominance), phenology (flushing, bud set, St. Johns sprouts) and disease resistance were assessed. Wild cherry is subject to two major diseases, viz anthracnosis, a fungal leaf disease caused by *Blumeriella jaapii* and bacterial canker caused by *Pseudomonas syringae* with three distinct pathovars, namely pv *syringae*, pv *morsprunorum* and pv *avii*. For each trait, the narrow sense heritability h^2_A as well as the general combining ability GCA was determined, allowing the construction of a selection index: $I = \Sigma_i (h^2_A.GCA)_i$ with $i = 1$ to n and n = number of traits.

Wild cherry is entomophilous, with bumble bees (*Bombus* spp.) acting as main pollen vectors. Bumble bees display a particular foraging behaviour, which leads one to suspect a small-scaled patch-like pollination pattern within wild cherry populations (Goulson *et al.* 1998, Heinrich 1976).

As a test-case, one of the above-mentioned multiclonal plantations was selected, consisting of 65 accessions and surrounded by eight 'natural' wild cherry populations situated at various distances. In order to unravel the pollen flow, i.e. assessment of internal mating patterns as well as pollen input from outside the plantation, a parenthood analysis was carried out using

11 microsatellite markers and the self-incompatibility genotype (DP = 0.98). The objective was to trace the father tree of 60 half-sibs randomly chosen within the offspring of 16 selected accessions, using the exclusion method. When determining the mating pattern, the allogamous nature of wild cherry was taken into account. This trait is governed by a single multi-allelic *S*-locus with gametophytic action which controls self-incompatibility (SI) and cross-incompatibility between individuals with the same SI-genotype. The SI-genotype is determined by concensus and allele-specific PCR (Sonneveld et al. 2001, Sonneveld *et al.* 2003).

Results and conclusions

Analysis of AFLP and microsatellite markers revealed four groups of identical genotypes. As wild cherry often regenerates through root suckering, plus trees selected within the same population are likely to be identical. Consequently, the basic collection was reduced to 152 genetically different accessions with a genetic distance ranging from 0.46 to 0.92.

The selection index I was determined for all accessions. No significant differences of the I-values were found between the 13 progeny trials. A total of 52 genotypes was finally selected, showing an I-value higher than the median value and a genetic distance varying between 0.72 and 0.89.

Parenthood analysis of the 960 half-sibs showed that 21 % of the sires was located outside the plantation. Tracing of external pollen donors revealed that 80 % of the pollen input came from sires situated within a wild cherry population located at a distance of 400 m from the plantation studied.

Assessment of the internal pollen flux revealed that 75 % of the sires was located within a distance of 10 m from the mother tree, i.e. twice the planting distance (Fig. 1). Paternal contribution was influenced by flowering period and *S*-(in)compatibility but not by flowering abundancy. Finally, the parent exclusion probability exceeded 0.99.

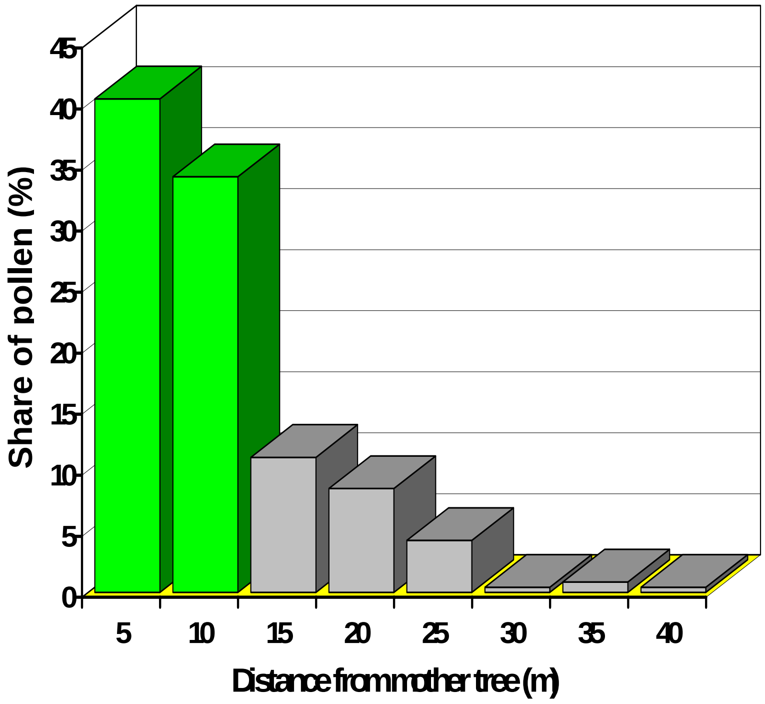


Figure 1. Relation between pollen contribution of sires and their distance from the mother tree in a multiclonal plantation of wild cherry.

As the paternity analysis revealed a small-scaled patch-like pollination pattern, neighbouring trees in the new generation seed orchards should be phenologically compatible, i.e. display an overlapping flowering period as well as gametophytically compatible, i.e. have a different SI-genotype.

Flowering phenology of the 52 selected genotypes was observed in four plantations in 2002 and 2004 by recording the share of fully opened flowers every 48 hours during the whole flowering period. When assessing the overlap between the flowering period of two accessions, it has to be taken into account (i) that fully opened flowers are only receptive for 38 hours and (ii) that the pollen in the pollen bags of bumble bees are viable during 12 hours. The flowering phenology of an accession was not influenced by its location within the populations. No significant differences were found between sites and the observations of 2002 and 2004 appeared to be concordant.

Observation of the flowering phenology resulted in a cross-tabulation of the overlap in flowering period for all possible combinations between the 52 selected accessions. When adopting a tresshold value of 25 % for the overlap in flowering period, 66 % of all combinations appeared to be phenotypically cross-compatible.

The self-incompatibility genotype of the 52 accessions was determined and confirmed by carrying out controlled crosses. In addition, six 'new' *S*-alleles, previously unknown in sweet cherry cultivars, were detected and numbered S_{17} to S_{22} (De Cuyper *et al.* 2005). Few of the genotypes (11 %) appeared to be cross-incompatible.

In view of the establishment of the seed orchard, selected genotypes were propagated vegetatively by grafting on the dwarfing rootstock “Gisela 5”. Furthermore, traditional pomological techniques are applied to increase fruit production, such as planting in espalier, use of bird nets and placing of several bumble bee nests. Early and abundant flowering is induced by adequate pruning, tearing and bending of branches, weed control and root pruning.

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PROSAD a tool for projecting and managing data about seed orchards

Vladimír Foff
LIA – Forestry Information Agency
ČSA 396, SK - 033 01 Liptovský Hrádok, Slovakia
foff@mag-net.sk

Elena Foffová
National Forest Centre, Centre of the Control of Forest Reproductive Material

Abstract

PROSAD is a database programme developed for projecting seed orchards and respective data management. It is also a useful tool for keeping data about clone archives, stool-beds and various types of field trials, including provenance plots. The programme provides powerful database functions for processing data about these objects and also various possibilities to export and import data into and from other data formats.

Introduction

Programme PROSAD allows managing and processing data on seed orchards and similar objects. Present product was built by combining previous system for seed orchards projecting and data keeping programme for seed orchards and plus trees, which were developed in 1992 - 1993 and updated in 1996. The programme is based on a relational database model, which is able to use free tables and provides a rich data management outfit for creating and managing tables in a relational database (RDBMS), generation of aggregated tables with help of the SQL language or creating output reports. It is developed in Microsoft Visual FoxPro 9.0 for desktop computers as a client – server application with supported web publishing.

Description of the programme

System requirements

The PROSAD programme has no special hardware demands. It can be installed on a computer with Intel or AMD processor under operating system MS Windows 2000 or later. CD or DVD drive and 15 MB free space on hard disk (without user data) are needed for installation. Satisfactory displaying unit is a display with minimum resolution 800 × 600 and minimum 256 palette colour is satisfactory to show the data.

Data management

A user communicates with the programme through working and dialogue windows by using built-in operating elements as buttons, options, switches, lists and pull down menus. Each working window manages a relational database (linked tables). There are several working windows in the system, which are used form managing data about administrative location, ownership etc.

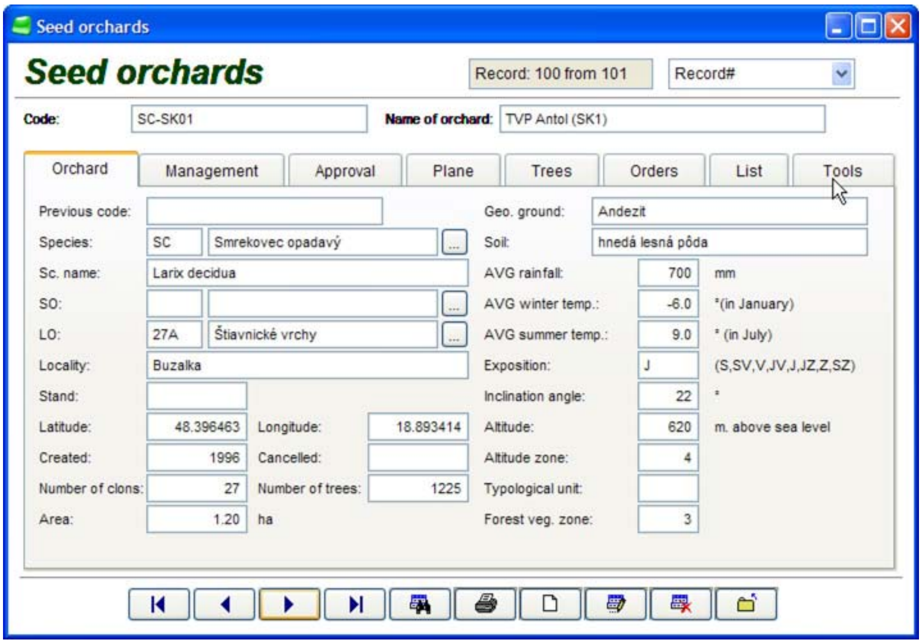
Seed orchards database

This working window (Fig. 1) is used for work with the database containing linked tables of the seed orchard, plus trees (parents) used for establishing the orchard and measures realised in the seed orchard. Data are split by topics and arranged on separate pages of the working window.

In the table of the seed orchard there are kept basic data, data about tree species, data about environmental conditions, geographical and administrative location and ownership of the orchard:

- code of the basic material, name of the seed orchard, number of clones, area, year of establishing and cancelling, name of the designer, nursery
- name, botanical name, number and name of the provenance region
- number and name of the forest region, altitude, altitudinal zone, forest vegetation zone, forest management type, average precipitation, average summer and winter temperatures, slope angle and exposition, ground

- locality, forest user unit, forest management unit, owner, user (manager), district, county, competent forestry office



Record: 100 from 101
Record#
Code: SC-SK01
Name of orchard: TVP Antol (SK1)

Previous code:
Species: SC Smrekovec opadavý
Sc. name: Larix decidua
SO:
LO: 27A Štiavnické vrchy
Locality: Buzalka
Stand:
Latitude: 48.396463
Longitude: 18.893414
Created: 1996
Cancelled:
Number of clons: 27
Number of trees: 1225
Area: 1.20 ha
Geo. ground: Andezit
Soil: hnedá lesná pôda
AVG rainfall: 700 mm
AVG winter temp.: -6.0 °(in January)
AVG summer temp.: 9.0 ° (in July)
Exposition: J (S,SV,V,JV,J,JZ,Z,SZ)
Inclination angle: 22 °
Altitude: 620 m. above sea level
Altitude zone: 4
Typological unit:
Forest veg. zone: 3

Fig. 1 Main working window of seed orchards database.

The table of parent's trees (plus tress, clones etc.) is linked to the seed orchard table by the code of the basic material (primary key). There are as well as in the table of seed orchard presented basic data, data about tree species, environmental characteristics, geographical and administrative location and ownership of the each registered parent tree completed by its, quantitative and qualitative description:

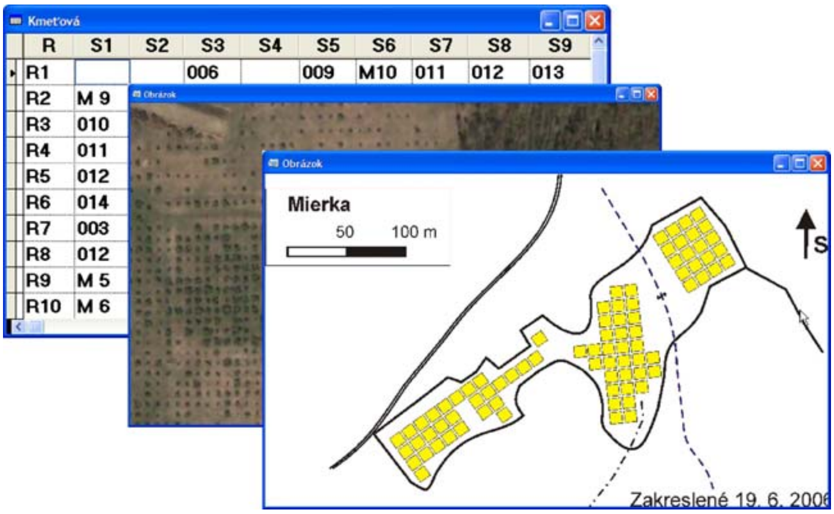
- age, diameter ($d_{1,3}$) and height of the tree,
- straightness, convergence and cleaning of the stem, width, length, density, shape and symmetry of the crown, position and thickness of the branches etc.

All interventions and actions in the orchard are registered in next linked table. Here are kept chronological records related directly with management of the seed orchard plot and with the administrative and organizational issues:

- date of the entry, detailed description of activity, flowering, amount of collected cones or seeds and costs connected with the particular activity.
- required actions, authority, responsible person.

It is possible to link additional information to the basic database, either in form of free tables or graphical information in vector or raster formats (Fig. 2). Dates are not saved directly into main database. Programme PROSAD registers only links to the files, where the data are placed. This helps to prevent overloading of the programme and simple updating of these linked data. To the database are linked:

- Plot of seed orchard, which is a table with maximal number of columns and rows, where is specified the position of each tree. The plot is product of the projecting part of the programme and has specified structure. It is possible to register archive seed orchard inventories from past years in several tables, as well as evaluations of the plot expressed by numerical or character values. In the system only this table is displayed, which is currently linked (it can be easily changed).
- Current inventory list created from the seed orchard plot with names (labels) of particular clones with concrete numbers of trees situated on the plot. For detailed statistical processing is possible to create a specific list with identification of each tree position (column, row and cell on the plot) in projecting part of the PROSAD programme.
- System can complete the documentation by displaying graphical data in vector formats WMF and EMF (charts of the plot) for projective scheme and other plans with identification point, line and polygon requirements. For shows of satellite, aerial and terrestrial pictures is possible use raster formats BMP, DIB, JPG, JPEG, JPE, JFIF, GIF, TIF, EXIF and PNG. In working window miniature of graphical data are showed with option of their enlargement (dependent on size and resolution of screen).



R	S1	S2	S3	S4	S5	S6	S7	S8	S9
R1			006		009	M10	011	012	013
R2	M 9								
R3	010								
R4	011								
R5	012								
R6	014								
R7	003								
R8	012								
R9	M 5								
R10	M 6								

Fig. 2 Different types of information in windows showed from additional files.

Tools for data tables and files management

In the system are included tools for processing of information on one record level, as well as for multiple processing data either in one table, or several (virtual linked) tables. Tools for processing one record are available directly in the working window. They allow:

- to display current position in the table,
- to move in the table (from the first to the last record) with immediate displaying data,
- to move in the table with displaying data, which fulfils specified criteria
- to print data into defined reports
- to remove records in two steps (to improve safety of this operation) with a possibility to back-up removed data into an external file,
- to sort data according to defined simple or multiple ordering criteria (sorting indices),
- to complete automatically dependent data during input and editing, which eliminates writing mistakes,

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- to move in the table (from the first to the last record) with immediate displaying data,
- to move in the table with displaying data, which fulfils specified criteria
- to print data into defined reports
- to remove records in two steps (to improve safety of this operation) with a possibility to back-up removed data into an external file,
- to sort data according to defined simple or multiple ordering criteria (sorting indices),
- to complete automatically dependent data during input and editing, which eliminates writing mistakes,

- to move a group of data from free tables with help of dialogue windows (e. g. information about tree species, owners or managers, administrative localisation of the plot),
- auto completing of expressions, when often repeated information is inserted

Multiple data processing tools are available through plug-in modules mediated by dialogue windows. These modules can be used universally in all working Windows. An advantage is automatic justification of the environment (opened databases and tables, defined sorting indices, setting of the system variables etc.). In a such case a programme user do not need to justify anything. Some of the various tools for multiple data processing used in the PROSAD programme are described below.

Browsing records in the tables and records, which are indicated to be deleted

This module is used for browsing data in the tables. It is accessible from the main menu and also from each working window. When called from the working window the environment will be automatically set up (opened database or free tables, their structure, sorting and system variables). Before browsing tables, the user is allowed:

- to select records displayed by defining selection criteria for each column in the table with help of operators for comparing characters or numbers. User can combine conditions by conjunction or joining to a maximum extent of 50 simple conditions or 3,600 characters.
- to select displayed columns and their ordering. He can also justify width of the displayed column and change its position in the table.
- to create more sophisticated filters for data, valid until they are cancelled by user or by ending the session,
- to set data sorting in the browsed table before or after displaying the table,
- to see the structure of the current used tables, number of records, number of columns, sorting index, size of the file and date of the last updating,
- to justify the vertical and horizontal size of the displayed table and the size of fonts. It is possible to display several tables. If a relation between tables was set up before, the displayed data will follow the rules for the relation.

Exporting and importing records

Export and import are joined in one plug-in module and allow the user to communicate with other programmes and create data in other data formats. The environment is set up automatically as well as in the previous module. Main functions and features:

- Export and import are supported in several standard formats dBase, FoxPlus, FoxPro, VisiCalc, Data Interchange Format, MS Multiplan, System Data Format, Symbolic Link Format, Lotus 1-2-3, Lotus Symphony, CSV, MS Excel, ASCII text, Framework a Paradox). All used formats are well documented.

- Possibility to set up several separators to isolate columns, when the data are exported into text files.
- Filter is available for selection and sorting exported records.
- The code page, in which the source data were created, can be set up for import.
- It is possible to export a table ordered by user defined sorting.

Creating tables by SQL language

The module for using SQL language is able to work with all opened databases and free tables. The environment is automatically set up according to the working window, from which the module was called. The tool allows creating various temporal tables, which can be subsequently exported in the above mentioned data formats. Main features:

- Optional column from the open databases or free tables can be chosen for the output. The columns are clear marked and ordered by their belonging to the tables. It is also possible to compute the aggregated data values (such as average, number, minimum, maximum and sum).
- Tables can be linked together by compatible columns. At the some time it is possible to define other conditions to create views for used columns or also for columns included in the source tables.
- It is possible to create associated views (data joined from two or several tables) or aggregated views (grouped by defined column eventually also by using mathematical functions).

Printing data

There are several tools available for output data on printer. Data are always printed into defined output reports, which can be prepared for simple tables or for tables linked by relations. It is possible to print single or summary values (average, sum, numbers etc.). Several sections with specific rules for printing data can be defined in an output report. The saved outputs reports can be modified later. Programme user can directly apply following tools:

- Module for direct usage of the output reports. The user can choose a report and printer or to set up parameters for the printing. Single pages can be displayed on screen before printing them. This module can be called from the each working window.

- Module for guided creating output reports. The system has three “wizards” for creating reports: first one for simple outputs, the second for reports from tables linked by relations and the third one for printing labels.
- There is also a special module to output data in HTML format for publishing on web sites.

Functions for experts

For processing big databases sometimes the functions are needed, which are not a standard part of the database programmes. On account of this a module for experts was included into the PROSAD programme. With help of the module an advanced user can manually open and close the databases and tables, change their structure, change the system settings and by single commands carry out many other changes.

Other useful tools are using simple programmes (scripts), where the user can subsequently write often used commands or creating composite conditions for filtering or getting a view on data by the SQL language. Users can exchange these conditions and so provide for uniform data processing, if they work on the same projects or in the same databases.

Projecting seed orchards

This part was developed for projecting of seed orchards (stool-beds, clone archives and various field trials, including provenance plots). The procedure works with regular distribution of the single trees on the plot in a rectangular spacing. The projecting tool can be started from the part of the working window with tools in the particular dialogue window.

Projecting is based on the defined plot and the list of the clones, available for stocking the plot. Main features of the projecting:

- There can be used an equal or unequal number of trees of the particular clones for stocking the plot. The programme PROSAD will distribute them in a well balanced way on the whole plot.
- Maximum number of the columns on the plot is 254, number of the rows is not limited.
- It is possible to choose number of the zones (one to three), in which the number of the current distributed clone will not occur (Fig. 3). Of course, with the growing number of such zones, there is also higher need to use more equal representation of the clones.

- In projecting process up to two schematic thinnings can be planned. In several steps the programme distributes the clones in such a way, which allows keeping on the plot after each thinning the same ratio of the clones as there was at the beginning. This procedure will prevent the dropping out any of the clones during schematic thinning.
- PROSAD can stock also irregular plots with eventual free area on it. It is assured by the generator for stochastic selection of the clones, by rules for uniform distribution of the clones on the plot, by definition of the zone without repetition of the clone and by number of the thinnings.
- In the projection process various distribution on the plots can be created and apply the most suitable of them.
- During the updating of the data, historical inventories of the plot and of the clone list can be kept.

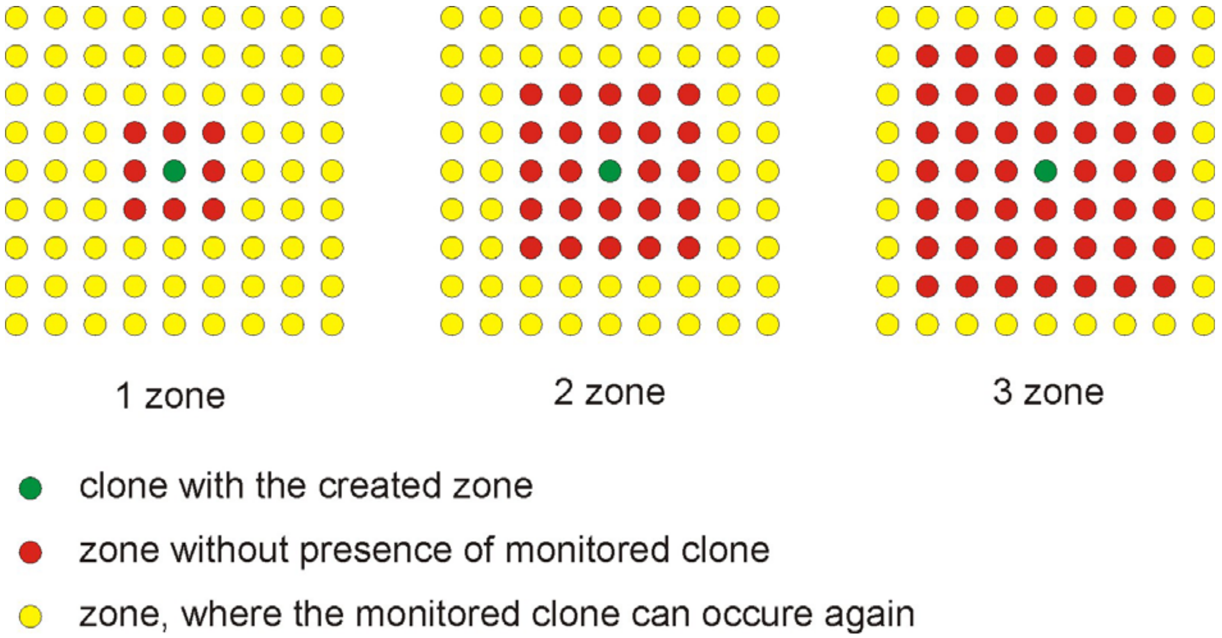

Fig. 3 Creating of isolation zones.

Processing data on the plots and processing the lists

This functions packet was included into PROSAD programme with the aim to have tools for processing data from the field trials and provenance plots. Basis for creating, updating and processing data is projected and stocked plot of the orchard or field trial. Assuming, that the plot will be evaluated in the future and the data will be processed in statistical programmes

PROSAD allows to keep updated copies of the plot table with the inserted character or numerical data. Advantage of such a way of data keeping is exact identification and positioning of each single tree on the plot and possibility to compare changes of its traits (expressed by numerical or character data types) in the subsequent time periods. Following functions support the data processing on the plots:

- Creating copy of the plot table prepared for inserting values in character or numerical form. Extent of the character values can reach from one to ten characters, numerical values can be inserted in interval of one integer and six decimal places to ten integers.
- Conversion of the evaluation table with character or numerical data to a list. In the list is specified automatically the position of the each particular value (cell, column, row) and the matching value from the evaluation table. It is possible to add one to five further columns with numerical or character format to the list, which allows detailed description of the plot (e.g. year or season of the evaluation, altitude, exposition, rainfall or other values useful for statistical evaluation).
- Converted lists can be effectively joined to the files suitable for simple or extensive statistical evaluation. The lists can be joined in horizontal direction (e.g. for correlation and regression analysis) or in the vertical direction (analysis of variance or other data comparisons). Joined table can be exported in various formats with help of tools described in the data management part.

Conclusions

Amount of data regarding basic material of forest reproductive material grows constantly. There is also a need to process such data to analyse amounts and quality of production in the changed ecological conditions.

The programme PROSAD is a part of database system for keeping data about basic material, evaluation of the seed quality, plants grown in the nurseries and about damaging agents in forests and forest nurseries.

This programme allows easy and effective projecting seed orchards and other similar plots.

It also serves powerful tools for keeping and processing relevant data about these objects and evaluating their growth, production, quality and other parameters.

Architecture of the data allows to export the kept or processed data to the other computer systems and also to adapt data from older programmes.

The Swedish Scots Pine Seed Orchard Västerhus

A study of linear deployment

Anders Fries[1,2], Dag Lindgren[2] and Bengt Andersson[3]

[1] anders.fries@genfys.slu.se

[2] Department of Forest Genetics and Plant Physiology
Swedish University of Agricultural Science, Umeå, SWEDEN

[3] Skogforsk Box 3, SE-918 21 Sävar, SWEDEN

Abstract

The Scots pine seed orchard Västerhus is a seed orchard in the 2nd round of orchards in Sweden, but since the clones were selected after progeny testing, it is genetically comparable to the 3rd round of seed orchards. It contains 28 clones which were selected from clones used in six plus tree seed orchards and used in different numbers. The clonal selections and deployed clonal proportions were worked out by Dag Lindgren and Bengt Andersson in an attempt to apply advanced designs in an operational orchard. The gain should then be increased while constrains would be kept on the genetic variation, a strategy later named linear deployment. In this deployment, the strategy was that the clones with the highest breeding values should be represented in higher numbers. In addition, flowering capacity was considered to predict gene contribution from each clone for the clonal deployment. The seed orchard was established 1991. In the summer 2007 16.1% of the grafts were dead and for 0.9% of the grafts, the root stock had replaced the grafted clone. The gain in breeding value by the linear deployment was initially estimated to 5.7-6.2% as compared to a comparable hypothetical seed orchard with equal clonal representation. The estimated gain by linear deployment at establishment did not change much due to loss of grafts. It is assumed that in this young seed orchard pollen contamination from the surrounding stands may have a larger and reducing effect on the genetic gain than loss of grafts and thus changed clone proportions.

Introduction

In the beginning of the Swedish forest tree breeding much progeny-testing was done by controlled crosses in seed orchards, and therefore the clones in the different seed orchards were tested in different sets. Utilizing selections from such progeny-tested clones makes Västerhus to the most advanced seed orchard of this age in Sweden. The areas where Västerhus according to Planter's guide (Hannerz and Ericsson 2007) seem to be among the best available seed sources, and more than around 12% better than optimally transferred stand seeds, are given in Table 1.

For Västerhus, six sets of clones corresponding to six seed orchards constituted the start material. The seed orchards and clone numbers are given in Table 2.

Table 1. Suitable areas for Västerhus seed orchard crop

Latitude (^{o}N)	Altitude (m.a.s.l.)
61	300-800
62	50-500
63	0-300
64	0-100

Table 2. Seed orchards from which the clones were selected

Seed orchard no.	Seed orchard	Tested clones	Available clones	Selected clones
412	Domsjöänget	52	42	7
410	Robertsfors	12	10	2
18	Brån	34	27	4
403	Nedansjö	40	32	5
411	Domsjöänget	50	40	7
426	Holm	20	16	3
	Total	208	167	28

Clonal deployment

An additional genetic strength and novelty at the time of establishment of the seed orchard is that the number of grafts of the different clones was deployed on the basis of the genetic capacity of the clones, i.e. their breeding values. Then, the genetically best clones were used in higher proportions. The clonal selections and deployed clonal proportions were worked out by Dag Lindgren and Bengt Andersson in an attempt to apply advanced techniques in an operational orchard, to increase gain while keeping constrains on genetic variation. This technique was suggested by Lindgren & Matheson

(1986), and was later named linear deployment. In addition to the breeding value has the flowering capacity been considered, and adjustments has also been made to reduce the risk for inbreeding. Although established in the 2nd round of seed orchards, having clones selected after progeny testing and furthermore deployed with the genetically best clones in higher proportions, Västerhus should be genetically better than the other 2nd round seed orchards, actually one of the most advanced seed orchard of this age in Sweden.

When determining the clonal proportions, it was assumed that the average breeding value in the six clone sets (one set per seed orchard) was equal. To reach this, the assigned breeding value for each clone was the expected value from a normal distribution for the rank of the clone within its set. In a set with many clones, the top ranked clone thus get a higher assigned breeding value than the top ranked from a set with few clones. The aim of this study is to analyze whether the genetic improvement that was obtained by the linear deployment remains after 16 years, or if losses of grafts had reduced the advantage. Another aim is to make a documentation of the status of a seed orchard under development. This is the initial stage of a project which molecular tools will study the pollination pattern and contamination in the seed orchard.

Material and methods

The study was performed in the seed orchard Västerhus (Tab. 3). During June and August 2007, survival in the seed orchard was registered. Occurrence of cases where the root stock had replaced the graft was also registered. Three data sets of number of clones and clone frequencies were obtained: *i)* the intended number and frequencies of grafts per clone when designing the seed orchard, *ii)* the actual number and frequencies at planting, and *iii)* the number and frequencies in August 2007. The second data set differ from the first due to non-successful grafting, availability of scions etc, and the third from the second due to death of grafts or root stocks replacing the graft. By comparing the clonal proportions and assigned breeding values, the change in breeding value of the seed orchard can be estimated.

Results

Table 4 shows for each clone the number and proportion of planted grafts, dead grafts and root stocks replacing the grafts. It also shows the proportion of grafts for a hypothetical seed orchard without linear deployment. This comparison is made at the approximate same effective population size, and this implies 20 clones in the traditional orchard. In the linear deployment orchard, 17.0% of the grafts were lost between planting 1991 and August 2007. Either the graft had died (16.1%) or the root stock had replaced the graft (0.9%). Breeding values in Table 5 are expressed as deviation from average of all 167 clones mentioned in Table 2 in units of the standard deviation of the breeding value among the clones available for selection (thus as a fraction of the root of the additive variance). Table 5 shows also the breeding value for the hypothetical seed orchard at the same effective population size. The contribution of the different clones in terms of their breeding values is illustrated in Fig. 1. In spite of the losses, the relative breeding value remains similar (1,600 and 1,657, respectively). The results indicate that that the loss of grafts has negligible influence on the genetic quality of the seed orchard.

Table 3. Facts about Västerhus

ID no: S23FP100T10, T10A

Location: 15 km NV from Örnsköldsvik, lat. 63°18’N, long. 18°32’E, alt. 5-25 m.a.s.l.

Establishment year: 1991

Number of clones: 28

Number of planted grafts: 4594 (plus 44 grafts of an unidentified clonal mixture).

Included clones: From six seed orchards in north central Sweden (Tab. 2). The clones were deployed mainly according to linear deployment, i.e. the clones with highest ranking in highest number.

Design: Spacing is 7 m between columns and 2.5 m between rows. Many plots are only partly filled with grafts.

In the comparison between the linear deployment in Västerhus and the assumed traditional design in a hypothetical seed orchard that undergoes the same development (mortality etc.), the linear deployment shows its advantage. The breeding value of the linear deployment seed orchard is 5.7% higher than an assumed traditional seed orchard, when comparing breeding values after adjustment for graft mortality and at the same effective population size (Ne=19.7) (Tab. 5).

Table 4. Numbers and proportions of the clones, intended according to linear deployment, at planting, in August 2007 and for an assumed seed orchard with equal number of clones per graft. Assigned breeding values based on the normal distribution (see text).

Clone	Assigned breeding value based on ranking (std dev)	Planted grafts (no.)	Dead grafts (no.)	Root stock replacing the graft (no.)	Number of grafts in August 2007	Clonal proportions (%)			Clonal proportion in an balanced seed orchard seed	
						Intention	At planting	August 2007	20 clones	After adjust- ments (see text)
AC1006	2,180	352	82	16	254	7,8	7,66	6,66	5.00	4,27
Y2005	2,161	370	60	0	310	7,7	8,05	8,13	5.00	5,28
Y4016	2,070	321	43	2	276	8,1	6,99	7,24	5.00	4,49
AC3056	1,998	408	69	1	338	7,9	8,88	8,87	5.00	5,64
Y4507	1,776	241	26	0	215	6,1	5,25	5,64	5.00	4,62
Z3007	1,766	242	19	2	221	5,4	5,27	5,80	5.00	5,36
Y3012	1,753	286	56	0	230	5,3	6,23	6,04	5.00	5,69
Z2081	1,647	240	28	2	210	4,7	5,22	5,51	5.00	5,86
Y3001	1,563	203	38	0	165	4,2	4,42	4,33	5.00	5,15
AC2064	1,542	207	36	1	170	4,1	4,51	4,46	5.00	5,43
Y3014	1,539	218	27	1	190	4,1	4,75	4,99	5.00	6,07
Y2004	1,517	96	32	1	63	4,0	2,09	1,65	5.00	2,06
Z4003	1,400	130	22	1	107	3,3	2,83	2,81	5.00	4,25
Z3009	1,370	107	16	2	89	3,1	2,33	2,34	5.00	3,76
Z4022	1,344	143	21	0	122	3,0	3,11	3,20	5.00	5,33
AC1075	1,306	91	27	3	61	2,7	1,98	1,60	5.00	2,96
Y4508	1,285	122	13	2	107	2,6	2,66	2,81	5.00	5,39
AC3040	1,232	143	16	0	127	2,3	3,11	3,33	5.00	7,24
X4203	1,217	79	12	0	67	2,2	1,72	1,76	5.00	3,99
Z3029	1,203	108	15	2	91	2,2	2,35	2,39	5.00	5,42
AC2047	1,115	77	22	0	55	1,6	1,68	1,44	0	0
AC3033	1,114	68	17	2	49	1,6	1,48	1,29	0	0
Z4032	1,083	40	5	0	35	0,8	0,87	0,92	0	0
Z4019	1,067	78	14	3	61	1,4	1,70	1,60	0	0
AC4221	1,009	73	8	1	64	1,0	1,59	1,68	0	0
AC3065	1,001	50	4	1	45	1,0	1,09	1,18	0	0
Y4103	0,990	46	5	0	41	0,9	1,00	1,08	0	0
AC3015	0,977	55	7	0	48	0,9	1,20	1,26	0	0

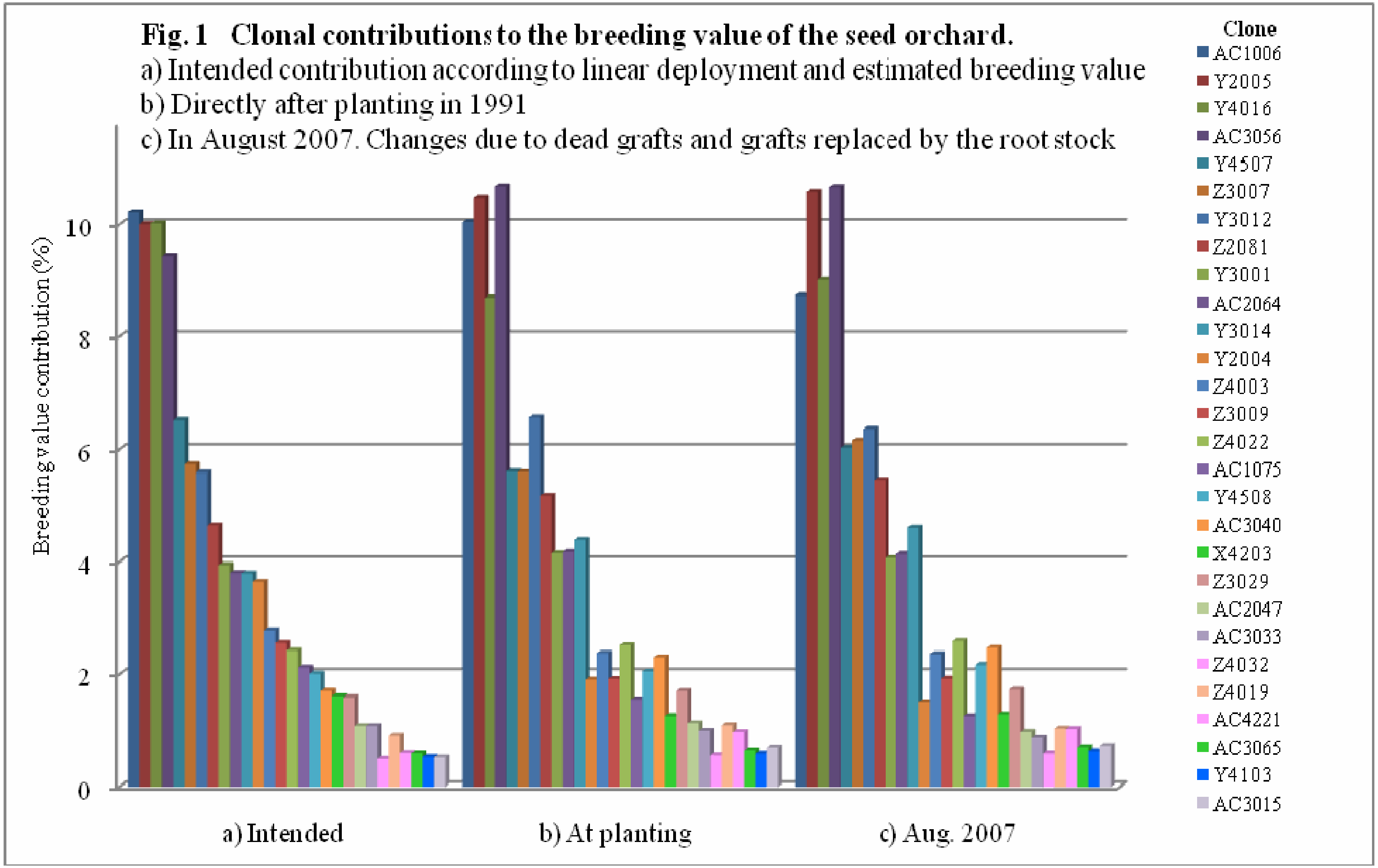

Fig. 1 Clonal contributions to the breeding value of the seed orchard.
a) Intended contribution according to linear deployment and estimated breeding value
b) Directly after planting in 1991
c) In August 2007. Changes due to dead grafts and grafts replaced by the root stock

Discussion

Breeding value of the seed orchard

Although clonal differences in mortality and in root stock dominance (8-33% and 0-4.5%, respectively) (Tab. 4), there is no decrease in genetic quality in the seed orchard (Tab. 5) and graft mortality seems thus not correlated with the breeding value of the clone. Linear deployment seems in addition to be a satisfactory efficient approximation for optimal deployment when considering occurrences of selfings (Prescher at al. 2006), and when thinning a balanced seed orchard linear deployment can optimize both production and genetic diversity (Prescher et al. 2007). The advantage with designing the seed orchard with linear deployment is therefore worthwhile.

Table 5. Clonal frequencies, breeding values and effective population sizes for the five seed orchard alternatives.

	Västerhus seed orchard with linear deployment			**Traditional seed orchard with truncation selection**	
	Intended according to linear deployment	At planting	In August 2007	20 clones in equal proportions (%)	Adjusted due to mortality (%) [1]
Clonal frequency (%)	0.8-8.1	0.9-8.9	0.9-8.9	5.0	2.1-7.2%
Breeding value (in standard deviations)[2]	1.665 (106.2%)	1.600 (105.9%)	1.657 (105.7%)	1.593 (101.6%)	1.568 (100%)
Effective population size (Ne)	19.93	19.75	19.68	20	19.7

[1] Adjustments of clonal proportions were made on the basis of registered graft mortality and root stocks replacing the graft in Västerhus, i.e. in August 2007.

[2] Breeding values are standardized so the average of the 167 available clones (Table 2) is set to 0 and their standard deviation for breeding value to 1.

Contaminating pollen

The quite large loss of grafts from plus tree progenies (17%) may currently be a larger threat to the genetic quality of the orchard than changed clonal proportions, due to a larger influence from contaminating pollen from adjacent stands with unselected native Scots pines in combination with Västerhus being a rather young seed orchard. Pollen counts in 2006 show however higher pollen production per graft in Västerhus than in other developing seed orchards of the same age (Wennström, pers. communication). The pollen production in Västerhus 2006 has been estimated to the magnitude of 20 kg pollen per hectare, which traditionally is considered as sufficient to reduce pollen contamination. Contamination problems may thus be less serious in Västerhus. That almost one percent of the grafts are root-stocks reduces the gain from the seed orchard, in particular if seeds are harvested from these trees. That root-stocks may play a role in the genetic composition of seed orchard seeds is an argument to use root-stocks of a genetic material adapted to similar climate as the seed orchard clones.

Contamination rates have been studied with isozyme techniques on Scots pine seed orchards. In the studies, they varied between years and between clones, but high values were found: 53-58% (Lindgren 1994), 17-39% with differences between years (Harju & Muona 1994), and nearly 70% (Yazdani & Lindgren 1991). Undetected contamination caused by the occurrence of the same isozyme pattern in the contaminating pollen as in the seed orchard pollen is then included. Those figures become thus somewhat unsafe. New molecular techniques will however be used to study contamination in Västerhus. Then clonal differences in contamination rate and spatial differences in contamination within the seed orchard can be analyzed. The technique can in addition more effectively separate contaminating pollen from seed orchard pollen.

Conclusions from the present study of Västerhus

- Grafts which die and root stock which replace the grafts seem to cause rather small problems for the genetic quality of the seed orchard.
- Linear deployment increases the breeding value by approximately 5-6% compared to a traditional design with equal number of grafts from each clone at the same effective population size.

• Acknowledgements

The here presented results and the coming studies are financed by the Swedish Tree Breeding Association (Föreningen Skogsträdsförädling) which hereby is acknowledged. The seed orchard is operated by Svenska Skogplantor AB, and we acknowledge their cooperation in allowing us to use the seed orchard and sharing some documentation. The seed orchard was planned and documented mainly by efforts by Skogforsk. We thank Johan Westin and Ulfstand Wennström for assistance with the electronic map of the seed orchard.

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COANCESTRY AMONG WIND POLLINATED PROGENIES FROM A *PINUS PINASTER* SEED ORCHARD IN A PROGENY TRIAL.

Maria João Gaspar[1]; Ana de-Lucas[2,6]; Santiago C González-Martínez[3,6]; Jorge Paiva[4]; Elena Hidalgo[2,6]; José Lousada[1]; Helena Almeida[5]

1 - Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas, Dpto Florestal, Universidade Trás-os-Montes e Alto Douro (UTAD), Portugal.
2 - Laboratorio de Diagnóstico Genético. Dpto. Producción Vegetal y R.F. E.T.S.II.AA. Universidad de Valladolid, Spain.
3- Departamento de Sistemas y Recursos Forestales, CIFOR-INIA, Spain.
4- Laboratório de Biotecnologia de Células Vegetais, ITQB/IBET Oeiras, Portugal.
5 - Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Centro de Estudos Florestais, Portugal.
6 - Unidad Mixta Universidad de Valladolid - INIA.

Introduction

Maritime pine (*Pinus pinaster* Ait.) is an important commercial species in southwest Europe. In Portugal, *P. pinaster* is one of the most important native species, covering 1 Mha. It is the only source of long fibre for pulp and paper and the main source for solid sawn timber industries. A tree improvement plan has been developed since the early 80s with the aim of increasing volume and stem form. A genetic improvement program management implies the knowledge of the genetic parameters, of the way these parameters affect the genetic gains, and how the progenitors transmit to the progeny the traits under improvement. The knowledge of the coefficient of coancestry is a requirement for estimating variance components and other genetic parameters for any quantitative trait. In populations where plus trees are typically selected, pedigrees are usually unknown, and it is assumed that all plus trees are genetically unrelated, as it was in the Portuguese *Pinus pinaster* improvement program. In forest trees, deviations from this assumption lead to greater inbreeding and loss of genetic gain. Thus, knowing the value of the coancestry coefficient among parents and within their progeny can be useful to improve the heritability estimation (additive *vs.* non additive variance) and to decide how to select the trees within the families in a combined selection scheme. The aim of this work is to estimate a mean value of the coancestry coefficient of the families present in a progeny trial originated from seed collected in a clonal seed orchard, and in what way this affects heritability estimations.

Material and Methods

To accomplish this objective 125 offspring from a sub sample of six families from a progeny test planted at Mata do Escaroupim were analyzed. Seeds for the progeny test were originated by open pollination of 46 plus trees maintained at Escaroupim Clonal Seed Orchard. Total genomic DNA was isolated from needles following Doyle and Doyle (1990) protocol, with some modifications. Offsrping were genotyped for five highly polymorphic microsatellite markers: two chloroplast microsatellite loci (Pt87268 and Pt1254) and three nuclear microsatellites (Itph4516, Ctg275 and Ctg4363). The amplification conditions for the different molecular markers are described in Robledo-Arnuncio *et al.* (2004) (cpSSRs), González-Martínez *et al.* (2002) (Itph4516) and Chagné *et al.* (2004) (Ctg275 and Ctg4363). Estimates of correlated mating within families were obtained following Hardy *et al*. (2004) by using the freely available software SPAGeDi 1.2 (Hardy & Vekemans 2002). Narrow sense heritability was estimated using ASREML (Gilmour et al. 1998).

Results and Discussion

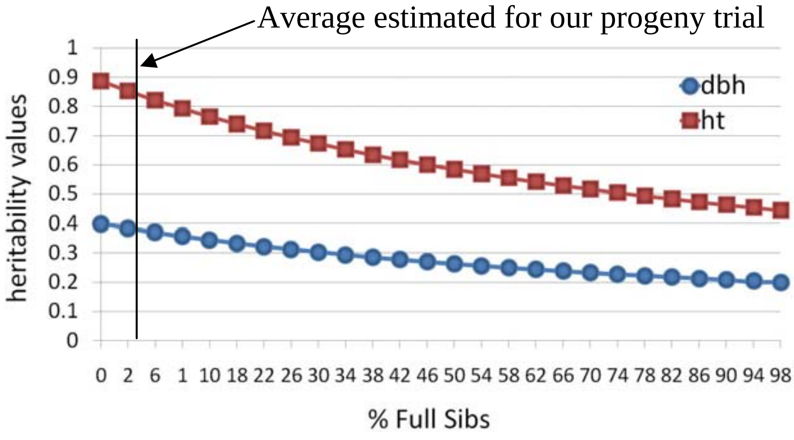
The percentage of full-sibs slightly differed among families, being 4% the average value within the families studied (Table 1) presents estimates of correlated mating (i.e. percentage of full-sibs) within families. The mean value of the genetic covariance coefficient of the families present in this progeny trial was then of 0.26. Adjusted heritabilities for different percentages of full-sibs found in an open-pollinated progeny trial are represented in Figure 1. Differences between the unadjusted and adjusted heritability values were more pronounced in total height (0.40 and 0.38, respectively) than in diameter (0.90 and 0.89, respectively), but they did not imply severe bias (<5%).

We can conclude that in a *Pinus pinaster* open-pollinated trial the associated error in heritability estimates due to the inclusion of full-sibs, when assuming a coefficient of relation amongst open pollinated sibs of ¼, is low.

Table 1: Marker-based estimates of correlated mating

Fam	nuSSR	cpSSR	Mean
50	0.0284	0.0614	0.0449
14	0.0282	-0.0319	-0.0018
32	0.0072	0.0840	0.0456
59	0.0416	0.0513	0.0464
15	-0.0032	0.1507	0.0737
General mean		0.0418	

Figure 1: Adjusted heritability for different percentages of full-sibs found in an open-pollinated progeny trial


Average estimated for our progeny trial

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Contribution of seed orchards to timber harvest in the short-run and in the long-run

Peichen Gong[1], and Ola Rosvall[2]

Summary: Seed orchards contribute to increasing forest growth through the production of genetically superior seeds. It is obvious that, other things the same, the increase in forest growth will lead to greater timber harvests in the long-run. When future forests grow faster than existing ones, it may be profitable to harvest and regenerate an existing forest a few years earlier than in the case when there is no difference in growth between the future and the existing forests. Therefore, access to genetically improved regeneration materials could also motivate a forest owner to harvest more in the short-run. By how much timber harvest would increase in each time period depends on, among other things, the current and future timber prices. It should be noted that normally seed orchards are established at such scales that sufficient amount of seeds are produced to meet the regeneration needs within large regions. When a large number of forest owners increase their harvests simultaneously, the price of timber would inevitably be affected. In order to assess the contribution of seed orchards to timber harvest, one has to take into account the impact of changing harvest level on the price of timber.

This study investigated the potential contribution of seed orchards to timber harvest in Sweden by comparing the optimal harvest volumes over time with and without access to genetically improved seeds from seed orchards. A timber market model was developed to determine the market equilibrium price and supply of timber over time. The model builds on an exogenous timber demand function, which is assumed to be constant over time. The supply curve in each period was determined using a managerial decision model which optimizes the harvest volume of individual forest owners conditional on a given time series of timber price. Market equilibrium timber price and supply in each year during a 200-year time horizon were determined by maximizing the present value of social surplus (the sum of forest owners' profits and consumer surplus).

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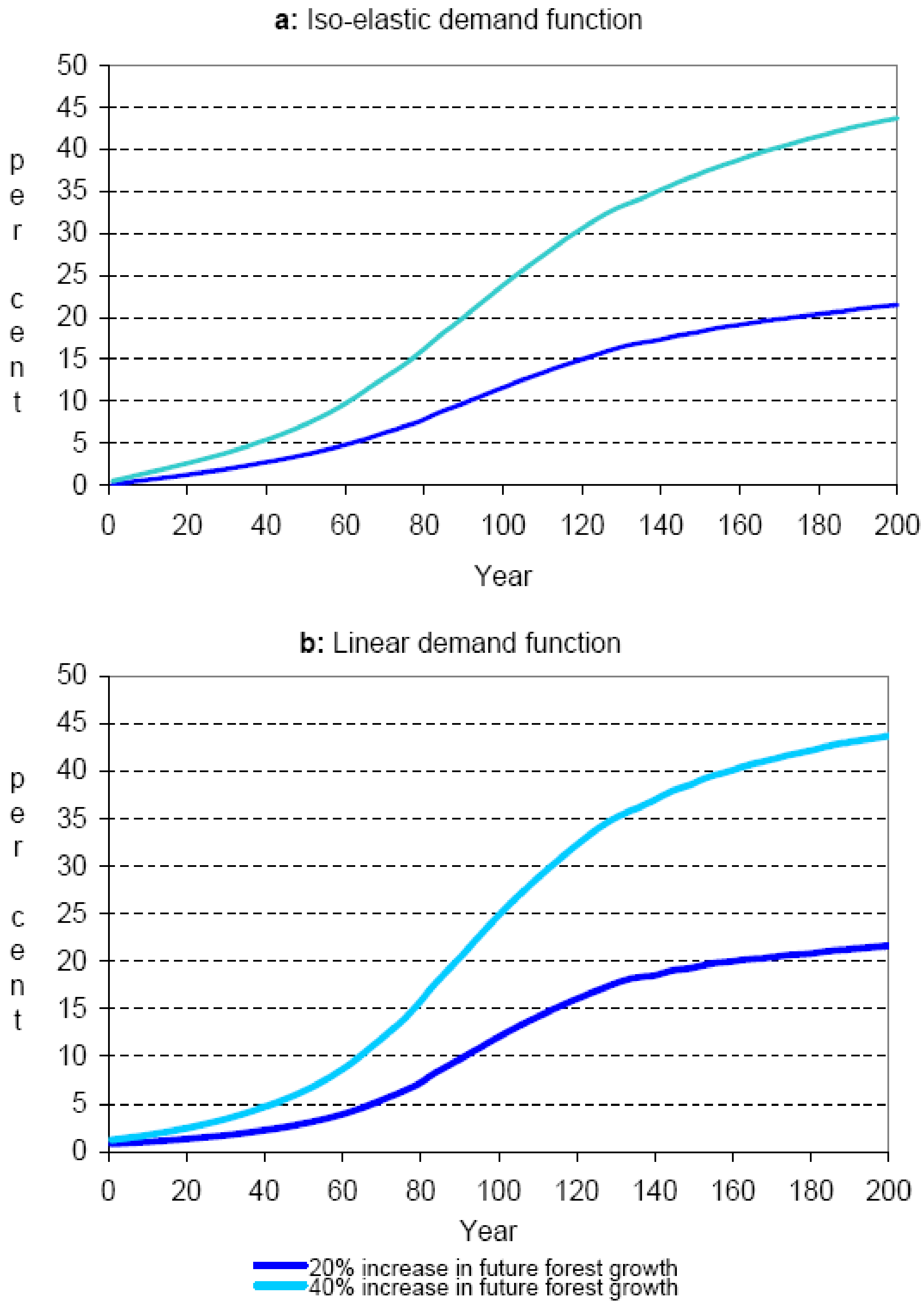
[1] Department of Forest Economics, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden. Email: peichen.gong@sekon.slu.se
[2] Skogforsk, Box 3, SE-918 21, Sävar, Sweden. Email: ola.rosvall@skogforsk.se



a: Iso-elastic demand function

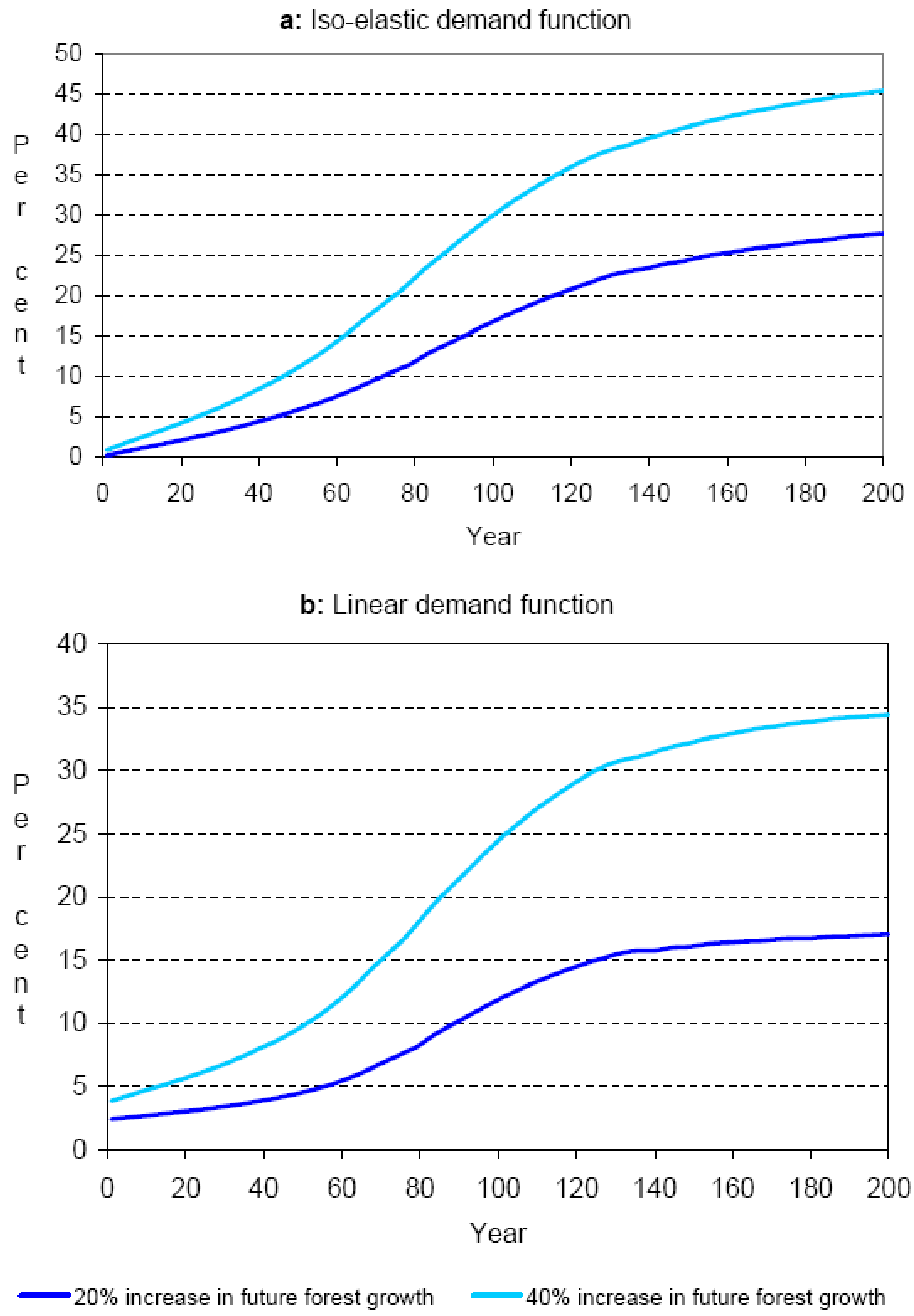
b: Linear demand function

Figure 1. The percentage increase in timber harvest resulted from the increase in Future forest growth.

The analysis was conducted with an iso-elastic and a linear demand function, respectively. The two demand functions are comparable in the sense that they give the same price (314 SEK/m^3) and have the same price elasticity (-0.6) at a harvest level of 80 million m^3 (which is roughly the current harvest level in Sweden). The total forest area covered in the analysis was 20 million ha (which includes all forests under the age of 120 years), divided into two soil productivity classes of approximately equal size. The age-class distributions of the forests were determined based on data from national forest inventory. Two levels of future forest growth improvement, resulted from the use of seeds produced in seed orchards, were evaluated. Specifically, it was assumed that future forests grow 20% or 40% faster (measured in terms of the maximum mean annual increment) than the existing ones. A real interest rate of 3% was used throughout the analysis. Other important assumptions underlying the analysis include: a) the growth effect of genetically improved seeds is known with certainty, b) seeds from seed orchards are available from now on and will be used in all future forest regenerations, and c) forest owners' objective is to maximize the profit of timber production.

The results show that the access to genetically improved seeds has relatively small effects on timber harvest in the short-run. During the first 60 years (that is, before forest owners can start to harvest the new forests), the increase in timber harvest is about 10% of the potential increment in future forest growth. The impact on timber harvest increases over time (see Figure 1). In the long-run (i.e. when the harvest effect stabilizes), the increase in harvest is determined by the increase in future forest growth. For example, if the new forests grow 20% faster than the existing ones, timber harvest would in the long-run rise by approximately 20%.

The price of timber becomes lower when genetically improved seeds are used in regeneration than in the case when such seeds are not used. Since the demand function is by assumption constant over time, the decrease in timber price induced by large scale applications of genetically improved seeds becomes larger as time goes (see Figure 2). With the iso-elastic demand function, the average timber price during the 200-year time horizon decreased by 18% if future forests grow 20% faster. A 40% improvement in future forest growth would lead to a 30% decrease of the average timber price. With a linear demand function the effect of improvement in future forest growth on timber price is smaller, but still significant.


a: Iso-elastic demand function

b: Linear demand function

Figure 2. The percentage decrease in timber price induced by the increase in future forest growth.

In short, an important consequence of widespread application of genetically improved seeds in forest regenerations is that more timber will be produced and traded at lower prices. Results from the current analysis show that forest owners' profits will drop by 5% if genetically improved seeds lead to a 20% increase in future forest growth. With a 40% increase in of future forest growth the profits of timber production would decrease by about 10%. Timber based industry, on the other hand, would benefit considerably from the use of genetically improved seeds in forest regenerations, which makes it possible for the industry to buy more timber at lower prices. The social surplus would increase by 15 billion SEK if future forests grow 20% faster, and by about 30 billion SEK if the increase in future forest growth is 40%. This result indicates that advances in tree improvements can generate significant benefits to society.

It should be emphasized that this study ignored several issues which are of importance when assessing the consequences of tree improvements. First, the analysis did not pay any attention to the non-timber benefits associated with the forests, except that about 3 million ha of the existing old forests were saved from harvesting. The effect of the non-timber benefits on the harvest decision may be different when genetically improved seeds are used in future regenerations than when such seeds are not used. Thus, explicit inclusion of the non-timber benefits in the analysis may lead to different results about the impacts of applying genetically improved seeds on timber harvest and price. A second issue is uncertainties in the growth effect of and the potential ecological risks associated with the use of genetically improved seeds. With consideration of such uncertainties and risks, genetically improved seeds may not be used in all future forest regenerations. To what extent genetically improved seeds will be used depends on the expected growth improvement and the degree of uncertainties and risks involved, as well as on forest owners' attitudes towards such uncertainties and risks. A third issue is the continuous progress in tree improvement. A fourth issue is the possible increase in future timber demand which may occur as a consequence of increasing forest growth. Progress in tree improvements not only lead to faster growing forests, but may also stimulate increasingly higher demand for timber. Increase in future timber demand would in turn affect the price and supply of timber. Further analyses need to be conducted in order to quantify the impacts of these factors on the effects of using genetically improved seeds on future timber harvest and price.

Planter's guide - a decision support system for the choice of reforestation material

Mats Hannerz [1] and Tore Ericsson [2]

Skogforsk (the Forestry Research Institute of Sweden)
[1] Uppsala Science Park, SE-751 83 Uppsala, Sweden
[2] Box 3, 918 21 Sävar, Sweden

A new web-based tool will help the forest owners and nursery managers to select reforestation material for the right planting site. The new programme "Planter's guide" will replace the existing "Val av skogsodlingsmaterial". The motives for the replacement, and the content of the tool will be shortly described here.

Reforestation material is big business in Sweden. In 2006, 332 million seedlings were sold from Swedish nurseries. Sixty percent were Norway spruce, and 35 % were Scots pine. A smaller part of the produced and sold seedlings were lodgepole pine or broadleaves. Of the spruce seedlings, 49 % originated from seed orchards. Corresponding figure for pine was 78 %. The remaining part originated from unimproved stand seed.

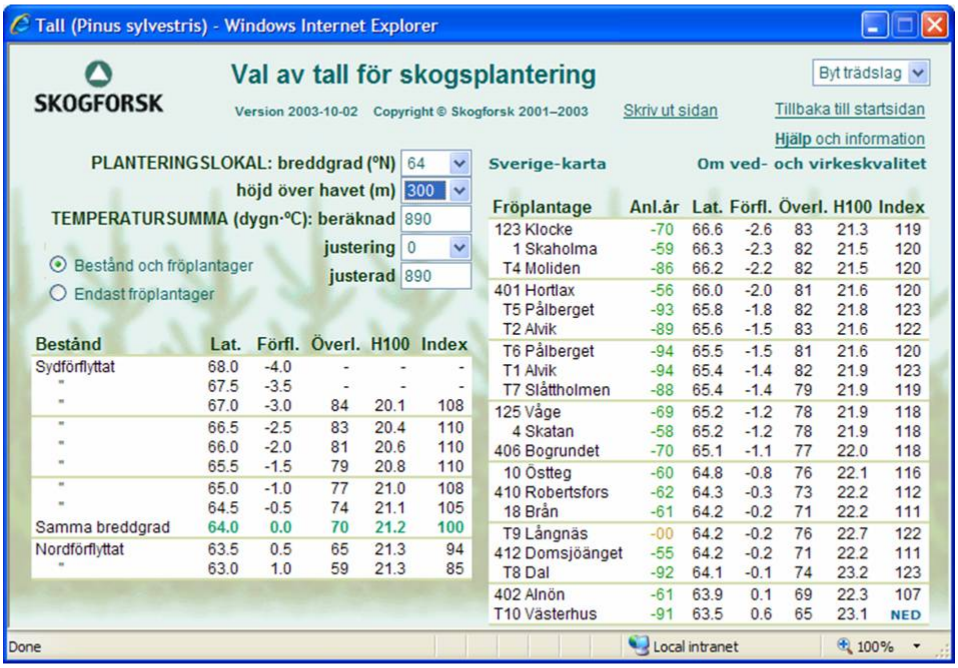
The choice of genetic origin of the seedlings has important implications for the growth in the forest. An inoptimal choice can easily make the forest owner loose ten or more percent in total yield over a rotation. A choice of seedlings with inappropriate hardiness can also lead to a total failure of the regeneration.

It is a delicate task to find the most suitable material for the right site. There are some typical questions which has to be answered in the practical forestry: Which reforestation material is recommended for my particular site, and why? If I possess a certain seed lot, or origin of seedlings, where should it be planted? How can I balance the risk of poor hardiness with growth potential, considering the trade-off between hardiness and growth in northern Sweden?

These questions can be tricky to answer only from the literature, since recommendations vary along latitudinal and altitudinal clines. The web-based tool can however give immediate answers, not only of which material to choose, but also of the expected growth potential and survival.

From Val av tall to Planter's guide

The first prototype of the tool dates back to the mid 1990's, when Bengt Andersson constructed "Val av tall i norra Sverige" (choice of pine for northern Sweden), an Excel-programme. The programme became publicly available on the web in 2000, and was extended and improved in the following years (Ericsson, 2001). The construction and maintenance of the programme has been in the hands of Tore Ericsson, but since 2003, no major revisions of the programme were made until the construction of the new interface in 2007.



PLANTERINGSLOKAL: breddgrad (°N) 64
höjd över havet (m) 300
TEMPERATURSUMMA (dygn·°C): beräknad 890
justering 0
justerad 890

● Bestånd och fröplantager
○ Endast fröplantager

Bestånd	Lat.	Förfl.	Överl.	H100	Index
Sydförflyttat	68.0	-4.0	-	-	-
"	67.5	-3.5	-	-	-
"	67.0	-3.0	84	20.1	108
"	66.5	-2.5	83	20.4	110
"	66.0	-2.0	81	20.6	110
"	65.5	-1.5	79	20.8	110
"	65.0	-1.0	77	21.0	108
"	64.5	-0.5	74	21.1	105
Samma breddgrad	64.0	0.0	70	21.2	100
Nordförflyttat	63.5	0.5	65	21.3	94
"	63.0	1.0	59	21.3	85

Fröplantage	Anl.år	Lat.	Förfl.	Överl.	H100	Index
123 Klocke	-70	66.6	-2.6	83	21.3	119
1 Skaholma	-59	66.3	-2.3	82	21.5	120
T4 Moliden	-86	66.2	-2.2	82	21.5	120
401 Hortlax	-56	66.0	-2.0	81	21.6	120
T5 Pålberget	-93	65.8	-1.8	82	21.8	123
T2 Alvik	-89	65.6	-1.5	83	21.6	122
T6 Pålberget	-94	65.5	-1.5	81	21.6	120
T1 Alvik	-94	65.4	-1.4	82	21.9	123
T7 Slåttholmen	-88	65.4	-1.4	79	21.9	119
125 Våge	-69	65.2	-1.2	78	21.9	118
4 Skatan	-58	65.2	-1.2	78	21.9	118
406 Bogrundet	-70	65.1	-1.1	77	22.0	118
10 Östteg	-60	64.8	-0.8	76	22.1	116
410 Robertsfors	-62	64.3	-0.3	73	22.2	112
18 Brån	-61	64.2	-0.2	71	22.2	111
T9 Långnäs	-00	64.2	-0.2	76	22.7	122
412 Domsjöänget	-55	64.2	-0.2	71	22.2	111
T8 Dal	-92	64.1	-0.1	74	23.2	123
402 Alnön	-61	63.9	0.1	69	22.3	107
T10 Västerhus	-91	63.5	0.6	65	23.1	NED

Figure 1. The interface of the old version "Val av skogsodlingsmaterial"

The new programme "Planter's guide" has used the entire content and data structures of the former "Val av skogsodlingsmaterial", but more functions have been added and the interface is completely renewed.

There are at least three major reasons for the construction of the new version: 1) The old programme was difficult to update and edit. The programme was constructed as a continuous Javascript code, and important seed orchard data was embedded in strings. Revisions of the content was very dependent on the programme constructor, and we foresee more frequent updates in the near future, since many new seed orchards are taken into production.

2) The development of the web and the computer environment has increased the demands for standardized format of programmes like this, in order to facilitate for programme makers, editors and IT administrators. The new program is based on the dotNET platform, and is uniform with other calculation tools on Skogforsk's web.

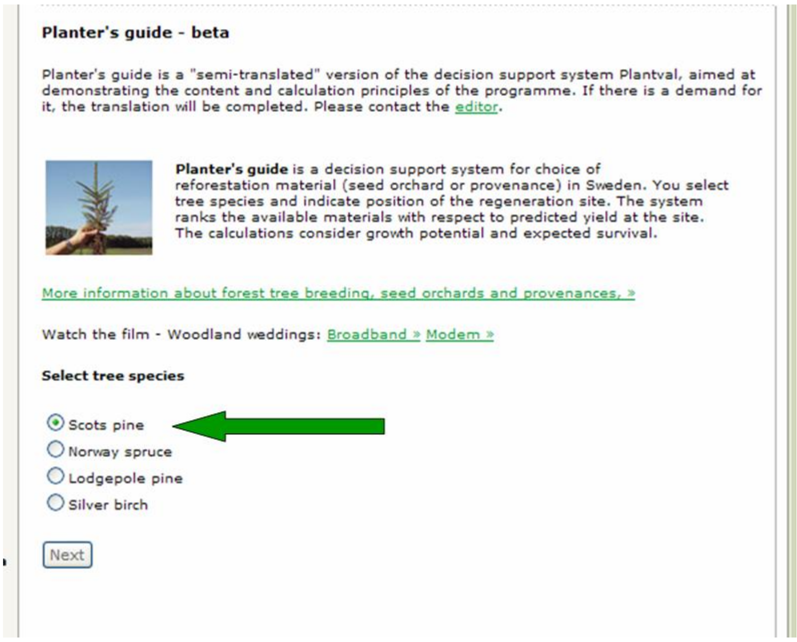
3) There was a demand to improve the usability of the programme. It was originally made to suit the needs of seed managers, and worked very well for that specialized target group. However, there is a demand for access to the information from other users, such as forest owners or forest servants responsible for silviculture. During our workshops with users, we have also learnt that there is a need for a more user-friendly version also among the expert users.

So what is the programme doing? Simply explained, it uses response functions of height growth and survival to photoperiod and temperature sum (Rosvall et al., 1998). Since the photoperiod and temperature sum are functions of latitude and altitude, these are easy to estimate if we know the coordinates of the reforestation site. The functions are based on 40 years of research on how growth and hardiness is affected by provenance transfer. The gains of seed orchard material is calculated with respect to basic genetic gain, clonal origin, seed orchard location, pollen contamination and selection gains. The programme presents an index for each material, which compares the overall production with that of a local, unimproved provenance. An index of 110 is thus a 10 % gain over a rotation.

The programme

Let´s look at the programme. It is part of "Kunskap Direkt", a web-based knowledge system in silviculture, targeted to forest owners and their advisers (Hannerz et al., 2005). The main programme (Plantval) is presented in Swedish, but there is also an English version (Planter's guide) with some restrictions.

The first step is to select species (Scots pine, Norway spruce, lodgepole pine or birch). This is a delicate task in itself, but not covered by the programme.



Planter's guide - beta

Planter's guide is a "semi-translated" version of the decision support system Plantval, aimed at demonstrating the content and calculation principles of the programme. If there is a demand for it, the translation will be completed. Please contact the editor.

Planter's guide is a decision support system for choice of reforestation material (seed orchard or provenance) in Sweden. You select tree species and indicate position of the regeneration site. The system ranks the available materials with respect to predicted yield at the site. The calculations consider growth potential and expected survival.

More information about forest tree breeding, seed orchards and provenances. »

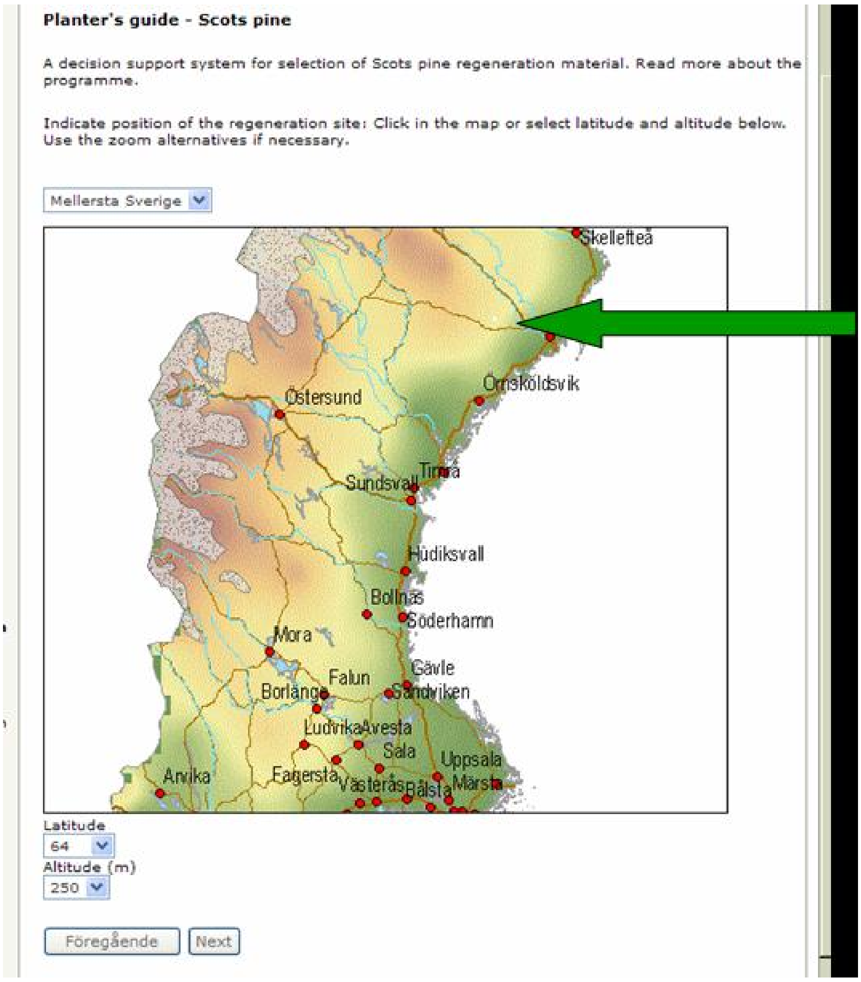
Watch the film - Woodland weddings: Broadband » Modem »

Select tree species

● Scots pine
○ Norway spruce
○ Lodgepole pine
○ Silver birch

Figure 2: The first step in Planter's guide is to select tree species.

Let us assume that the aim is to plant Scots pine. The next step is to identify the coordinates of the reforestation site. We have learnt from our workshops and test groups that this task needs to be simplified. Many forest owners do not know their coordinates, for example their latitude. Therefore, we use a simple map function, where a click in the map gives sufficient information both about the latitude and the altitude. These, and the derived temperature sum, are thereafter possible to adjust more in detail. The temperature sum can naturally vary depending on the specific conditions of a site. Therefore, it might be suitable to reduce the temperature sum for e.g. harsh north slopes, or increase it for warm southwestern slopes.



Planter's guide - Scots pine

A decision support system for selection of Scots pine regeneration material. Read more about the programme.

Indicate position of the regeneration site: Click in the map or select latitude and altitude below. Use the zoom alternatives if necessary.

Mellersta Sverige

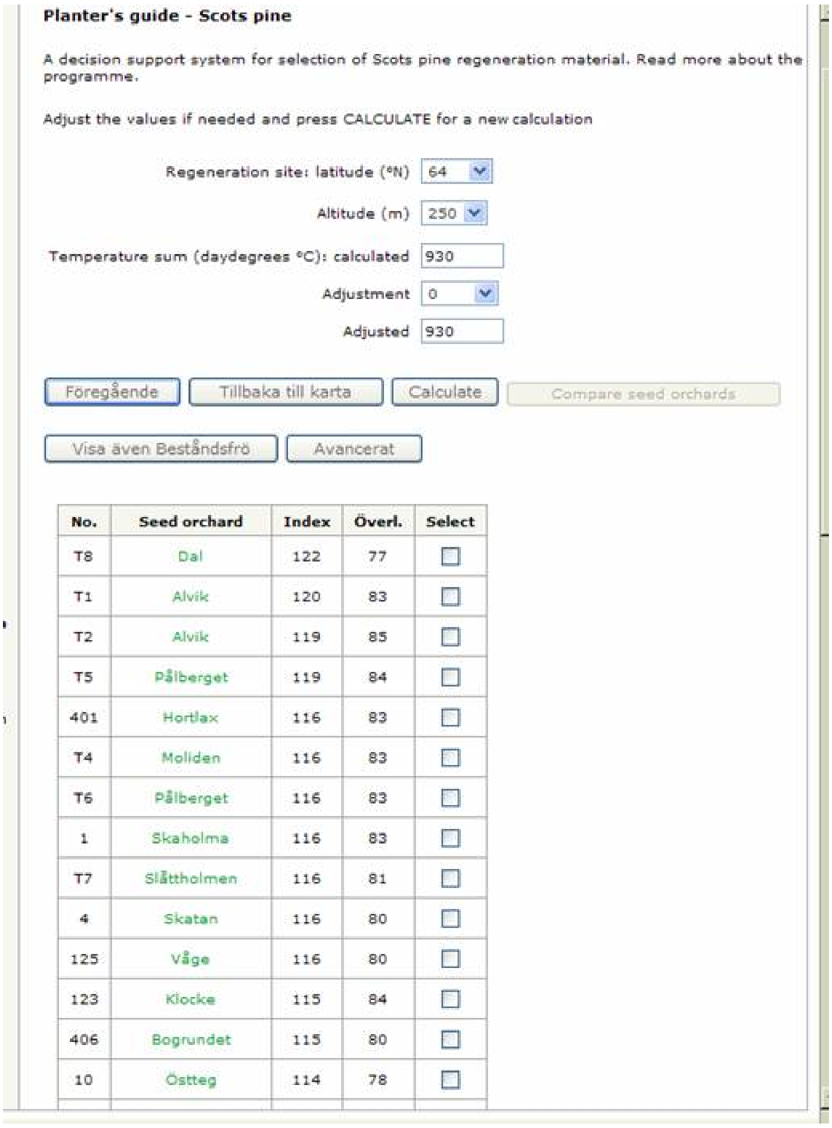
Latitude: 64

Altitude (m): 250

Figure 3: The second step is to find the coordinates of the reforestation site, which is done by clicking the map. The map includes a database with altitude information.

Once the coordinates are fixed, we are given the results. The first view presents seed orchards in descending order with respect to index. Survival is also given in this view. The index is the product of height growth and survival. Thus, it is understood that a high-index-material with low survival has a high growth potential, and vice versa.

A click on the button shows us a view with stand seed, i.e. unimproved provenances. This gives us a chance to compare the seed orchard alternatives with transferred stand seed. The local provenance has an index of 100, by definition. In the example case, index will be increased by transfer from more northern latitudes. The reason is that survival is higher for those provenances. The index from southtransferred provenances is still far below those of the best seed orchards.



Planter's guide - Scots pine

A decision support system for selection of Scots pine regeneration material. Read more about the programme.

Adjust the values if needed and press CALCULATE for a new calculation

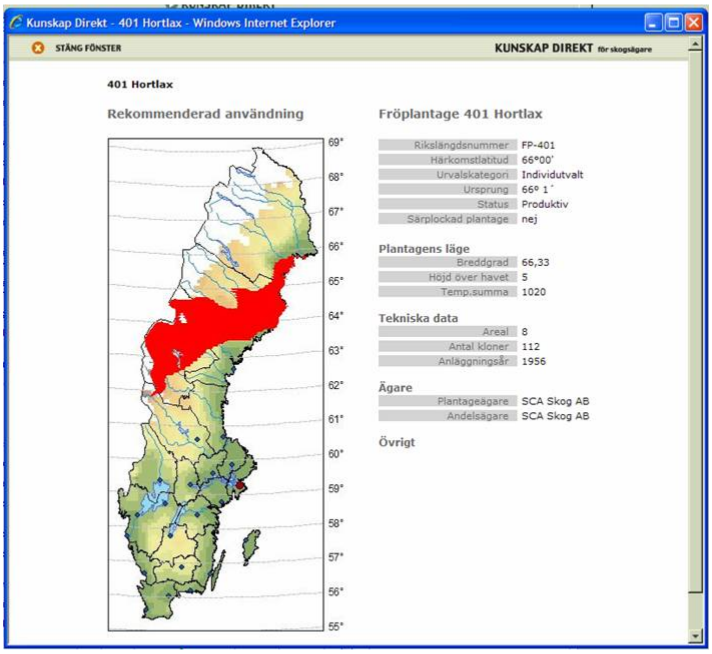
Regeneration site: latitude (°N) 64
Altitude (m) 250
Temperature sum (daydegrees °C): calculated 930
Adjustment 0
Adjusted 930

No.	Seed orchard	Index	Överl.	Select
T8	Dal	122	77	☐
T1	Alvik	120	83	☐
T2	Alvik	119	85	☐
T5	Pålberget	119	84	☐
401	Hortlax	116	83	☐
T4	Moliden	116	83	☐
T6	Pålberget	116	83	☐
1	Skaholma	116	83	☐
T7	Slåttholmen	116	81	☐
4	Skatan	116	80	☐
125	Våge	116	80	☐
123	Klocke	115	84	☐
406	Bogrundet	115	80	☐
10	Östteg	114	78	☐

Figure 4. The results are first given in a simple view with seed orchards ranked by index. Survival is also presented in this mode. By clicking the buttons, it is also possible to see results of transferred provenances, or a more advanced view with more detailed information about the orchards.

There is also an advanced view with more information, such as age of the seed orchard, yield translated to site index, and an indicator if seed orchard is old enough to produce seed.

In the simple view, it is also possible to open up more information about each seed orchard. Information presented here is a map of recommended use, technical information about the orchard, and data about who owns the orchard. The owner is thus an indicator to where seedlings can be purchased.



401 Hortlax

Rekommenderad användning

Fröplantage 401 Hortlax

Rikslängdsnummer	FP-401
Härkomstlatitud	66°00'
Urvalskategori	Individutvalt
Ursprung	66° 1'
Status	Produktiv
Särplockad plantage	nej

Plantagens läge

Breddgrad	66,33
Höjd över havet	5
Temp.summa	1020

Tekniska data

Areal	8
Antal kloner	112
Anläggningsår	1956

Ägare

Plantageägare	SCA Skog AB
Andelsägare	SCA Skog AB

Övrigt

Figure 5. Separate windows shows information on recommended area for planting, technical information, and information about ownership of the seed orchards.

In the simple mode, it is also possible to click the checkboxes of the materials we are particularly interested in. In a format familiar to many shopping sites, these seed orchards are compared in a new window.

The tool that is present on the web now is a beta version, which will be tested and revised during the autumn 2007. We also plan to add more functions, such as a simple "translator" to yield in cubic metres, and a visualization of the effects of transfer with respect to growth and survival.

The address to the tool is www.kunskapdirekt.se/plantersguide.

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Ericsson, T. 2001. Knowledge system for choosing regeneration stock. Skogforsk, Resultat nr 19, 2001. 4 p. (In Swedish with English summary.)

Hannerz, M., von Essen, M. & Johansson, S. 2005. Knowledge Direct – an internetbased guide for forest owners. Skogforsk, Results no. 2, 2005. 4 p.

Rosvall, O., Andersson, B. & Ericsson, T. 1998. Species-specific guidelines for choosing forest regeneration material for northern Sweden. Skogforsk, Redogörelse nr 1, 1998. 66 p. (In Swedish with English summary.)

POMOTECHNICAL TREATMENTS IN THE BROADLEAVE CLONAL SEED ORCHARDS

Davorin Kajba[1], Nikola Pavičić[2], Saša Bogdan[1] and Ida Katičić[1]

[1]Faculty of Forestry University of Zagreb, 10 000 Zagreb, Croatia
[2]Faculty of Agriculture University of Zagreb, 10 000 Zagreb, Croatia
Email:davorin.kajba@zg.htnet.hr

Abstract

For several decades management of most economically important forest tree species has faced problems of natural regeneration, repair planting on partially naturally regenerated areas and afforestation of open sites. Over these decades, the periodicity of seed crops has not been regular, which has limited natural regeneration and artificial planting. In order to increase genetic quality and reduce seed crop irregularity, it was decided to establish clonal seed orchards. Pedunculate oak (*Quercus robur* L.) and narrow leaf ash *(Fraxinus angustifolia* Vahl) are two of the most important and the most valuable forest tree species. Three clonal seed orchards of pedunculate oak were established between 1996 and 2001 in three seed regions (total area from 15 to 26 ha). The grafts were formed by the oval spindle training system and with spacing 10 × 8 m. The clonal seed orchard of narrow leaf ash was established in 2005 in the area of 3.5 ha. The grafts were planted with 4 × 4 m spacing and formed by the spindle pyramid training system. The clonal seed orchard of wild cherry was established in 2001 in the area of 2 ha and it contains 27 clones. Grafts were planted with 6 × 3 m spacing and formed by the spindle bush training system. The orchard is further supplemented by new grafts. In one of the earlier experimental clonal seed orchards of pedunculate oak root cutting was used to produce higher yield. All clonal seed orchards have been regularly maintained by pruning and other agrotechnical treatments.

Background

Highly disturbed natural balance of forest stands and lack of their regular natural regeneration have called for special measures in seed and nursery production in Croatia with application of expert forest knowledge about restoration, preservation and enhancement of forest resources.

In the last decades management of most economically important forest tree species has faced problems of natural regeneration, repair planting on partially naturally regenerated areas and afforestation of open sites. There is no regular periodicity of seed crops in stands and the time period between mast yield years is prolonged. The seed is collected from natural and seed stands as well as seed orchards (clonal seed orchards).

Even though the Croatian forests are supposed to be naturally regenerated it cannot be fully conducted. The most common reason is lack of yield in those stands that should be naturally reforested. The seed amount of important forest tree species required in a year is higher than its yearly production. To reduce or annul this negative difference between the required and the collected seed and to organize the production of genetically improved seed clonal seed orchards were established.

Seed stands and clonal seed orchards in Croatia

The total area of 17 612 ha (Table 1), managed by Hrvatske Sume limited liability company, has been selected as registered seed stands in the category of special purpose forests. Their main purpose is the production of seed needed for the production of seedlings and stands underseeding. The seed yield of every species produced in seed stands is not always sufficient for the underseeding of stands (as a way of enhancement of natural regeneration) or for open sites afforestation. Only in a year of mast crop there is sufficient productivity on the above stated area of seed stands. However, the yield periodicity for almost all tree species is between two and five years or even more. The clonal seed orchards were establish to reduce the lack of seed in the years when there is either no crop or low and insufficient seed crop (Table 2).

Table 1 Seed stands in Croatia

Species	Type	No.	Total area (ha)
Conifers			
Abies alba	PSS/ISS	14	418
Larix decidua	PSS	1	15
Picea abies	PSS	13	280
Pinus brutia	PSS	3	21
Pinus halepensis	PSS/ISS	5	185
Pinus nigra	PSS/ISS	15	302
Pinus nigra ssp. *dalmatica*	PSS	1	57
Pinus nigra ssp. *laricio*	PSS	2	29
Pinus pinaster	PSS	4	50
Pinus pinea	PSS/ISS	3	9
Pinus sylvestris	PSS	5	69
Taxodium distichum	PSS	1	1
Total	PSS/ISS	76	1 436
Social Broadleaved species			
Fagus sylvatica	PSS/ISS	25	1 603
Quercus ilex	PSS	3	85
Quercus petraea	PSS/ISS	35	2 044
Quercus pubescens	PSS/ISS	3	129
Quercus robur	PSS/ISS	106	10 094
Quercus robur var. *tardissima*	PSS	5	99
Total	PSS/ISS	180	14 054
Noble Hardwoods species			
Acer pseudoplatanus	PSS	1	22
Alnus glutinosa	PSS/ISS	4	50
Carpinus betulus	PSS/ISS	4	132
Castanea sativa	PSS	1	23
Fraxinus angustifolia	PSS/ISS	25	1 603
Fraxinus excelsior	PSS	1	22
Juglans nigra	PSS/ISS	3	56
Tilia tomentosa	PSS	2	42
Tilia platyphyllos	PSS	3	172
Ulmus minor	PSS	3	GS
Total		47	2 122
Subtotal		303	17 612

PSS = registered seed stands
ISS = selected seed stands
GS = group of trees

To supplement natural regeneration of forest stands every year certain amounts of forest tree seed should be ensured. The need for the seed of important large seed producing broadleaved trees such as pedunculate oak, sessile oak, narrow leaf ash, beech tree, etc. is evident not only because of their yield periodicity but also because their seed cannot be stored. For example, on average around 900 tonnes of pedunculate oak and 170 tones of sessile oak is harvested in a year.

It is especially important to provide enough forest seed necessary for the nurseries managed by Hrvatske sume that lately have to grow and deliver large amounts of forest seedlings. Seed production in seed orchards should provide better solutions for regular yield of high quality and genetically improved seed as production of forest seedlings in nurseries (an average year production is between 18 and 26 million seedlings).

Table 2: Clonal seed orchards in Croatia

Species	No. of orchards	Total area (ha)	No. of clones
Pinus sylvestris	2	3,00	30
Pinus nigra	2	1,50	41
Larix europea	2	2,50	28
Quercus robur	4	47,00	150
Tilia cordata	1	0,72	13
Alnus glutinosa	2	1,70	61
Fraxinus angustifolia	1	3,50	56
Prunus avium	1	3,00	26
Total	15	62,92	

First clonal seed orchards in Croatia were established a few decades ago. Those orchards had scientific purpose and were used for practical training (Kajba et al 2006). They were experimental seed orchards on small areas mostly of coniferous and only few of broadleaved species. Newer clonal seed orchards were established on more productive sites in larger areas (from 15 ha up to 26 ha) in the period between 1996 and 2006 (pedunculate oak, narrow leaf ash, wild cherry). The establishment of clonal seed orchards of late flushing pedunculate oak (*Quercus robur* var. *tardissima*) and sessile oak (*Quercus petraea*) is in process. Three clonal seed orchards of pedunculate oak have been established in three provenance regions: “Central Sava river“, established in 1996 in the area of 15 ha including 40 clones, “Upper Sava river“

established in 2000 in the area of 26 ha including 53 clones and a “Lower Posavina“ established in 2001 in the area of 25 ha including 57 clones. The clonal seed orchard of narrow leaf ash was established in 2005 in the area of 3.5 ha and it includes 56 clones. The grafts were planted with 4 × 4 m spacing.

The clonal seed orchard of wild cherry was established in 2001 in the area of 3 ha. The orchard includes 27 clones with 517 grafts and it is further supplemented by new grafts.

Along with the above stated clonal seed orchards of pedunculate oak and narrow leaf ash the progeny trials of selected plus trees were established to test their genetic quality (Bogdan et al 2004).

Training shapes and forms

The purpose of successful fruit techniques is maintaining the balance between the vegetative and generative activity. These techniques are also being applied on forest tree grafts in clonal seed orchards.

The forming pruning is used to bring the grafts into the required training shape whereas pruning for higher yield maintains the adequate balance between the growth and the seed production.

Tree pruning and training started right after the planting with a goal to gain desired canopy shape with well-deployed scaffold branches in the next 7 or 8 years.

Grafts were reduced to the desired canopy height. Some of the branches were pruned for inducing further branching from the side buds.

The main function of pruning is the removal of competing shoots to enforce the growth of remaining desired ones. Pruning intensity, i.e. the relation between vegetative and generative buds in the canopy determines the tree’s condition, density and yield. The balance between vegetative and generative buds can only be achieved by appropriate underground and aboveground tree parts pruning.

Knowing the production morphology of each tree species is very important (oak, ash and cherry). For instance, oak- the fruit called acorn are on long stem of terminal buds on one-year branches. Ash flowers in dense clusters inflorescences. Cherry fruits on one-year branches of various length, long shoots or short bearing branches.

Characteristics of training systems

Training systems should produce a strong framework and good light penetration in canopy. Different training system is applied to each tree species.

Pedunculate oak – oval spindle training system (Figure 1).
The spacing is 10 × 8 m. The total height of the training shape is up to 7 m with six scaffold whorls (figures of trunk height and branch angle within every scaffold whorl): 0.90 m/45°, 1.30m/30°, 1.20m/25°, 1.00m/20°, 1.00m/10°.

Narrow leaf ash – spindle pyramid training system (Figure 2).
The spacing is 4 × 4 m. The total height of training shape and form is 4.00 m with five scaffold whorls (the trunk height and branch angle: 1.20m/45°, 1.00m/60°, 0.90m/70°, 0.50m/80°, 0.40m/90°).

Wild cherry – spindle bush training system.
This training system ensures a strong framework and a good light penetration. The spacing is 6 × 3 m. The trunk height is 90 cm and the central leader ends with the upright terminal shoot. There are four to eight lateral skeleton branches spirally shaped 20 to 40 centimetres apart in height. The unneccesery shoots that are competing with scaffolds are cut off. Skeleton shoots are spirally spreaded out around the central leader and are two times thiner than the leader.

Fig. 1 Pedunculate oak **(***Quercus robur***)** – oval spindle training system

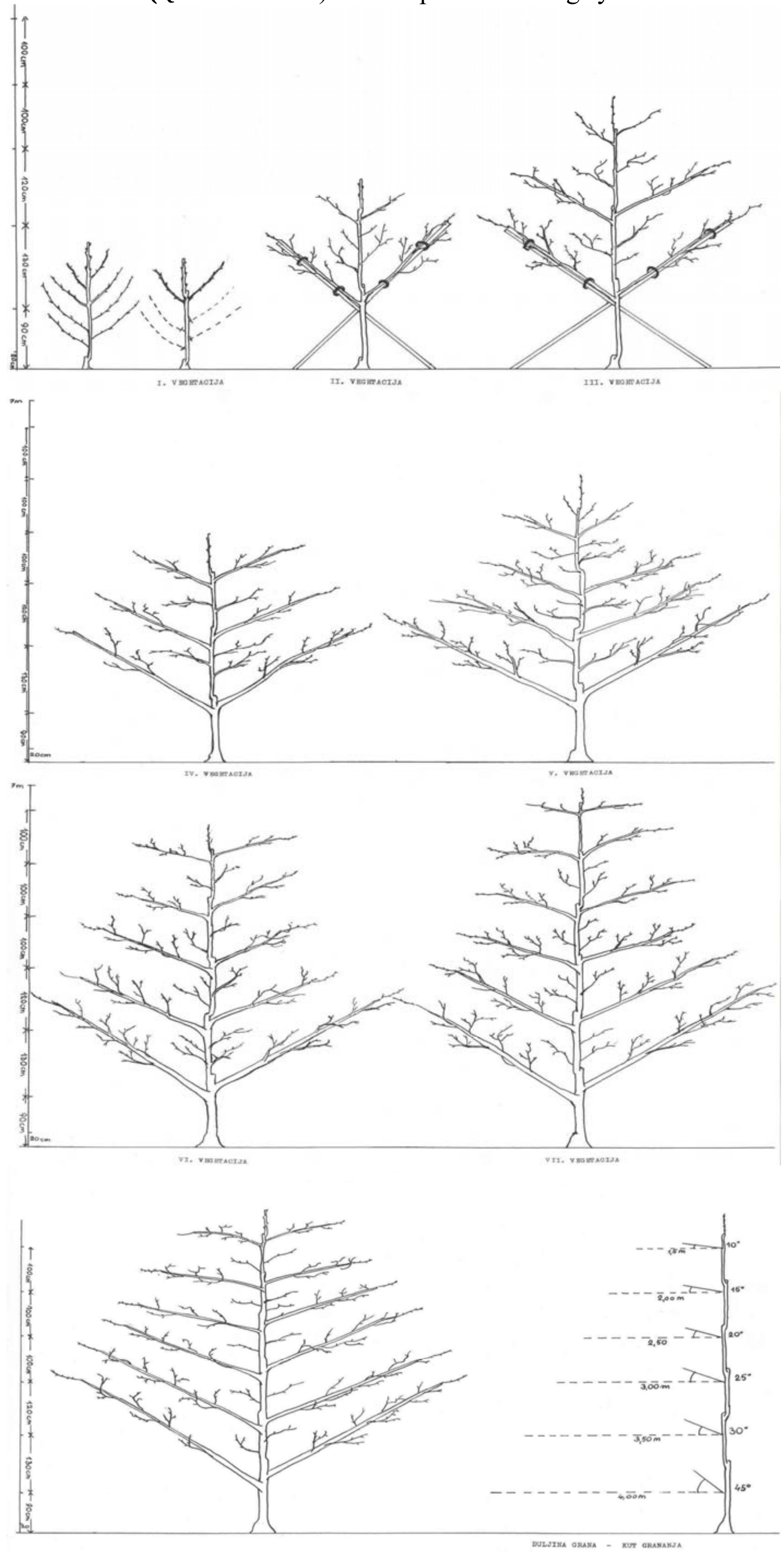
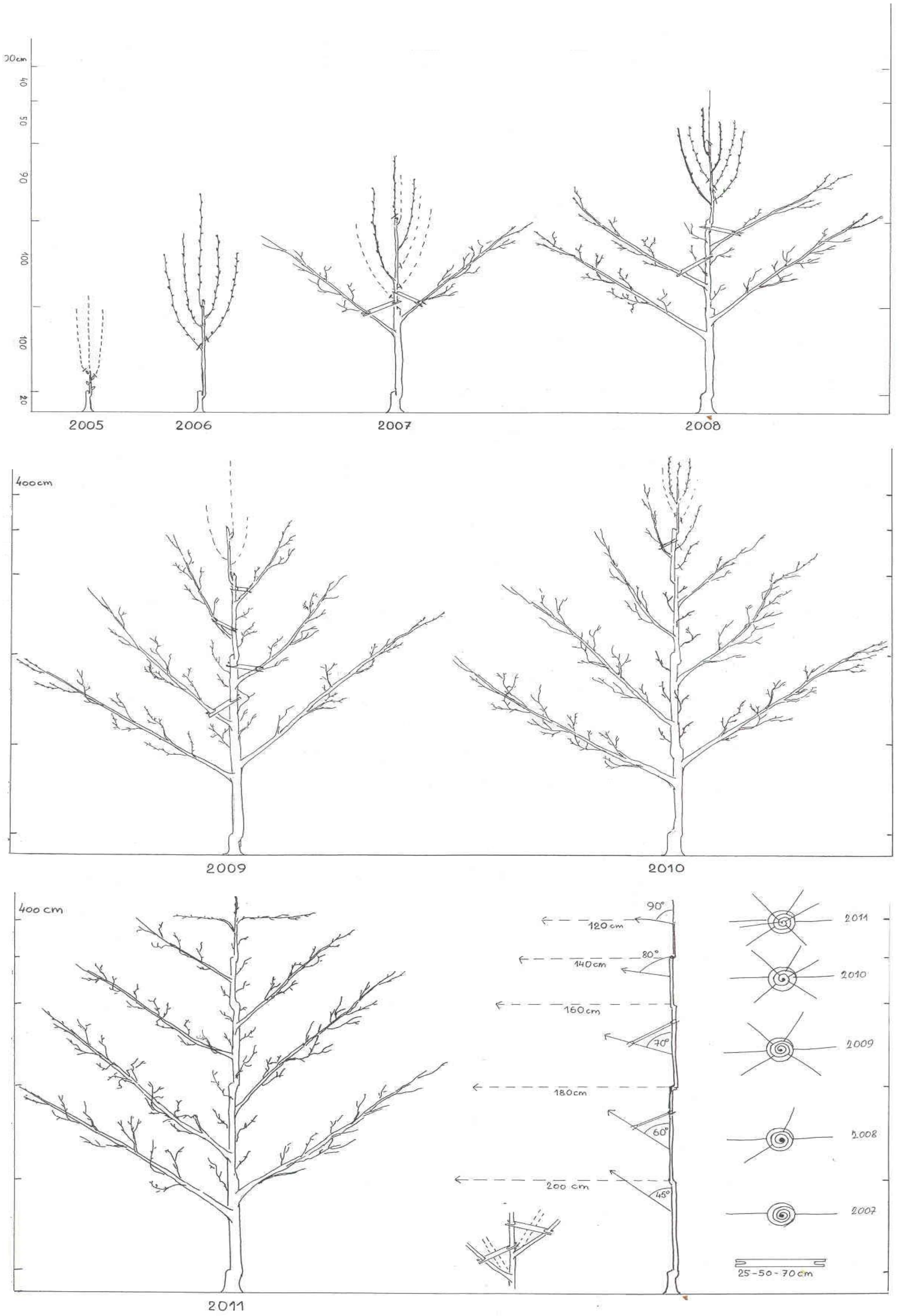


Fig. 2 Narrow leaf ash (*Fraxinus angustifolia*) – spindle pyramid training system



In the vegetative growing season of the second year shoots should be headed at the height of 25 cm to increase the yield.

On grown scaffold shoots only the best lateral branches are left whereas all the rest branches in the scaffold are cut off. In the third year there is a selection of branches in a scaffold and side shoots are cut down to 25 cm. When trees are older and have been shaped and formed the only method used is that of heading to thin and clean the canopy.

Root cutting

The first experimental clonal seed orchard of pedunculate oak was established in 1991. It consisted of 36 clones in the area of 1.00 ha. The spacing in that orchard was 6 × 6 m, which was too close and it was increased to 10 × 8 m in newly established production orchards (situated in the area of 14 to 26 ha). There were some clones that started blooming in the first and the second year upon planting.

Practical training and experience in training systems and pruning methods affecting the yield were gained in this experimental seed orchard. It also served as a mother plantation for grafts and as a site where flowering phenophases and various methods for soil treatment and protection were studied.

Root cutting in one line in the orchard was done on 13 May, 2006 on fifteen years old trees. The roots were cut at 120 cm from the grafts trunk and to a depth of 90 to 100 cm.

Root cutting can annul the negative effect of yields to flower buds differentiation and can also increase yield efficiency. Root cutting for some fruit-trees proved to be efficient method for the shortening of vegetative growth, but for some trees it had a negative effect on yield size and harvest. It has also been established that the effect of root cutting done once a year is not the same every year.

Root cutting increases the activity of cytokinin thus annulling a negative effect of giberelin**.** Knowledge gained so far shows that root cutting or other methods of limiting vegetative growth can affect fruit and higher yield and can increase the canopy of vigorous tree species.

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Mixing of seed crops from different years is an effective management strategy for enhancing effective population size in Eucalyptus seedling seed orchard crops

R. Kamalakannan and M. Varghese

Institute of Forest Genetics and Tree Breeding, Coimbatore - 641002, India
Email: mvarghese1@rediffmail.com

Abstracts:

Seed orchard trees contribute more equally to the seed crop if the seed crops from consecutive years are mixed. Fertility variation among trees and effective population size were estimated for two successive years in four seedling seed orchards (eight-nine year old) of *Eucalyptus camaldulensis* and *E. tereticornis* established in two distinctly different locations, a dry (Pudukkottai in Tamil nadu) and a moist (Panampally in Kerala state), in southern India. Fertility variation estimated as the sibling coefficient (Ψ), was high (Ψ = 5-13) in the orchards and varied between two successive years except in the *E. camaldulensis* orchard (Ψ = 2 & 3) established on the moist site. Mixing of seed crops from two successive years reduced the fertility variation considerably. The relative effective population size increased in all orchards by 31 – 48% and the variance effective population size by 36-55%. Since the relative contribution of trees varies between years the composition of the seed crops vary and gene diversity of the seed mix would be equal to or more than that of the best crop. Diversity of seed from orchards of the same origin at different locations could vary since the fertility of families vary between locations but mixing of seed would affect the performance at either site as the seed from an orchard is best suited to a similar site.

Key words: gene diversity, fertility variation, *E. camaldulensis*, *E. tereticornis*, genetic drift

Introduction:

India is one of the largest planters of eucalypts in the world with around 8 million hectares of planted area (FAO, 2000). Industrial plantations of eucalyptus are now a major resource for production of high quality wood pulp in India. The demand for quality planting stock of eucalyptus has increased several folds in recent years. First generation seedling seed orchards were established with selections from natural provenances, to supply quality seed as per a breeding program for *E. camaldulensis* and *E. tereticornis* in southern India (Doran *et al*., 1996).

Seed orchard crops represent a generation of new recombinants and genetic variation is transmitted from one generation to another. Actually seed orchards are the “link between breeding programs and reforestation through the delivery of consistent, abundant yields of genetically improved seed” (Kang, 2001). Seed orchards are expected to produce vigorous high yielding plantations because they provide offspring from mating between carefully selected superior trees. Modern seed orchard management research is the “science, business and art of managing and conserving genetic resources for continued economic, social and environmental benefit” (Kang, 2001). Genetic gain can be manipulated through orchard management alternatives (e.g. selective seed harvest, genetic thinning), while ensuring that reasonable gene diversity is maintained. The current study pertains to the analysis of diversity and management strategies in first generation unpedigreed orchards of *Eucalyptus* in different locations based on fertility observations made for two consecutive years.

Materials and Methods:

Two unpedigreed seedling seed orchards each of *E. camaldulensis* and *E. tereticornis* located in a moist site, Panampally (Kerala state) and an arid location, Pudukkottai, (Tamil Nadu) in southern India were used for the study. Edaphic and climatic details of the orchard sites are presented in Table 1. The seedling seed orchards were established in 1996 using bulked seed from more than 500 selected trees of eleven and sixteen natural provenances of *E. camaldulensis* and *E. tereticornis* respectively, as a short-term strategy for meeting the immediate seed requirement. The same seed lot bulks were used for establishing the seed orchards at both the locations. After initial establishment at 3 x 1.5 m spacing the stands were subjected to two thinnings by four years of age to achieve a final spacing of about 5 m between trees at the time of the study (Hegde and Varghese, 2002). Growth evaluation and thinning are usually completed in eucalyptus orchards by fourth year, when the orchard is normally ready for operational seed production (Meskimen and Francis, 1990). Fertility of the trees was assessed at eight and nine years of age using the methodology of quantifying the flowers and fruits produced by each tree (Varghese *et al*., 2002).

Since the seed used for establishing the stand originated from a wide base of more than 500 parent trees from wild natural stands, the orchard trees were considered to be unrelated and non-inbred. The Sibling coefficient (ψ), Group coancestry (Θ), Status number (N_s), Relative population size (N_r), Variance effective population size ($N_e^{(v)}$) and Gene Diversity (*GD*) were estimated based on the fecundity estimates made in different stands (Kang and Lindgren, 1999; Lindgren and Mullin, 1998; Lindgren *et al*., 1996). -

Constrained seed collection and mixing of seeds:

Two management strategies were considered, namely constrained seed collection and mixing of seed crops from two consecutive years to reduce the impact of fertility variation on diversity of seed crop. Constrained seed collection (selective harvest) was done by collecting equal number of fruits from each parent genotype (Kang *et al*., 2003; Kang and Lindgren, 1999; Bila, 2000; Varghese *et al*., 2006).

Results:

Mixing of seeds from two consecutive harvests helped to enhance the overall fertility and diversity parameters in the seed crop than that of either harvests. Generally this management strategy resulted in enhanced values of status number, relative population size, variance effective population size and gene diversity in all the four orchards.

The *E. camaldulensis* orchard in the moist site had comparatively lower sibling coefficient and group coancestry than the other orchards since this orchard had greater proportion of flowering trees. Mixing of seeds helped to further reduce the sibling coefficient to 2.21 from the first and second year values of 2.24 and 3.19 (Table 2) thereby enhancing the status number and gene diversity in the orchard. The relative contribution of trees increased from 31% to 45% and the variance in effective population size also increased substantially from 83 to 150 in the orchard. At the same site, the *E. tereticornis* orchard had very low proportion of fertile trees resulting in high sibling coefficient (11&13), high group coancestry (0.3) and low relative population size (0.08). Mixing of two seed crops helped to reduce the sibling coefficient by 44% and relative population size by 57%. The variance effective population size increased from 15 to 24.

At the arid site (Pudukkottai), both species had more or less similar flowering trend with almost twice the proportion of fertile trees in the second year compared to the first year. Since there was almost three times more stocking in both species, in the arid site compared to the moist site, the status number and group coancestry of the orchards were on par with those of the moist site. Mixing of seeds further helped to reduce the sibling coefficient by 36% and enhance the status number by 56% in *E. camaldulensis*. In *E. tereticornis* orchard in the same site, the sibling coefficient was reduced by 50% and status number by 95%. The relative contribution of trees almost doubled and the group coancestry decreased by 50% when successive seed crops were mixed.

When seed crops of two years was mixed the cumulative contribution in *E. camaldulensis* orchards increased (Fig. 1a, b) in both moist (by 19 %) and arid sites (by 12 %). In *E. tereticornis* the cumulative contribution increased by 32 % in the moist site and by 7% increase in arid site when seed crops were mixed (Fig. 1c, d). Constrained seed collection would substantially reduce the sibling coefficient and enhance the status number in all the orchards. The gene diversity and relative population size would also increase in the orchards with this strategy.

Discussion:

Selective harvest and genetic thinning and a combination of both are orchard management options that can be used to increase genetic gain while maintaining genetic diversity in seed orchards (Lindgren and El-Kassaby, 1989; Bondesson and Lindgren, 1993). The practice of selective harvest improves only the genetic contribution of seed parents, while both seed and pollen parents are improved with genetic thinning. When fertility variation is very high in orchards, genetic thinning should be done only after fertility evaluation of the trees. Constrained seed collection is very effective in reducing the fertility variation but is often not very feasible when large quantities of seed are required.

Mixing seed crops from different harvests, is an easy option which does not require much technical inputs for implementation. Seed orchard trees contribute more equally to the seed crop if the seed crops from consecutive years are mixed. This strategy would be very effective in species where the trees show alternate bearing tendency (Varghese *et al.*, 2007). Since the relative contribution of trees varies between years the composition of the seed crops vary and gene diversity of the seed mix would be equal to or more than that of the best crop. This strategy will be very beneficial in domesticating a newly introduced exotic species as in the case of *E. tereticornis* in tropical humid regions (Varghese *et al.*, 2002). A similar observation was made by Kang *et al.* (2005) in seed orchards of *Pinus thunbergii* where fertility variation for the combined seed crops was lower than that observed for any single year, implying that the genetic diversity of seed crops could increase if seeds collected from different years are pooled. Diversity of seed from orchards of the same origin at different locations could vary since the fertility of families vary between locations as seen in the case of *E. tereticornis* orchards in the current study. Mixing of seed from different orchards would however affect the performance at either site as the seed from an orchard is best suited to a similar site.

Acknowledgment

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Table 1. Location, edaphic and climatic details of *Eucalyptus camaldulensis* and *E. tereticornis* seedlilng seed orchards

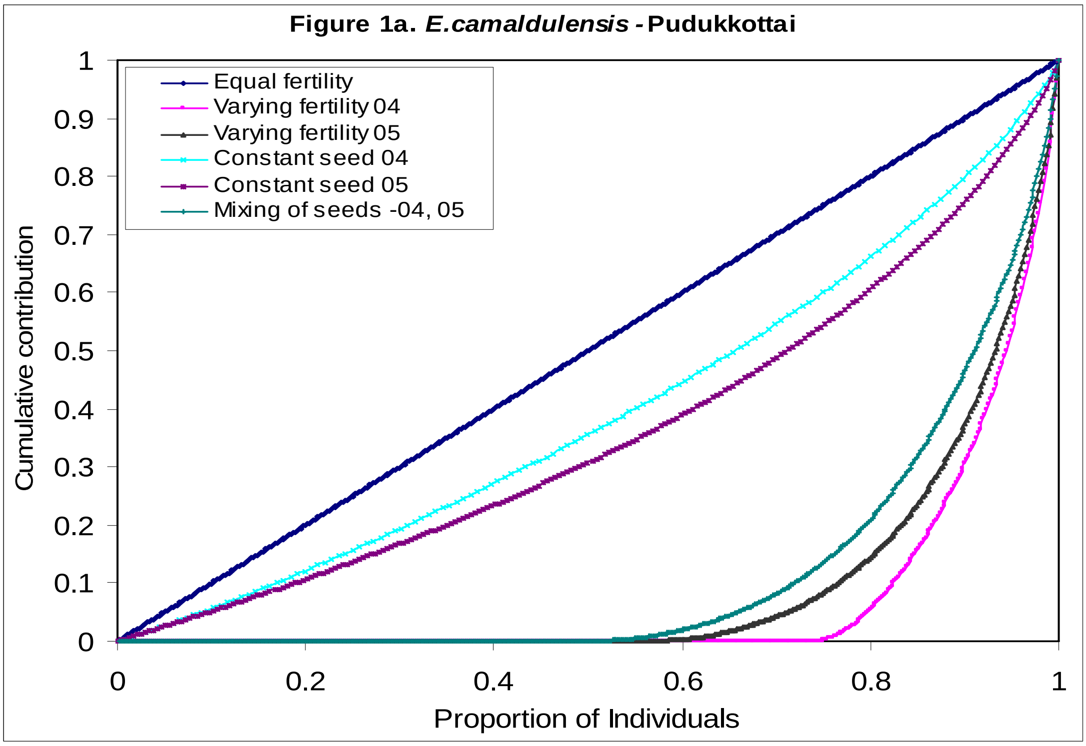
Location	Panampally	Pudukkottai
Latitude	10°52’ N	10°53’ N
Longitude	76°46’ E	78°49’ E
Annual Rainfall (mm)	1400	650
Altitude (m)	400	180
Soil type	Clay loam	Red Sandy Loam
Annual Temp(°C) range	22-39	21-42

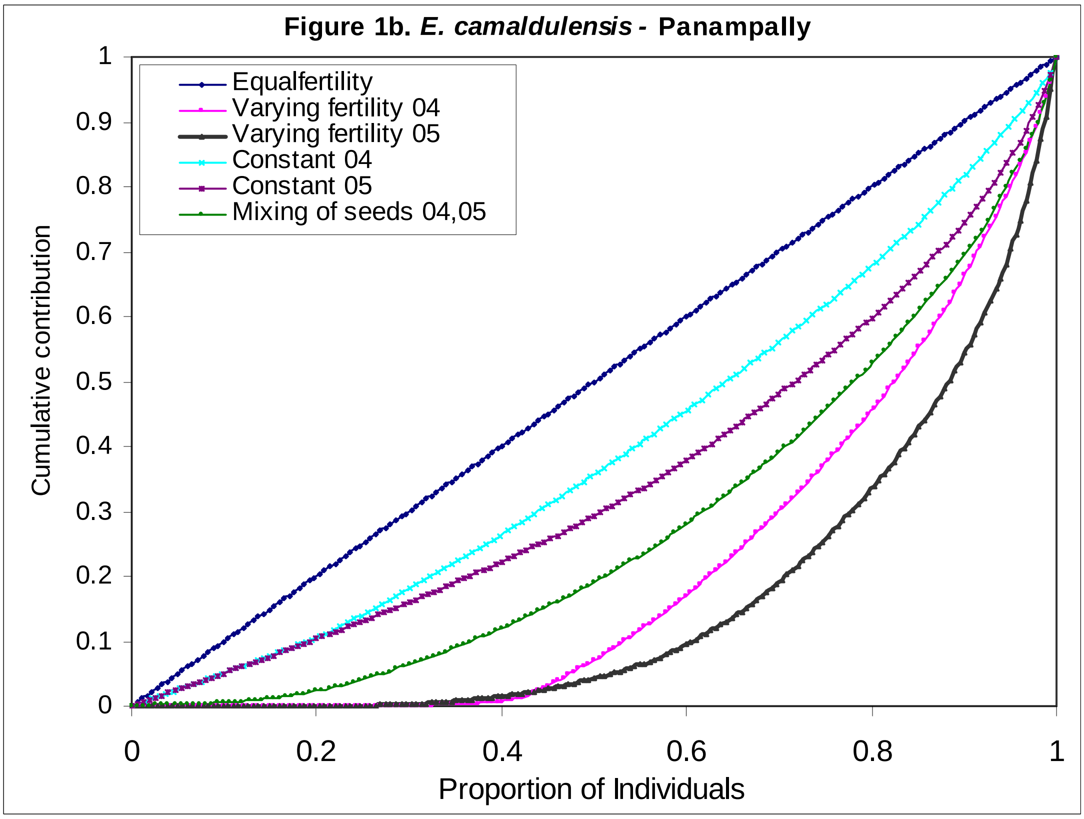
Table 2. Fertility status and the impact of management strategies on fertility variation, effective & variance effective population size and gene diversity in four seedling seed orchards of eucalyptus

Species	*E. camaldulensis*				*E. tereticornis*			
Location	Panampally		Pudukkottai		Panampally		Pudukkottai	
Years	1	2	1	2	1	2	1	2
				Varying fertility				
No. of trees (N)	182	182	525	525	192	192	505	505
Fertile trees (%)	73.1	82.4	25.9	44.6	23.4	15.1	28.3	50.5
Average DBH (cm)	15.43	-	12.57	-	14.3	-	12.47	-
Fruits/tree	3939	5114	1195	764	328	227	1674	978
Ψ^1	2.24	3.19	6.72	5.43	13.39	11.70	8.47	5.22
Ns^2	81.08	56.99	78.18	96.59	14.34	16.41	59.6	96.74
Θ^3	0.006	0.009	0.006	0.005	0.035	0.030	0.008	0.005
GD^4	0.994	0.991	0.994	0.995	0.965	0.970	0.992	0.995
Nr^5	0.445	0.313	0.149	0.184	0.075	0.085	0.118	0.192
$Ne^{(v)6}$	146.46	83.02	91.79	117.83	15.420	17.47	67.49	118.94
				Constrained seed collection				
Ψ^1	1.59	1.71	4.57	3.04	6.55	7.89	4.77	2.79
Ns^2	114.65	106.54	114.78	172.62	29.33	24.33	105.94	180.98
Θ^3	0.004	0.005	0.004	0.003	0.017	0.021	0.005	0.003
GD^4	0.996	0.995	0.996	0.997	0.983	0.979	0.995	0.997
Nr^5	0.630	0.585	0.219	0.329	0.153	0.127	0.210	0.358
$Ne(v)^6$	309.79	256.95	146.89	257.17	34.62	27.86	134.06	282.07
				Mixing of two years seeds				
Ψ^1	2.21		4.27		8.99		4.38	
Ns^2	82.449		122.991		21.348		115.392	
Θ^3	0.006		0.004		0.023		0.004	
GD^4	0.994		0.996		0.977		0.996	
Nr^5	0.453		0.234		0.111		0.228	
$Ne^{(v)6}$	150.735		160.619		24.018		149.568	

1 - Sibling coefficient, 2 - Status number, 3 - Group coancestry, 4 – Gene Diversity, 5 - Relative effective population size, 6 - Variance effective population size

Figure 1. Cumulative fertility contribution (varying, constrained seed collection and mixing of seeds) in seedling seed orchards at different locations (1 a-d)





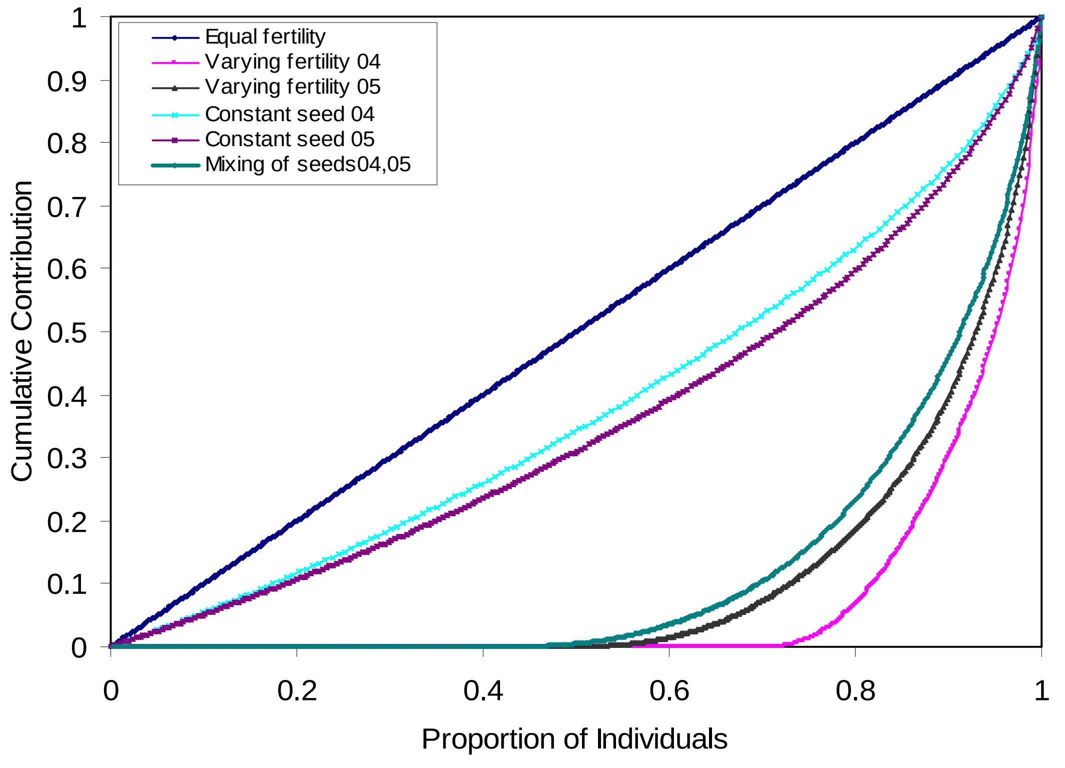


Figure1c. *E. tereticornis* - Pudukkottai

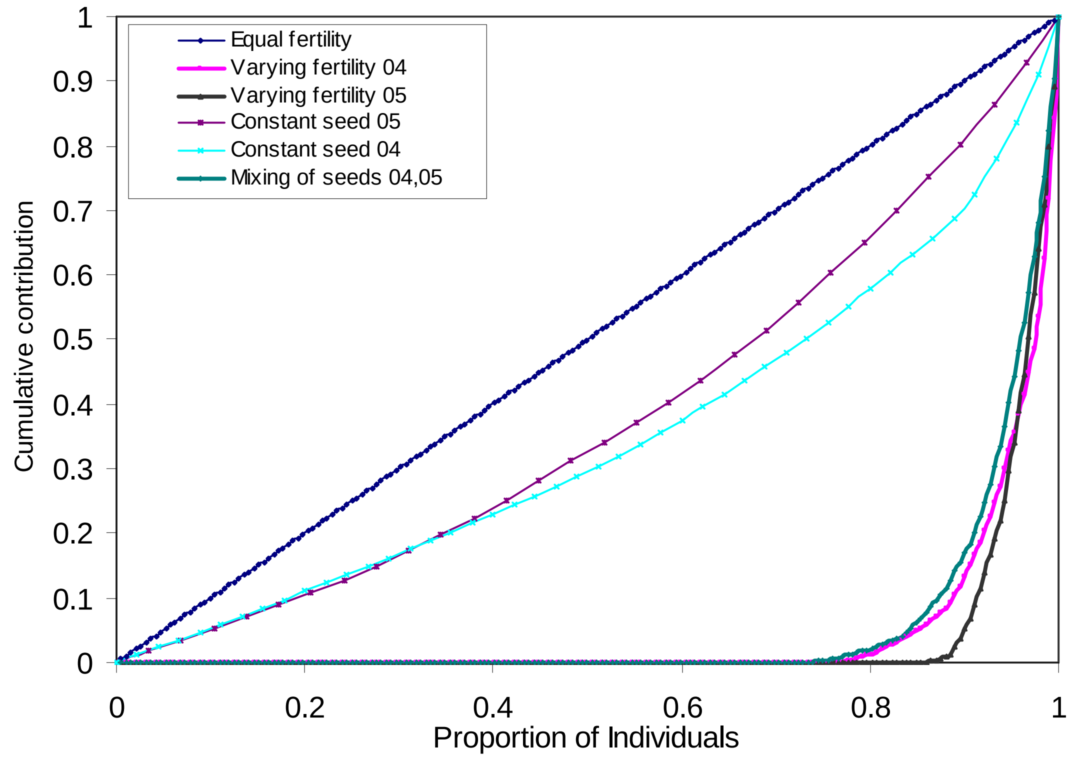


Figure1d. *E. tereticornis* - Panampally

Management of Seed Orchards considering Gain and Diversity and how it is Applied in Korea

Kyu-Suk Kang (kangks@foa.go.kr) and Chang-Soo Kim
Korea Forest Research Institute, 44-3 Omokcheon, 441-350, Suwon, Republic of Korea

Genetic gain and diversity, expressed by status number, of seed crops from a Korean seed orchard were estimated considering selection, fertility and pollen contamination, and compared for different management alternatives (selective harvest, genetic thinning, and combination). Management variables included the proportion of clones left after selective harvest and/or genetic thinning. The impact on genetic gain and diversity of seed crops was quantified as a function of the quantity and quality of gene flow from outside the seed orchard.

Selective seed harvest, genetic thinning (50% and 75%) and combination of both options increased genetic gain over the initial orchard condition. The increase was, however, coupled with a decrease in status number. Genetic gain was highest and diversity (status number) lowest in the alternative with 75% thinning intensity under both gene migration scenarios.

Pollen contamination affected both genetic gain and diversity. With no pollen contamination, all alternatives showed higher genetic value but lower status number, compared to the scenario with pollen contamination. Before thinning (selective harvest), a gene migration rate of 15% increased the status number by 22.2%, but after genetic thinning, regardless of intensity, the same level of gene migration raised the status number by 27.8%.

Relative gain from orchard management varied with the proportion of selected and/or thinned clones. The increase in genetic value was not linear relative to the proportion of selected and/or thinned clones in selective harvest and genetic thinning options. Genetic thinning gave greater gain than selective harvest at the same intensity, but this was accompanied by a greater loss of status number.

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Gene Conservation Through Seed Orchards – A Case Study Of *Prunus spinosa* L.

Jörg R.G. Kleinschmit*, Ludger Leinemann**, Bernhard Hosius¶

* Northwest German Forest Research Institute, Department of Forest Genetic Resources, Germany; ** Department of Forest Genetics and Forest Tree Breeding of the University of Göttingen; ¶ ISOGEN, Göttingen, Germany;

Contact: joerg.kleinschmit@nw-fva.de; Prof.-Oelkers Str. 6, 34346 Hann. Münden, Germany

Summary

In Germany plantations of indigenous shrub species with regionally harvested propagation material become more and more important to compensate for the encroachment of natural habitats. The use of such material is based on the expectation of its high adaptedness and vitality. The urban sprawl and land use make it difficult to identify populations which correspond to such expectations. For some species gene flow between populations and hybridization with cultivars blur potential patterns of adaptation. Results of systematic provenance trials are missing for indigenous shrub species. Thus the use of regional material is a strategy to safeguard against unintentional change of potential patterns of adaptation.

To contribute to this discussion we analysed 13 natural stands, two conservation seedling seed orchards and two seed lots of *Prunus spinosa* L. using isozyme gene markers.

This paper studies the following questions:

1. Are naturally occurring populations of *P. spinosa* L. genetically differentiated?
2. Is there any evidence for a link between genetics and spatial proximity?
3. Do the existing seed orchards of *P. spinosa* L. represent their source populations?
4. Are imported seed from southeast Europe (Hungary) significantly differentiated?
5. Do genetics support the hypothesis of naturally arisen source populations?

The natural stands are genetically differentiated. Evidence exists for a link between genetic differentiation and spatial proximity. The genotypes of the seed orchards represent the gene pool of the natural stands well. Minor genetic differentiation exists between the German study material and the seed lot from Hungary. Cluster analysis supports the hypothesis of naturally arisen source populations.

Introduction

According to considerations of nature conservation plantations of indigenous shrub species with regionally harvested propagation material become more and more important to compensate for the encroachment of natural habitats in Germany. Over 150 million trees and shrubs are produced annually in German nurseries for plantations in urban areas and the open landscape. On top of this amount a considerable quantity of propagation material is imported from other countries for the same purpose. There exist no legal regulations on harvest and procurement of these species. Especially widely distributed species like blackthorn (*Prunus spinosa* L.) have a considerable economic relevance. But the costs for harvesting seed especially from shrub species are much higher in Western Europe than in other countries. Thus 50 to 80% of the plant material is imported from low income countries (Spethmann 1995, 2003). During the last years a debate arose concerning this practice. Two partially conflicting objectives have to be reached: avoiding risks for nature and landscape by planting potentially maladapted material and allowing economically interesting production of woody plant species.

For indigenous shrub species results of systematic provenance trials are missing. Nevertheless first exploratory studies show some disadvantage of material which has been transferred over wide geographic distances (Liesebach et al. 2007). In terms of nature conservation the use of regional material is supposed to be a strategy to safeguard against unintentional change of potential patterns of adaptation. Thus the federal Ministry of Food, Agriculture and Consumer Protection published recommendations for provenance regions for shrub and minor tree species (BMVEL 2003). The use of local material is based on the expectation of its high adaptedness and vitality (McKay et al. 2005). Unfortunately the urban sprawl and land use make it difficult to identify populations which correspond to such expectations. The human as "migration factor" might alter naturally occurring adaptation processes. For some species gene flow between populations and hybridization with cultivars blur potential patterns of adaptation and might even have the potential to endanger species (Allendorf et al. 2001).

Seed orchards of shrub species were established during the last decade to overcome the shortage of reproductive material originating from local populations and to allow an economically interesting seed production. Within the process of conservation of forest genetic resources populations of indigenous tree and shrub species were identified, which according to historical records evolved without artificial introduction of plant material (Paul et al. 2000). These populations should be separated from artificially planted material of the same species.

A sample of those populations was used as source population to set up conservation seed orchards for different shrub species. This paper describes the example of a blackthorn (*Prunus spinosa* L.) seedling seed orchard.

Example of blackthorn (*Prunus spinosa* L.)

Black thorn (*Prunus spinosa* L.) is a character species of hedges along forest edges and tracks as well as on open farm land (Scholz and Scholz 1995). The phenotypic appearance of *P. spinosa* L. is quite variable. The species is widely planted in the open landscape. The taxonomic status and possible hybridization with *Prunus domestica* ssp. *Insititia* is discussed in Hegi 1995. The species is tetraploid (4n=32). Blackthorn propagates vegetatively through root suckers in nature. As an allotetraploide species *P. spinosa* is supposed to be self fertile (Hanelt, 1997). Experimentally Guitán et al. (1993) could not support this hypothesis. Population genetic studies have been carried out by Leinemann et al. 2002 and Schmitt (2003).

Two seedling seed orchards have been established with 15 seedlings of each source population in the year 2000. One seed orchard is supposed to represents natural populations of the lowlands in the north of Lower Saxony the other those of the hilly region in the south. Even though some degree of spatial isolation to neighboring populations of the same species was sought gene flow from outside cannot be excluded.

In the light of the above mentioned concerns the following questions have to be answered:

1. Are naturally occurring populations of *P. spinosa* L. genetically differentiated?
2. Is there any evidence for a link between genetics and spatial proximity?
3. Do the existing seed orchards of *P. spinosa* L. represent their source populations?
4. Are imported seed from southeast Europe (Hungary) significantly differentiated?
5. Do genetics support the hypothesis of naturally arisen source populations?

Material

In total we analyzed 17 different objects consisting of 13 natural stands which are the source populations of the northern *P. spinosa* L. seedling seed orchard (SPL-HSF), two seed orchards and two seed lots, one originating from the northern seed orchard, the second coming from Hungary. Buds were harvested in autumn 2006 and seeds were obtained from one private enterprise and the tree seed center of the Lower Saxony State Forest. As a rule 70 samples per source population were analyzed (except DAN-ST: 36 and NIE-96: 26), 80 samples of each seed lot and 100 of the southern and over 300 of the northern seed orchard, respectively. Figure 1 shows the locations of the different source populations and the seed orchards.

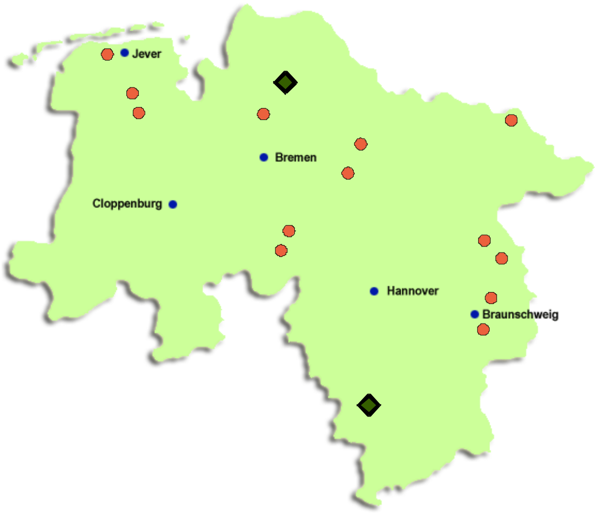

figure 1: Map of Lower Saxony, Germany with the location of source populations (red dots) and seed orchards (green romboids)

Source populations	**abbreviation**
Danndorf	DAN-AK
Danndorf	DAN-ST
Göhrde	GDE-BL
Harsefeld	HSF-HE
Neuenburg	NEU-109
Neuenburg	NEU-137
Neuenburg	NEU-UP
Nienburg	NIE-67
Nienburg	NIE-96
Rotenburg	ROT-AL
Rotenburg	ROT-DI
Wolfenbüttel	WOL-HO
Wolfenbüttel	WOL-KA
Seed orchards	
Grohnde	SPL-GR
Harsefeld	SPL-HSF
Seed lots	
Harsefeld	S-HSF
Hungary	S-UNGA

Methods

Five polymorphic enzyme systems, representing seven gene loci were analyzed (table 1). The methods for isoenzyme analysis of blackthorn are described in Leinemann (2000), Leinemann and Bergman (2000) and Leinemann *et al.* (2002). Data analysis was carried out using the program Tetraploide, Version 1 (Decarli and Leinemann 2003). The dendrogram illustrating the genetic distances between populations is based on the software NTSYS, Version 2.01, Applied Biostatistics. The enzyme systems and their E.C. numbers are described in table 1.

table 1: Analyzed enzyme systems, zones and number of observed alleles

Enzyme system	gene loci	number of alleles
Alcohol-Dehydrogenase	Adh-A	4
E.C. 1.1.1.1		
Malat-Dehydrogenase	Mdh-A	4
E.C. 1.1.1.37	Mdh-B	3
Isocitrat-Dehydrogenase	Idh-A	3
E.C. 1.1.1.42		
6-Phosphogluconat-Dehydrogenase	6-Pgdh-A	5
E.C. 1.1.1.44	6-Pgdh-B	3
Phosphoglucose-Isomerase	Pgi-B	5
E.C. 5.3.1.9		

Results

Genetic variation within samples

Three to five alleles were observed at each enzyme locus. The seed orchard Harsefeld comprises all the alleles, which were found in the source population, with the exception of three rare alleles: Adh-A3 was found only in GDE-BL, 6Pgdh-B1 was found only in WOL-HO and WOL-Ka and Pgi-B5 was found only in NEU-109.

The seed orchard Harsefeld has the allelic multiplicity of 23. The average over all studied objects is 17.9. The seed orchards show the highest degree of variation within the studied objects (figure 2) followed by the seedlot originating from Harsefeld. Thus the seed orchards

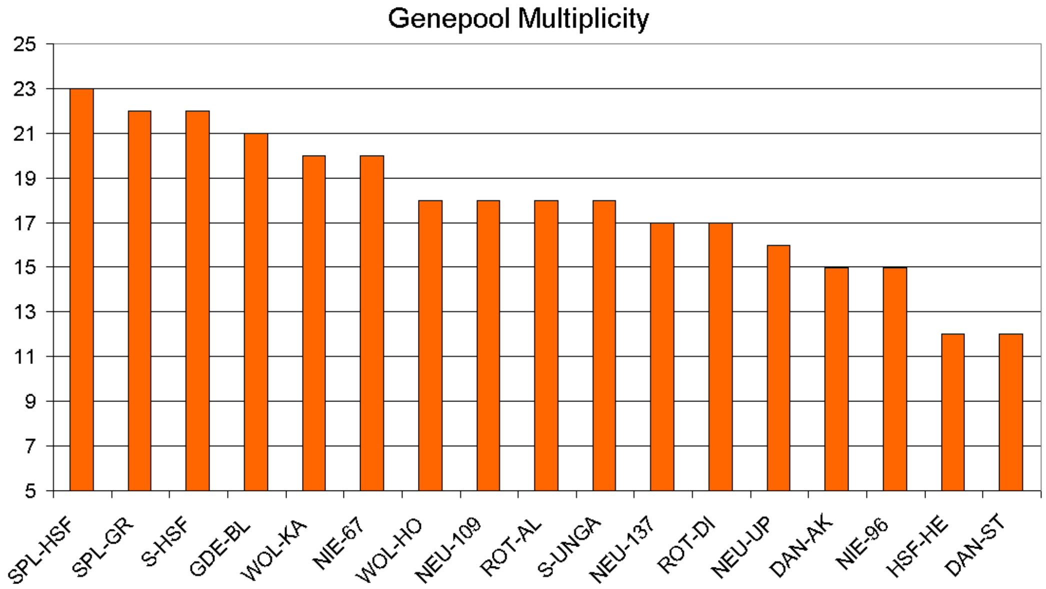

figure 1: Genepool multiplicity

represent artificial populations, which comprise more variation than the natural populations. This variation is represented in the produced seed as well. Multiplicity is influenced by sample size. In small samples there exists the chance of losing rare alleles. This might be the explanation for the values of 12 and 15 of the populations represented by small samples (DAN-ST: 36 and NIE-96). Nevertheless HSF-HE was represented by the same amount as GDE-BL for example but the first has low multiplicity of 12, the later one of 21 respectively.

The allelic diversity as measure takes the frequencies of alleles into account. The average diversity is v = 1.24. Least diverse are the sample DAN-ST with v = 1.10 and the seed lot from Hungary (S-UNGA) v = 1.16. The sample NIE-67 has the highest diversity v = 1.38.

The sampling in the source populations was done along a line. The changes of multilocus genotypes along these lines were analyzed. The maximum estimated extent of a clone is 72 meters (average 20 meters). This is an indication for naturally arisen populations as compared to plantations, where we expect different multi locus genotypes next to each other. The later situation is given in the two seed orchards.

No association exists between the number of multi locus genotypes per source population and its size. Thus small populations potentially harbor high amounts of genetic variation as well as big populations.

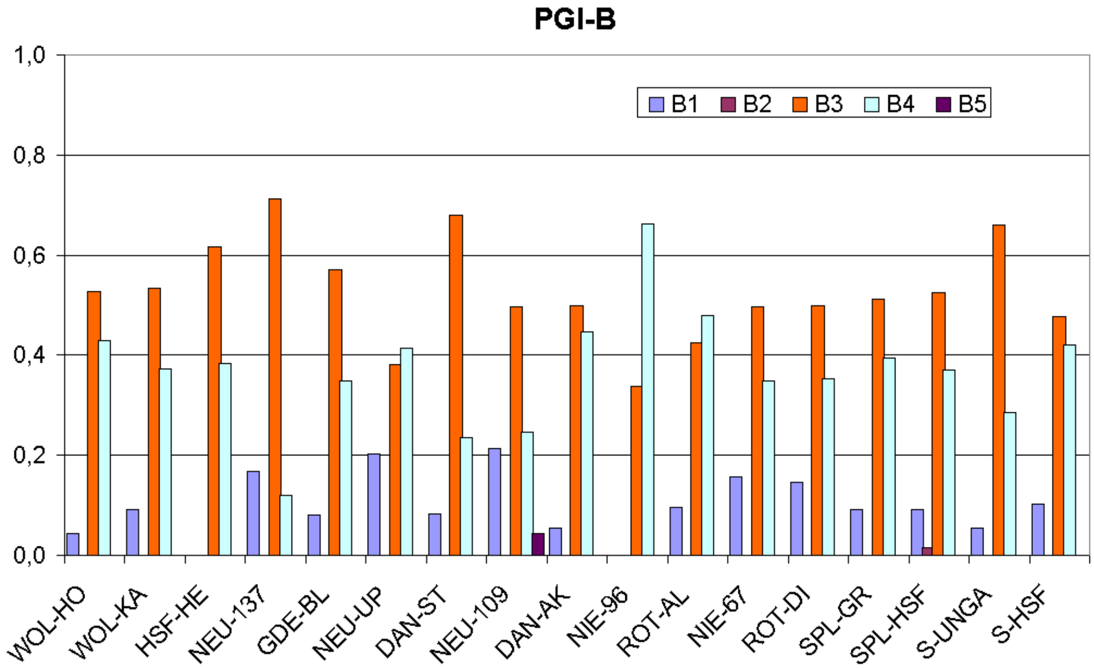

figure 2: Allelic frequencies at enzyme gene locus PGI-B

Genetic differentiation between samples

The average genetic distance (Gregorius 1974) of the gene pool is d_0=0.16. This is an extraordinarily high value for isoenzymes. The biggest allelic distance between the source populations was found for PGI-B ($d_{0\_Nie-96\_Neu-137}$=0.54) (see as well figure 3).

The neighbor joining dendrogram of average pair wise genetic distances (figure 4) shows an association between genetic and spatial proximity for some of the source populations (Wolfenbüttel and Neuenburg). The two seed orchards are grouped together joined on the next level by the seed lot from the seed orchard Harsefeld.

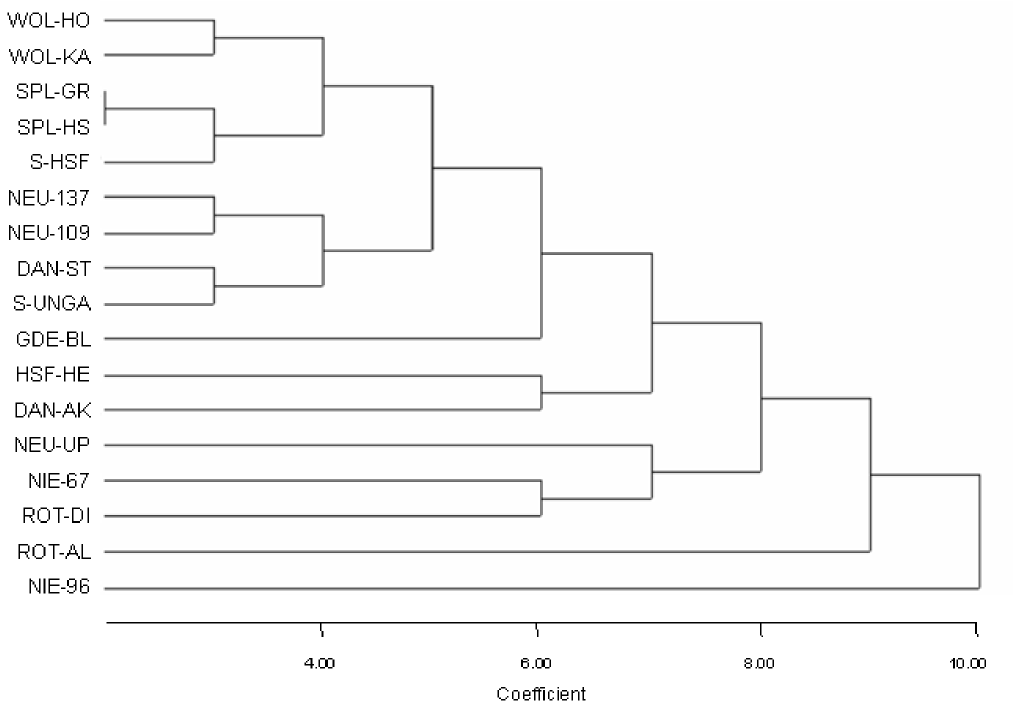

figure 3: phylogenetic (neighbourjoining) tree based on pair wise genetic distances between source populations

The subpopulation differentiation D_j (figure 5) extends the concept of genetic distances between two to multiple samples. According to Gregorius (1984) and Gregorius and Roberds (1986) D_j measures the genetic distance between a sample and its complement (the average of all other samples). The sample with the lowest value of D_j represents best all the rest of the samples. Samples with high values of D_j might represent special genetic information linked to genetic processes like adaptive differentiation.

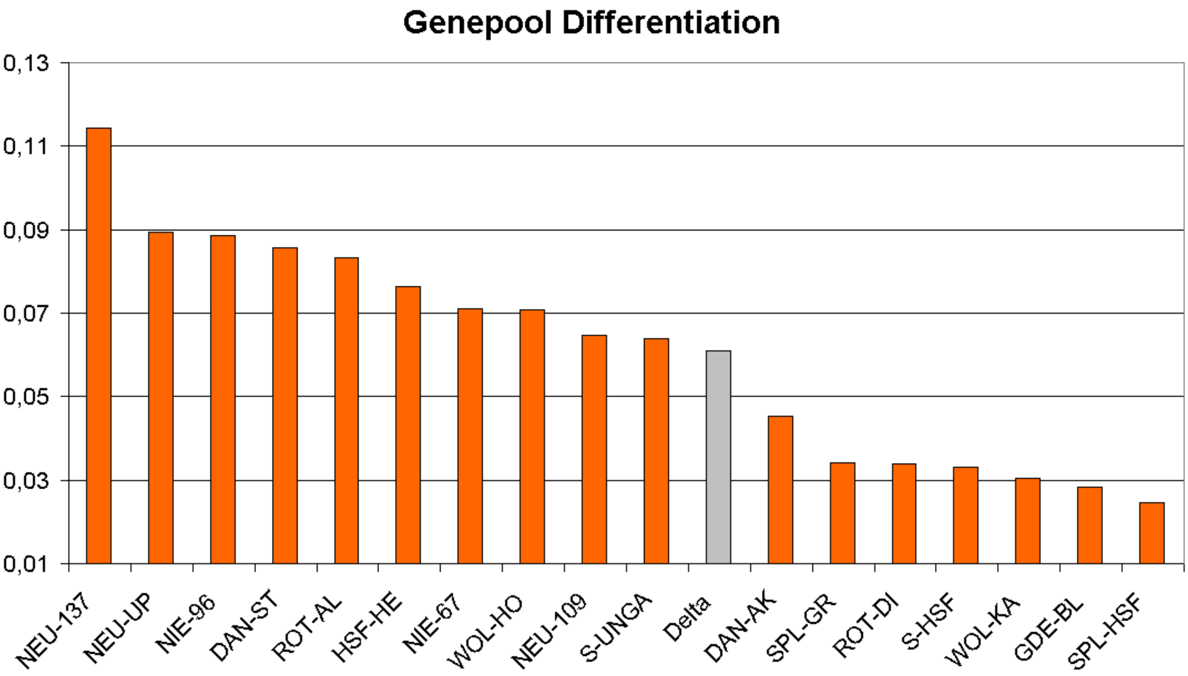

figure 4: subpopulaition differentiation of the genepool; the gray column represents the average differentitation

The mean differentiation $D_j = 0.065$ (gray bar). The population NEU-137 shows the biggest differentiation from all other populations as compared to the seed orchard Harsefeld (SPL-HSF) with the lowest value of differentiation. The later is especially representative for the rest of all samples. The seed lot of Hungary (S-UNGA) does not show a remarkable differentiation.

Discussion

Prunus spinosa L. shows a high degree of genetic variation based on isoenzyme gene markers. Seeds from 13 natural stands are represented in the seed orchard Harsefeld (SPL-HSF). The natural stands are genetically differentiated. There is now evidence for strong gene flow into the natural stands. Otherwise we would have found new allelic variants in the seed orchard.

Setting up a seedling seed orchard (SPL-HSF) to represent the variation within the natural populations was successful. The comparison between the genetic structures of the seed orchard Harsefeld (SPL-HSF) and the seed orchard Grohnde (SPL-GR) gives no evidence for the assumption of genetic differentiation between lowland’s and hilly region’s populations at the isoenzyme level. But physiological studies point out the possibility of such adaptive differentiation (see Liesebach et al. 2007). Therefore it can be seen as a strategy to safeguard against unintentional change of potential patterns of adaptation to keep two separate seed orchards for the lowlands and the hilly region as long as no results of studies of adaptive traits are available.

Evidence exists for a link between genetic differentiation and spatial proximity. Thus an adaptive significance of the observed genetic differentiation cannot be excluded. The use of different types of markers is discussed in Holderegger et al. (2006). The authors advocate for a combination of adaptive and neutral marker types.

The genotypes of the seed orchards represent the gene pool of the natural stands well. Minor genetic differentiation exists between the German study material and the seed lot from Hungary. The seed lot of Hungary (S-UNGA) is less genetically diverse than the seed lot (S-HSF) originating from the seed orchard Harsefeld.

The size of clonal structures within the stands supports the historical records that these populations were in place for already long time. The source populations probably existed before the period when intensive planting of shrub species began in the open landscape. The cluster analysis of the natural stands according to their genetic distances supports the hypothesis of naturally arisen source populations. Genetic markers represent a useful tool to analyze the different types of reproductive strategies (vegetative vs. generative propagation) of blackthorn.

For future seed procurement the seed orchards represent the chance to harvest seeds economically while safeguarding genetic variability. Genetic analysis of potential source populations prior to the establishment of seed orchards would allow to optimize the genetic variability and representativeness of the seed orchard material (e.g. Hosius et al. 2000).

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

Jan Kowalczyk
Forest Research Institute, Department of Genetic and Forest Tree Physiology
Sękocin Stary, Braci Leśnej Str. no 3, 05-090 Raszyn
e-mail: j.kowalczyk@ibles.waw.pl

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. The 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 influences 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 tress 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

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

$W_f = h_{F1}^2 d_{F1} E_1 + \ldots + h_{Fk}^2 d_{Fk} E_k$ - 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:

$W_T = W_f + W_t$ - Genotypic value of the tree

were:

$W_t = h^2_{w1} d_{w1} E_1 + ... + h^2_{wk} d_{wk} E_k$ - genetic index value of a tree within its family

d_{wk} - selection differential for trait *k*

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

$V_w=\sigma^2_E- \sigma^2_E /n$ - within family variance (Falconer, Mackay 1996)

X - trait value

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

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

Status number (Ns) 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 bye explained by the family structure. Heritabilites 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 planed on the part of block 1. Tees selected to cut were marked with the red colour, trees marked with green colour were protected even if they planed 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 planed. 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

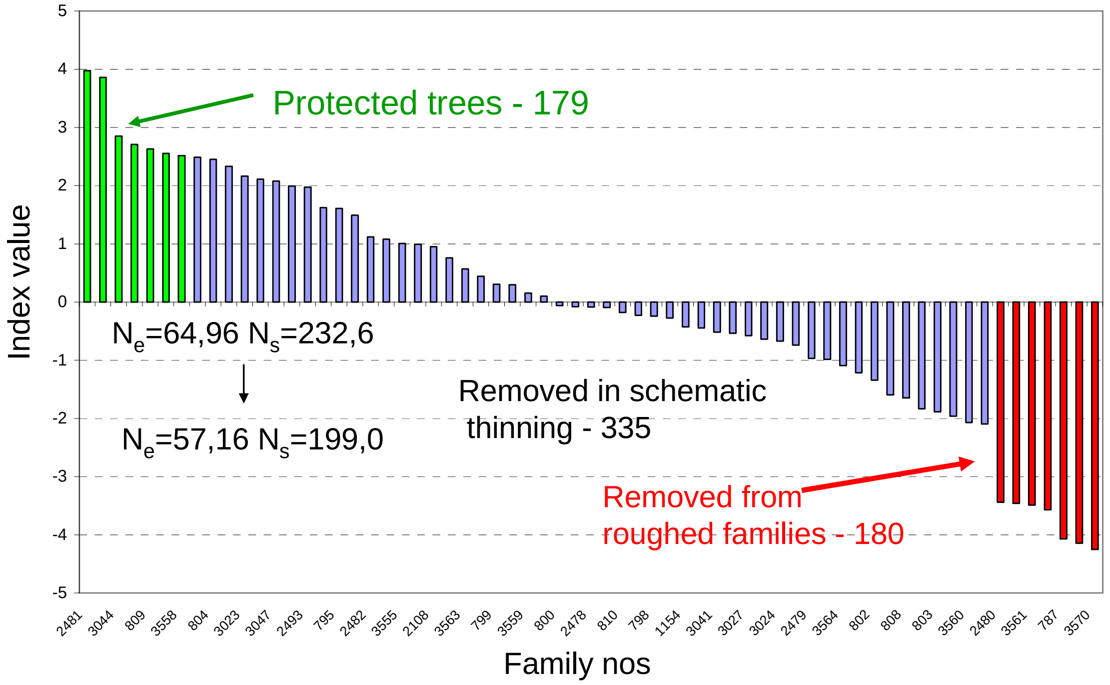
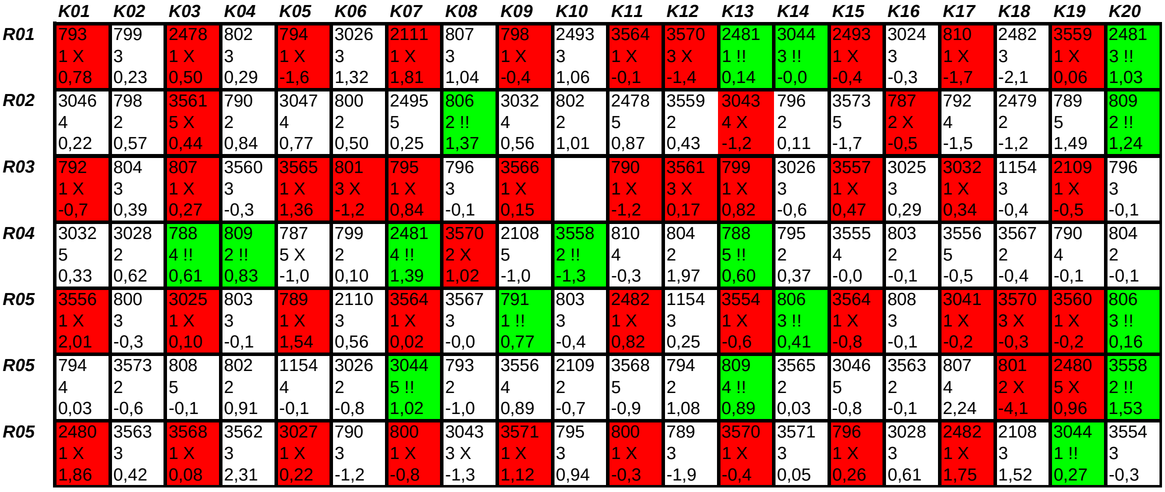


Fig 1. Estimated index values of families and thinning description.



	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 - tress to be removed firs do 5 – destination trees). On the bottom of each cell is written tree index value. Trees marked on red color are planed to 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 (Ne). 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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Deployment of clones to seed orchards when candidates are related

Dag Lindgren [1] and Darius Danusevičius [2]

[1] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden. E-mail: dag.lindgren@genfys.slu.se

[2] Lithuanian Forest Research Institute, Liepu 1, Girionys, LT-53101, Kaunas district, Lithuania. E-mail: darius.danusevicius@takas.lt

Abstract

This paper deals with deployment of clones to seed orchards in situations when the candidates are relatives. Possible strategies comprise finding the best solution by an advanced computer algorithm, restricting against relatives and neglecting relatedness. Linear deployment versus truncation was considered. The value of application of a strategy has to be quantitatively defined to make comparisons among strategies; here it was defined as "Net gain", considering predictions of breeding values, inbreeding depression, gene diversity (status number) and effective number. The efficiency of the strategies was studied in a (ideal) population of candidates composed of half-sib families. Results showed that, if the number of families available is considerable higher than the number of desired selections a close to optimal and simple strategy is to restrict against any relatedness and deploy the single best individual from the best families by linear deployment. Otherwise a more sophisticated algorithm is suggested. Here it is suggested that the simpler procedure can be used when the status number of the candidate population is 8 times higher than the status number of the clone deployment suggested. It is suggested that the effect by relatedness on gene diversity is more constraining on the extent of relatives in seed orchards than the inbreeding depression following mating of relatives.

Introduction

When tree breeding has passed its infancy, the option arises to select related clones to seed orchards. One way out of this dilemma is to structure the breeding population in unrelated compartments and select one clone from each (Lindgren and Gregorius 1976). However, this strategy has disadvantages. It is a restriction on selection and the best related selection is likely to have a much larger breeding value than the best unrelated. Inbreeding is a problem in the breeding population, it is an additional cause of variation and it reduces the variance available for selection. The consequences of restricting against relatives in seed orchards become more severe and annoying as breeding continues over generations. A limited number of relatives in a seed orchard has only a small and may be neglectable negative effect (Olsson 2001).

This study deals with how the deployed proportion of each candidate clone can be decided at the establishment of a seed orchard when the breeding values are available for each candidate in a population of half-sib families. Conventional seed orchard deployment strategies relied on simple truncation selection: selecting the candidates with breeding values above a certain threshold and deploying the candidates in equal proportions. Lindgren (1974) showed that deployment of candidates proportionally to their breeding value is a more efficient approach. If the candidates are unrelated, the deployment of clones in proportions linearly related to their breeding value is the most efficient strategy to maximise genetic gain (Lindgren and Matheson, 1986). In advanced breeding cycles, however, the candidates tend to be related and the linear deployment strategy does not guarantee an optimum solution. The emphasis on breeding value of related candidates may increase relatedness, hence inbreeding in the orchard to harmful levels (Olsson, 2001; Wang Tongli *et al.* 2003). One simple approach to cope with the negative effects of relatedness is to introduce constraints on relatedness, such as restricting candidates to say, the one top-ranking individual per family and then use linear deployment for the ramet number. Is there a better deployment solution?

The aim of the present study is to develop and investigate procedures to deploy tested, related genotypes to clonal seed orchards with variable numbers of ramets across a range of scenarios with simple relatedness patterns. Simulation procedures are used based on artificial data generated for half-sib families that provide the candidates pool for seed orchards. Results may help to guide forest tree breeders about the potential and application of the method over a range of circumstances.

Material and methods

The following deployment strategies were compared: (a) truncation selection by selecting the clones with the breeding values exceeding certain threshold and deploying equal number of ramets (*Truncation* strategy); (b) truncation selection by selecting only one best individual within each family (*Truncation unrelated*); (c) maximizing gain at a given effective clone number (*Linear deployment*); (d) linear deployment by selecting one best individual within each family (*Linear deployment unrelated*) and (e) maximizing net gain at a given gene diversity (*Optimal proportions*).

Net gain was the target parameter to be maximized and was calculated as as the average breeding value of seeds produced from the orchard with a deduction for the expected inbreeding due to matings between related clones:

$$BVI = (1 - ID * \Theta_P)\sum p_i g_i \text{ , [4]}$$

where:

BVI is the predicted average breeding value of the ramets adjusted for the expected inbreeding (ignoring selfing).

$(1 - ID*\Theta_P)$ is a factor reducing the breeding values for inbreeding;

ID is the inbreeding depression "coefficient", which converts pair-coancestry to adjust for the expected inbreeding depression to the same scale as the breeding values. In this study, we assume no difference in inbreeding depression between orchard ramets and so set $ID = 1$, which is a reasonable value for forest tree applications and can be interpreted that production will be zero if there is complete homozygosity (if $ID= 1$ and pair-coancestry = 1, then the term (1-ID*pair coancestry)= 0 and $BVI = 0$).

Although in principle it is desirable to use the optimal procedure for all cases, in practice it may be difficult to impellent because specific software and expertise are needed.

The study focus on “linear deployment unrelated” and described its advantages and characteristics for a number of possible typical cases. The genetic gain adjusted for predicted inbreeding depression (*Net gain*), gene diversity and effective clone number were considered as the main ranking criteria. The data used in this study were simulated breeding values for unrelated half-sib families, which are considered as candidates for deploying in a seed orchard.

Order statistics were used to generate the “true” family and within family breeding values, expressed as standard deviations from the total mean set to 0 (Lindgren and Nilsson, 1985). The breeding values were expressed as "units of coefficient of additive genetic variation (CV_A)". The optimization was carried out according to the main and alternative scenarios of the key-parameter values (*Table 1*). When testing an alternative value of a parameter the values of the other parameters were kept at the main scenario values. Status number (N_{sd}), the number of unrelated and not inbred individuals desired in the seed orchard was used as the diversity measure. Group coancestry can be interpreted as the loss of the gene diversity in the wild forest by the implementation of tree breeding (Lindgren and Kang, 1997; Lindgren and Mullin, 1998 and Rosvall 1999). The first term expresses the self-coancestry, which depends on the number of ramets of a clone. The second term depends on the relatedness among different clones (pair-coancestry) and their occurrence in the orchard. *Net gain* was considered as the parameter to be maximised. In our study, net gain refers to the difference between the average breeding value of the ramets in the orchard adjusted for expected inbreeding depression and the average breeding value of the test, which is set to 100 to allow interpretation of the numbers as percentages. The *Net gain* is calculated as the average breeding value of seeds produced from the orchard with a deduction for the expected inbreeding due to matings between related clones. The number of half-sib families, the number of individuals from each family as well as number of ramets of each individual (expressed as proportion) to be included in the seed orchard were optimized to achieve maximum net at a desired status number in the seed orchard. For the calculations MS EXCEL 2003 was used.

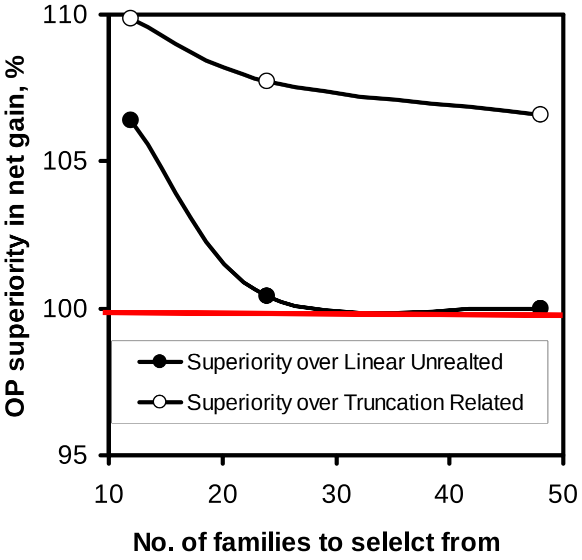

Figure 1. Superiority of Optimal strategy over the second ranking Linear Deployment unrelated (filled circles) and the worst Truncation related strategy.

Results and discussion

The *Optimal proportion deployment* strategy returned the highest *Net gain* at all scenarios considered here (Figure 1). The *Optimal proportion* strategy produced 5% greater *Net gain* than simple truncation selection for all scenarios except when there was no inbreeding depression and many candidates from many families were available (Figure 1). Thus, there are better strategies than simple truncation selection for breeding value as supported by Lindgren and Matheson (1986). The *Optimal proportion deployment* strategy was most efficient at the scenarios. *Linear deployment unrelated* was the second best approach in most cases (Figure 1). The efficiency of the *Linear deployment unrelated* strategy was especially low when the number of families dropped to 12 at the desired N_s of 12.

In certain situations, the efficiency of the *Optimal proportion deployment* strategy was not markedly different from that of the *Linear Deployment unrelated* strategy (Figure 1). A technical disadvantage of the *Optimal proportion* strategy is that it requires a more complex software than practical breeders usually operate and the optimization procedure is more difficult to understand if compared with the *Linear deployment* strategy. Thus, it seems worthwhile to closer examine the cases where the *Linear Deployment unrelated* may replace the *Optimal proportions* deployment. The basic difference between these two strategies is that *Linear deployment* considers the breeding value only, whereas, *Optimal proportion* is searching for the combination of genetic gain and relatedness to maximize the *Net gain* and in this way it takes account the relatedness of the candidates. Therefore, in cases when there were more families to select from, there was no marked difference between *Optimal proportions* and *Linear deployment unrelated* strategies, because both tended to select one top ranking individual in each family (Figure 1). However, fewer available families (low genetic diversity available for deployment) forced the selection of relatives and the *Optimal proportion* strategy was more efficient to optimize the numbers of the related half-sibs.

Inbreeding had generally rather small effect on net gain, even in the most drastic cases analyzed (selection of a status number 12 seed orchard from 6 half sib families by truncation selection of the best) inbreeding depression reduces net gain only about 2 percent, and if there was much diversity in the candidate population inbreeding had almost no importance and it turned out favorable to select unrelated clones anyway. Comparing 6 and 12 families of size 40, the presence of inbreeding depression reduced the superiority of selecting from 12 from 5.0 to 3.9, indicating that diversity considerations was a more severe constraint for gain than inbreeding considerations. Thus the demand for a certain status number among seed orchard ramets forces to go further down in the list to clones with a lower breeding value rather than to avoid inbreeding.

Another point where optimization of deployment may be made is family number and family size under constant test size in the tests from which the selections to the orchards are made. Figure 2 illustrates that if demand for diversity is high, it is an advantage to test more families of small size versus few but large families. However, if there is high diversity to choose from (the number of half sibs available is larger than the desired status number), the number of families and their size ratio is of little importance.

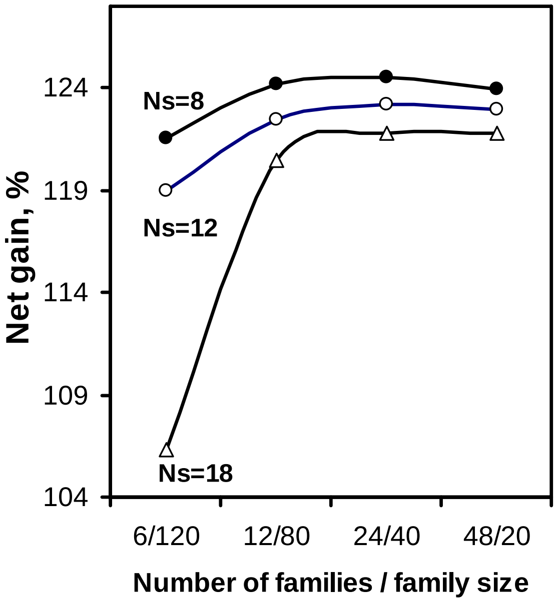

Figure 2. Effect of variable combinations of family number and family size at a fixed test size on net gain from Optimal proportions strategy for the three diversity levels desired in the seed orchard (expressed by status number (Ns).

The total number of clones deployed was never higher than 40. The largest number of clones was obtained in the scenarios where high diversity requirement forced selection more families (scenario with 48 families for N_s of 24). For a more realistic scenario with the desired N_s of 12, the optimum number of clones varied at about 20. At constant Ns of 12, doubling the family number form 24 to 48 did not affect the optimum number of the deployed clones. Thus, if N_s of 12 can be accepted as sufficient diversity level in the new seed orchards, then not more than 20 clones are needed. Lindgren and Prescher (2005) optimized number of tested clones in the seed orchards and suggested a similar figure of 20 clones for the first cycle of orchards comprising tested clones given similar conditions as in our study.

In conclusion this study shows that if there is a large number of half-sib families available for deployment to seed orchards (e.g. status number available among candidates is at least 8 times greater than the status number desired in the seed orchard), the best advice is to use linear deployment unrelated strategy: take the single best candidate from the best families and deploy the ramets linearly according to the individual breeding values. The number of families to select from can be selected to satisfy the desired status number. If such large reduction of diversity is not tolerable or the candidates tend to be related, optimization with the *Optimal proportions* strategy is recommendable.

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Swedish seed orchards for Scots pine and Norway spruce

Dag Lindgren[1], Bo Karlsson[2], Bengt Andersson[3], Finnvid Prescher[4]

1) Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden E-mail: dag.lindgren@genfys.slu.se
2) Skogforsk, Ekebo 2250, SE-268 90 Svalöv, Sweden
3) Skogforsk, SE-910 36 Sävar, Sweden
4) Svenska Skogsplantor AB, Seed production, SE-340 14 Lagan, Sweden

Summary

Clonal seed orchards of Scots pine and Norway spruce has been established since almost six decades. Plustrees were phenotypically selected in forests. Initially most of the selections were made in natural mature forests, but since 1980 most selections were done in middle-aged plantations. The selections were grafted and the grafts planted in seed orchards. The last decades many orchard clones have been selected based on testing. Testing is often progeny-testing, earlier with complicated mating designs, but more recently often with wind-pollination. Testing for spruce often means testing with cutting-propagated clones. Currently more than 60 percent of all planted plants come from seed orchards and the figure is increasing as new orchards start producing seeds. The additional production of planted forests from seed orchards today is estimated to around ten percent, and is rising. Establishment of a new round of seed orchards has been launched, which will lead to almost complete seed orchard supply for plant production with considerable higher genetic quality than what is used today.

Introduction

This paper describes Swedish seed orchards for Scots pine and Norway spruce. There are seed orchards of other species, but Norway spruce and Scots pine dominate forest plantation. In 2005 the numbers of available forest plants in Sweden were: 125 million Scots pine; 194 million Norway spruce; and 11 million of all other species. The statistics origin from the Swedish Forest Authority and it should reflect planted plants in Sweden, even if some plants which do not do it to the actual plantation are included. Import is included with about 40 million plants; the intention is that exports should be excluded. Clonal plants of spruce are used, but much less than a million. Seed orchards are almost the only way to get tree improvement out into the forest, and even if clonal forestry is technically and biologically possible, it is not seen as an economic option and no nurseries has currently any intentions to scale up its use.

The annual growth of Norway spruce in Sweden is larger than that of Scots pine and the rotation time shorter since spruce generally grow on more fertile soils. However, the species occupy approximately equal size of forest land area, spruce somewhat more in south and pine somewhat more in north. Emphases of breeding have been similar for spruce and pine, and the effort is also basically rather similar in different parts of the country. There are no seedlings seed orchards for the two major species and vegetative propagation has been done by grafts, but recently cuttings have been used to a greater extent for Norway spruce. Most grafts are propagated in nurseries and planted, but field grafting is sometimes applied.

It is practical to describe the development of the Swedish seed orchard program as three distinct rounds at different epochs, even if the real World is less simple. There were several decision makers and the exact circumstances are case specific, and ideas change over time. The moment of the establishment of a seed orchard is often not exact and not exactly compatible for different seed orchards. There is a planning period, there are ground preparations, grafts take time to prepare, some orchards are first planted with root stocks and grafting is done subsequently in the field, the whole seed orchard is seldom planted in a single year and fill in or complementary plantations may occur, thus “establishment year” has usually character of an average and the exact definition varies sometime in records. Thus “epochs” do not have distinct borders and all events are not typical for the epoch. The three rounds are here chronologically associated to years: the first round ≈1949-1972; the second round ≈1981-1994; and the third round ≈2004-onward, although this is not to be interpreted as exact limits.

The first round (≈1949-1972)

The concept of grafted conifer seed orchards has a long history. The first development in Scandinavia was done by Syrach Larsen in Denmark about 70 years ago (Larsen, 1934). The idea was promoted in Sweden by Holger Jensen at Ramlösa nursery, and a large seed orchard program was launched mainly according to these ideas. To make grafts in a large scale was a slow procedure, the selected plus trees in the natural forest were old and could not be harvested for many scions, and the scions harvested were typically not very vital. It was more feasible - but very time consuming - to first graft a few primary grafts and then make many secondary grafts. A number of small graft archives and experimental seed orchards were established. The development in Denmark was hampered and downgraded because of the occupation during the war and the urgency to rebuild after the war, while Sweden was not much affected by the war and could accelerate the already initiated tree breeding program immediately after, stimulated by the large request of timber for rebuilding Europe, where Sweden was the major unharmed supplier. The first full industrial scale conifer seed orchard in Scandinavia was initiated 1949 in Drögsnäs at Brunsberg in central Sweden (lat. 59˚37’ N) with planting of Scots pine grafts. Another early industrial scale effort occurred around Kratte Masugn (Anonymous 1963). Still today the outlines first suggested by Larsen (1934) are usually applied when establishing new seed orchards in Sweden and elsewhere.

The mature seed orchards established before ≈1972, which now supply most seed orchard based forest regeneration material, are now successively taken out of production. The following was rather typical for this first round of orchards:

- Selection of plus trees in mature forests. For Scots pine it was mostly naturally regenerated Swedish forests, while for Norway spruce in south Sweden some of the selections has been done in plantations with either indigenous or continental European origin or selected in Poland.
- The plus trees were carefully described and compared to measured comparison trees. There was a formal approval process.
- Scions were harvested and the plus-trees were multiplied as grafts. Clonal seed orchards were established using such grafts.
- Most plus trees were placed in seed orchards (1300 pine and 900 spruce clones).
- A typical seed orchard comprises 40 clones.
- The seed orchard program was associated with progeny tests of the clones in the seed orchards. Progeny tests were often done by controlled crossings in the seed orchards, when the grafts produced flowers. Thus progeny-tests established before 1980 are usually associated to specific seed orchards. Progeny tests were often performed with many (5 or more) matings per parent, large progenies (200) and replications on many sites (5).
- The seed orchards were usually established foreseeing a 50% systematic thinning. Genetic thinning, which was planned for a later stage, has been implemented sometimes but seldom. A typical object was established with 400 grafts per hectare and 40 clones in the orchard. At maturity usually somewhat less than 200 grafts per hectare remain.
- Several Norway spruce seed orchards were established to benefit from hybrid effects when provenances with rather diverse origins were brought together (Swedish and continental), but the

progeny appeared disparate and thus these seed orchards were thinned so only one component remains. Thus the idea of hybrid seed orchards failed for Norway spruce.

- Today changes in the set up of many of the mature seed orchards have occurred compared to initial plans and maps. E.g. it happens that bad clones are removed and better ones are filling their original planting space.

To optimize genetic gain more parents with less offspring each should have been tested. Further, the use of wind pollinated offspring from the plus trees in the forest would have decreased the time lag for testing. However, at that time genetic parameters and ways of inheritance were basically unknown and the large trials with dialells were (and still are) a valuable source for deriving basic data.

The first round of seed orchard program stopped around ≈1971, few seed orchards were established in the following decade. Some of the first round seed orchards are now cut down as they are replaced by newer units and more will be taken out of production soon.

The second round (established ≈1981-1994)

1982 a new nationally coordinated seed orchard program was inaugurated, the seed orchard establishment costs was paid by the state.

Since ≈1980 the results of the trials established in connection with the first round started to become evaluated and breeding values for plus-trees started to become available. Partly this material was used in the second round.

The selected plus-trees in the first round were regarded as an insufficient base for long term breeding and the tested trees were too few to support the second round program more than to a limited extent.

The main principles applied in plus tree selection and seed orchard establishment were:

- New selections were carried out in young culture stands (typically 20-40 years old). For spruce in southern Sweden it was often plantations of foreign origin. Reasonable aged plantations with known history was less frequent at the early plus tree selections, mainly because planting was less common, and did often not use what we now consider as “appropriate” provenances. Reasons for making selections in rather young plantations were:

  - It is in the correct geographic and climatic environment (provenances move somewhat initially)
  - It is in the correct silvicultural environment (planted and not natural regenerated)
  - Imperfections in especially the stem and branch quality in the most valuable bottom trunk of stem are not hidden inside the trunk.
  - Heritability can be expected to be higher in a uniform plantation than a natural regeneration, which may be unevenly aged.
  - More vital grafts from younger ortets, and as more plus-trees were selected, the requirement of grafts per tree could be relaxed.

- Emphasis on many selections (instead of accuracy of the individual selections, no comparison trees, except a few "unselected" checks). Around 5000 new plus trees of pine and spruce were selected. As many selections were done and genetic thinning was planned it was also natural to have many clones in seed orchards, typically more than a hundred.

- After selection, scions for grafting and wind pollinated seeds were harvested from individual trees and used for progeny testing. Thus the connection between specific seed orchards and the progeny test was relaxed. It was seen as too time consuming to wait till controlled crosses could be made on grafts, but still it was necessary to make grafts to store the selections and produce secondary scions for production of grafts for the seed orchards. When seeds were not available from open pollination in the selection stands, seeds from archive trees were also used for progeny testing.

- Seed orchards were often established foreseeing genetic thinning based on progeny test. However, the foreseen genetic thinning has until now rather seldom been realized.

- For some plus trees growth rhythm of progeny in green house or nursery has been guiding.

- Many seed orchards used some tested material and a few were established with only tested material. Three of the second round Scots pine seed orchards are based on result from short term adaptation/autumn frost hardiness tests. Also plus trees from the Finnish orchard program was tested, selected, and included. Some Norway spruce seed orchards in south Sweden were established after phenological test of the clones in the nursery.

- A few Norway spruce seed orchards are based on clones tested in clone tests (not progeny-tests), these are among the second round seed orchards with highest predicted gain, but have not yet reached the national list.

- The design of seed orchards become more variable than in the first round and depending on special circumstances, in particular the availability of bred material. It was also an intentional effort to try different models to widen the experience. As a consequence the clone number of second round seed orchards varies among ten and several hundreds.
- Almost half of the second round pine seed orchards on the National list (Table 1) are regarded as tested, while none of the spruce seed orchards. In spite of that the calculated gains is some percent higher for the spruce seed orchards than the pine seed orchards (Figure 1). This is probably as many of the second round spruce seed orchards which have (clone-)tested clones are not yet in the National list.

Table 1. Number of seed orchards in Sweden. The seed orchards where seeds are marketed or planned to be marketed soon are in the Swedish National List (read 2006) of approved basic materials. There exist new seed orchards, which are not on the list, as the seeds are not on the market yet. Old seed orchards are taken out of production, but seeds can still occur on the market. The category "Qualified" means phenotypically selected plus trees and the category "Tested" in the Table means progeny-tested Scots pine plus trees (sometime the test may be short-term). If only a minor part of seed orchard trees are tested it becomes "qualified". The number of seed orchards in the list is compared to all seed orchards.

	In Swedish National List of Approved Materials				All established	
Species	Qualified	Tested	≈ -1980	≈1981-2000	≈1981-2000	2004-
Scots pine	61	9	50	20	24	2
Norway spruce	27	0	22	5	12	3

When the second round coordinated program was initiated 1982, the intended area was 510 hectares of pine and 550 hectare spruce orchards, what actually became established was 350 hectares of pine and 200 hectares of spruce (Table 3). The largest reduction was for spruce in southern Sweden, which will lead to a lack of improved seeds decennia ahead.

This second round program can be seen as completed around 1994 even if a few seed orchards were established 1994-2003. Establishment of seed orchards was supported by governmental funds 1982-1994. This funding was discontinued 1992/93. The funding was derived from a tax on forest land, which was removed, and when also the benefits connected to it. The socialistic idea with a tax is that the state can use the money earned by forest owners and companies wiser than the they can do themselves. It took a decade for the Swedish forestry to find an administrative solution for paying the costs for seed orchard themselves after the money became available by releasing the tax from the shoulders of the forest owners, but in the end they did it and the third round of seed orchards started.

Swedish Scots pine seed orchard life time. There are 48 seed orchards on the national list established before 1971 and only 15 later 1971-1990, and most young seed orchards have not yet reached full production yet. Even when a seed orchard is established, the clones may be selected long time earlier. That probably means that much of the seedlings planted today originate from seed orchards harvested more than 35 years ago. A life time of Scots pine seed orchards above 40 years seems likely, once they are reasonable well established. Recently El-Kassaby et al. (2007) argued that Scots pine seed orchards become genetically outdated when they are aged 30, if the long term breeding functions as well as predicted. It seems desirable that Swedish Scots pine seed orchards are replaced more aggressively.

The third round (established 2004-?)

A national coordinated seed orchard program without governmental financial support was negotiated during the first years in the current millennium and launched 2003. Its structure and goals are described by Rosvall and Ståhl (2008). All genetic material is planned to be selected based on testing in one way or another. Some, but not much, will be offspring to tested clones. Until 2007 two pine seed orchard and four spruce seed orchards have been planted or planting has been started, but two of these orchards can be seen as single owner orchards, which would have been established even without the coordinated program.

Since most of the available large number of clones in the latest round of seed orchards is tested, the genetic gain will be considerably larger than in the first two rounds. Rosvall et al (2002) calculated the average ideal (no reduction for contaminating pollen etc.) possible gains to be 33 and 37% for pine and spruce respectively. A more realistic estimate is suggested to be 24 and 26%.

Retrospectively, the Swedish seed orchards have been described in terms of three distinct rounds. The cause for these phases is mainly administrative changes in the funding system by the Swedish political system, there is no "scientific" justification, and it is not rationale. The future will hopefully not mark out a distinct “Fourth round” of Swedish seed orchards after the end of the decided third round. Hopefully, from now on a smoother and more continuous program will be driven by genetic progress in the long-term breeding (Prescher 2007). Another argument for a continuous seed orchard establishment is the different breeding generation times. Southern populations will have a faster rotation turn over due to faster testing. A rolling front within the breeding population will also influence timing. Hopefully the forestry actors will establish new orchards when there is significantly improved material available. The size of required improvement will vary with economic resources and predicted demand of wood. Seed orchard themselves will probably turn more rolling front where parts are updated and replaced rather than starting from scratch. One argument for that is to get an early improved and better known pollen cloud rather than contamination.

The impact of seed orchards on Sweden

Table 2. National statistics for percent seed orchard seedlings in Swedish plantations.

	Percentage of seedlings from seed orchards				
	1975	1990	1995	2001	2006
Scots pine	60	60	Most	62	78
Norway spruce	Small	15	14	38	49

Since 2001, the statistics is based on enquiries to plant producers by the Swedish Forestry Authority, earlier statistics is more subjective estimates based on the potential of seed production and may be slightly overestimated. Since the mid 80s, plant production has decreased from more than 500 million plants per year to about 320 millions. Furthermore, pine plantation has decreased in southern Sweden (due to game injuries) and spruce plantation increased.

For Scots pine plant production, most seeds came from seed orchards the last three decades in southern and middle Sweden. In the central part of Sweden, i.e. latitude 62-64° N, there is still a lack of seed orchard seed. The most significant lack is for the harshest areas in the north, and seed orchards better adapted to that area now comes into production, thus the seed orchard use may be expected to rise to 90% in a few years. The seed orchard use of Scots pine will probably rise steeply soon as the second round of seed orchards has started to produce significant amounts of seeds the last years. However, even the second round of orchards was not established to cover the whole need for plant production in the north, and the limited increase between 1975 and 2001 give reason for some worries.

The situation for Norway spruce is different than for Scots pine. The first and second round spruce seed orchards were established in the end of the period and the area was insufficient for both rounds. The fructification of Norway spruce starts about five years later than for Scots pine, and thus seed from the orchards become available much later than for pine. In the late 70s and early 80s small crops were harvested, but it took until 1989 before the first big seed crop. Swedish foresters were conservative and cheap and rather good imports were available, and thus the marketing of spruce seed orchard seed was not easy when they first become available. Today the demand for seed is higher than the production capacity from the orchards in southern and northern Sweden. Unfortunately the lack of improved spruce seed will continue some decades; the withdrawn funding for establishing new orchards in the second round, mainly had impact on spruce in southern Sweden. These orchards were planned to be established in the end of the period because the progeny tests intended to be used for selection of clones, were going to be evaluated some years later. However, the changed funding situation totally stopped the establishment program of spruce orchards before it was complete. It is required that seed orchards only a few years old or not established reach full production to come close to 100% seed orchard use, and that will not occur the next decade.

Table 3. Reasonable successful established area of seed orchards (hectares)

	First round ≈-1972	Second round ≈1981-1994	Third round ≈2004- (2006)
Scots pine	575	350	28
Norway spruce	230	200	50

The genetic gain of seed orchards

Genetic gain considerations and estimates for Swedish seed orchards were presented by Rosvall et al. (2002). Gain estimates can be interpreted as gain in value production or gain in volume production at a constant quality. The gain of the early seed orchards can be said to have three sources: 1) the clones placed in a seed orchard origin from different populations and therefore trees are less related than in a stand, thus seed orchard seeds suffer less from inbreeding depression and may benefit somewhat from hybrid vigor. The gain by this is assumed to be 2%. 2) Some seed orchard phenomenon can be seen as equivalent of a gain, mainly “epigenic” effects ("aftereffects", one reason is heavier and more developed seeds and faster starting seedlings, the effect of that may hang on into productive ages) but also that it is easier to produce a more uniform and predictable plant crop in the nursery. This appears as a "genetic gain" which is assumed to be 2%. 3) The most important generator of gain is the artificial selection of superior trees. The selection gain when choosing plus trees in the forest was estimated to 6%. These generalized gain estimates build on experimental results with comparisons between artificial plus-tree crossings and commercial checks.

Considerable larger gain is achieved where selection is based on progeny or clone testing. Genetic gain after genetic thinning is rather small, but has some importance for some seed orchards.

The predicted gains of the second round seed orchards serving different areas of Sweden has been indicated on the target areas on maps of Sweden in Figure 1. These figures are based on estimates of the breeding values of the clones in the seed orchards and assume reasonable provenance transfer, thus that the seed orchard seeds are used in the intended area.

A gain thief is pollen contamination, probably only about half of the fertilizing pollen originates from seed orchard trees. Efforts to reduce this loss of gain have had only limited success. The contamination is an argument against small seed orchards, but seed orchards are anyway usually rather large for administrative and operative reasons. It is also an argument not to move seed orchards too far to the south compared with the origin of the clones. The location of the third round of seed orchards for harsh northern areas will probably be located a little more northern than the current seed orchards as the impact of the pollen contamination was not fully accepted during the second round program. Some of the valuable forest production in a stand origins from non-planted non-improved plants ("volunteers"), the currently used estimation is that on an average 80% of the forest production in plantations originate from the planted plants. Estimates of gain considering these two gain-reducing factors indicate that on average second round seed orchards of Scots pine increase forest production by 9.4 % and for Norway spruce 11.6 % compared to the production if seed orchard seeds were not used. If seed orchard seeds are used further away from where they are targeted compared to stand seeds that may reduce the realized gain somewhat, no estimates have been done.

Selfing and lack of diversity is predicted to reduce the possible gain from seed orchards, but only to a similar extent as stand seeds, and are thus not regarded as negative factors in the calculations in the study, at least not if the number of clones is not dropping lower than predicted for the third round of seed orchards (Prescher 2007). Selective harvesting where the offspring of the best clones is used for plant production has been used in a few cases and an expanding use is predicted, but the contribution to realized gain is still small.

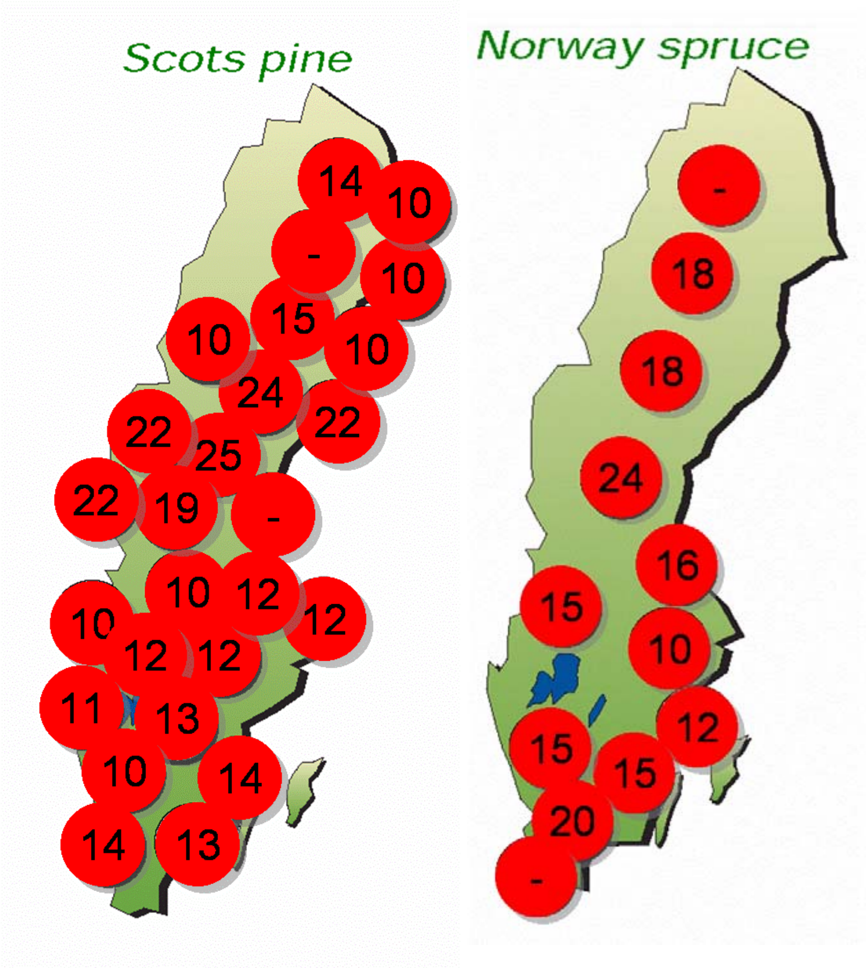

Figure 1. The genetic gain for the most modern existing seed orchards of Scots pine and Norway spruce established before 1998 (Rosvall et al 2002). The gain is an estimated percentage production advantage compared to stand seeds assuming only seed orchard progeny in the regeneration.

The added allowable harvestable timber in Sweden of seed orchards according the most probably scenario in accordance with current decisions and predictions was calculated by Rosvall (2007, personal communication). In Figure 2 this was compared with the impact of other suggested realistic methods to increase forest production (fertilization, clone forestry (SE), drainage, conversion of agricultural land to forest; contorta pine, better regeneration techniques) in a scenario considering economy, administrative constraints, likely technological development, environmental concerns,. As a reference level, the possible harvest was based on the forest maintainance and silviculture used during the 1990:s (SKA03) (Skogsstyrelsen 2004). The mentioned improvements were added to the reference level.

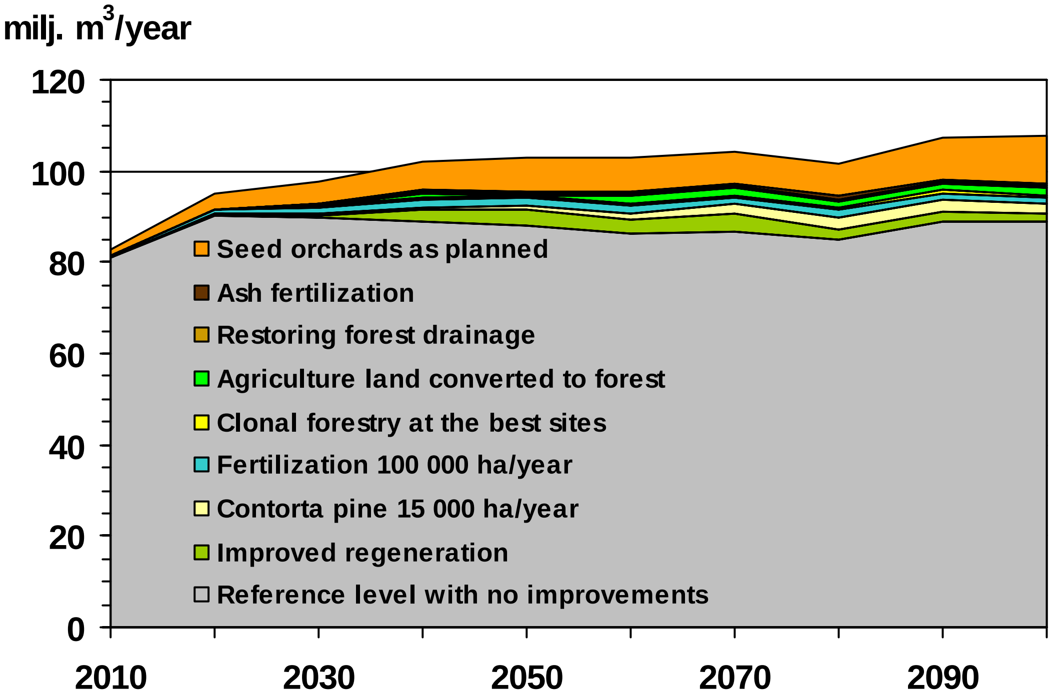

Figure 2. Allowable sustainable timber harvest in Swedish forests under different scenarios to increase production. For seed orchards it is the difference including the planned program compared to no seed orchards. The gain by seed orchards is considerable larger than all other options together.

Interaction and coordination of government, seed orchard owners, forest owners and research

The situation 2007 is that seed orchards or nurseries are not directly owned, controlled or paid for by the government or decisions under governmental control. The state controls formally Svenska Skogsplantor AB via shares in Sveaskog, but the ambition is to get it to work as a private company. Sveaskog owns about 35% of the plant production capacity and about 55% of the seed orchards. Other owners are large companies, forest owner associations, the church, and persons or companies in the plant business. Typically there are several owners to one seed orchard, but a single operative manager. The owners share seeds and costs. This shared ownership is managed in separate ownership groups for each seed orchard, and works well. A reason for shared ownership is that risks are spread; instead of one orchard per owner, the owners have shares in several orchards for the same utilization area, another reason is to get sufficiently large seed orchards and a third reason that many operators in many part of Sweden are to small to support own seed orchards.

For the second round of seed orchards (1982-1994) the coordination was lead by the government, and the establishment (but not the running costs after the first 5 years) was fully financed by governmental funds on condition that it was a part of the coordinated program. The grants originated from a forest tax, and that forest tax was discontinued 1992 and so the benefits derived from it like establishment of seed orchards. It took most of a decade till a similar coordinated program was initiated without state support.

For the third round three committees of interested organizations have been established for the three distinct geographic regions: north, middle and south Sweden. These groups meet currently around two times a year to discuss issues on the third round seed orchard program. Skogforsk has played an important role for getting a nationally coordinated third seed orchard round implemented. The state pays about half of the cost for the backing up long term tree breeding, which the current seed orchard program benefits from, through SkogForsk. The state also contributes to seed orchard research in a similar way.

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Advanced-Generation Seed Orchard Designs

Milan Lstibůrek[1] and Yousry A. El-Kassaby[2]

[1]Department of Dendrology and Forest Tree Breeding, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, 165 21 Czech Republic

[2]Department of Forest Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

Abstract

Over 30 years of seed orchards establishment and management, the spatial configurations of clones remained unchanged creating a paradoxical state. This is characterized by static seed orchard designs in an environment of constant changes driven by advancement in breeding programs with the majority entering their advanced generations phase. Additionally, the vast accumulation of knowledge pertaining to reproductive biology, ecology and output have changed our perception and assisted in rejecting of some seed orchards' myth. Advanced generations breeding programs feature complicated relatedness structures, combinations of backward and forward selections, desire to capitalize on proportional deployment or exploiting assortative mating etc. We propose two novel seed orchard designs applicable to first and advanced generations where these issues are considered. The first design combines the benefits of randomization and systematic arrangement of ramets and their clones after considering their regional proximity to neighboring clones from the same parent (called Randomized, Replicated, Staggered Clonal-Row (R^2SCR)) facilitating easy crop harvest. The second design (Minimal Inbreeding) utilizes global assignment of clonal ramets within the entire orchard with the objective to minimizing potential inbreeding in the seed crop. Many modifications to both designs are available, making them flexible, yet efficient in delivering genetic gain to operational forestry.

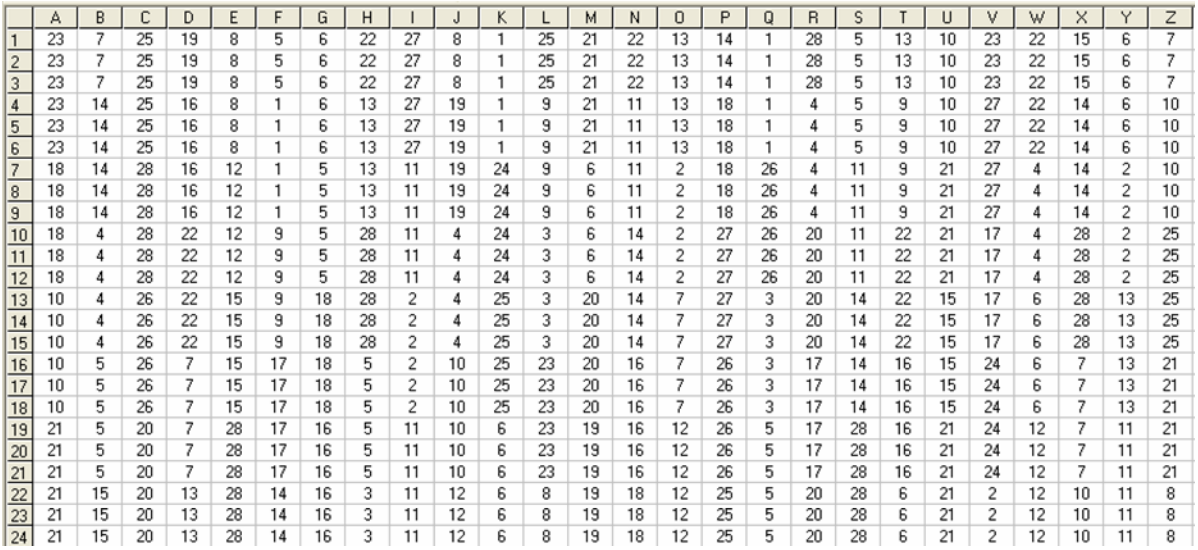
Introduction

During the initial phase of forest tree breeding, seed orchards have evolved as special-purpose plantations to facilitate mass production of genetically improved seeds. During the same period, number of special orchard layouts has been developed (see Giertych 1975 for a review) with a primary goal – to maximize the gain-conversion efficiency between breeding programs and operational forestry. At this initial stage, breeding programs started out with initial unrelated individuals' selections from wild stands, thus relatively simple orchard layouts served well its purpose. Fifty years later, the genetic statuses of breeding programs' have changed considerably with increased relatedness and inbreeding coupled with greater selection differentials and the emergence of new ideas for seed orchard structures such as proportional clonal deployment to maximize gain while managing diversity (Lindgren and Matheson 1986) and clonal-rows facilitating ease of harvesting without appreciable build-up of inbreeding (El-Kassaby 2003; El-Kassaby et al. 2007). Multi-generational breeding programs offer the opportunity to select material across generations, supported by the development and implementation of efficient genetic evaluation techniques with interested desire in combining specific parents with desirable properties. Additionally, advances in reproductive biology (e.g. Erickson and Adams 1989, Adams and Birkes 1991, Perry and Knowles 1990, Xie et al. 1991, Mitton 1992, El-Kassaby et al. 1994, O'Connell et al. 2004), including better understanding of mating systems, pollination biology, fertility variation, and contamination as well as the recent emergence of new breeding strategies (called "Breeding-Without-Breeding") that integrates classical breeding and pedigree reconstruction methods (El-Kassaby et al. 2006) with its specialized seed orchards (breeding arboreta) designs that maximizes the outcrossing rate among parents. All these factors individually and in concert made it possible to consider new seed orchard designs.

In order to develop appropriate seed orchard design suitable to advanced generations, one needs to identify relevant criterions, such as the acceptable overall outcrossing rate and/or the desire to perform crosses among numbers of specific parents, integration of related and inbred trees originating either from the same or different generations, separation of specific parents, different orchard's shapes/configurations, proportional deployment etc. In the same time, it is obvious that neither design will be optimal in every situation, due to year-to-year variability in reproductive output; however, some species-specific reproductive patterns (e.g. pollen dispersion) and environmental conditions (e.g. prevailed wind direction) should be considered during the establishment of advanced generation seed orchards.

Regional Assignment

El-Kassaby & Lstibůrek (in preparation) developed the "Randomized, Replicated, Staggered Clonal-Row (R^2SCR)" design that combines the advantages of systematic and randomized designs. This design allows each clone's rows to be separated by a predetermined radius, thus minimizing mating among ramets in a specific row with others of the same clone and maximizing mating among unrelated clones through: 1- staggering of rows (i.e., each clone is surrounded by 4 different neighbors), and 2- changing the neighbors at each replication. Additionally, a predetermined isolation zone could be created to separate genetically related individuals (i.e., exclusion zone among related clones). This design evolved from earlier work by El-Kassaby (2003) and El-Kassaby et al. (2007) that aimed to: 1- maintaining a relatively simple layout, thus facilitating selective seed harvest by individual clones and 2- reducing the effective selfing rate and correlated mating (Figure 1).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
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2	23	7	25	19	8	5	6	22	27	8	1	25	21	22	13	14	1	28	5	13	10	23	22	15	6	7
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24	21	15	20	13	28	14	16	3	11	12	6	8	19	18	12	25	5	20	28	6	21	2	12	10	11	8

Figure 1. An example of the "Randomized, Replicated, Staggered Clonal-Row (R^2SCR)" seed orchard design showing the separation among replicated clonal-rows of the same clone (i.e., regional separation) and the staggered nature of rows (see position occupied by clone #7).

A user-friendly computer program was developed to implement this scheme under a specific scenario without the need to understand the mathematics of the model. As stated above, regional clonal assignment requires the selection of specific separation zones prior to the randomization and the length of row (must be even to allow perfect overlap between rows). A number of feasible solutions is generated by the program along with the relevant fit-statistics and this number is correlated with the number of initial iterations selected (i.e., the higher the iteration number, the higher the resultant solutions). Input parameters include the number of clones, seed orchard size, length of rows, separation zones, physical configuration and either balanced or proportional deployment option is desired. A computer program is available from YAE.

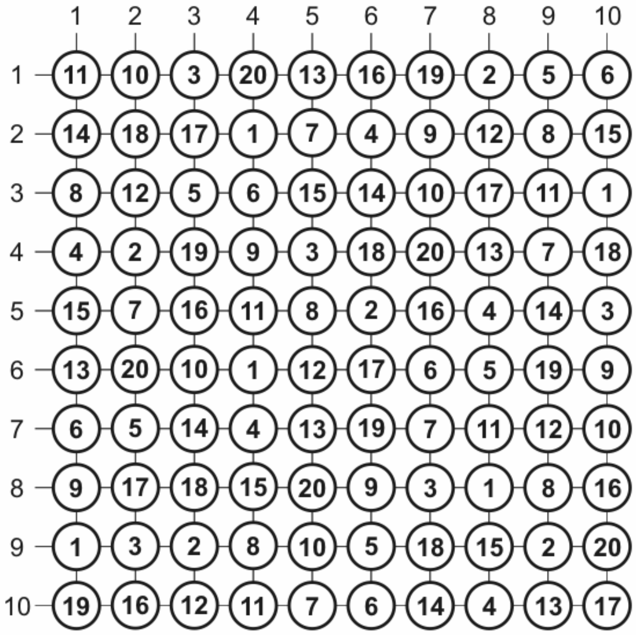
Global Assignment

Theoretically, this represents the most efficient seed orchard design with respect to the minimization of inbreeding in seed orchard crop ("Minimum-Inbreeding" design) (Lstibůrek and El-Kassaby, in preparation). There are no separation zones in this design since a given location of a particular ramet is considered in relation to all other locations in the orchard. In some cases apparent inbreeding is not completely avoided (proximity between two ramers of the same clone) but the resulting layout leads to a minimum inbreeding in seed orchard's crop considering all other feasible layouts (Figure 2).

Optimum layout is a result of separating clones and their ramets on the orchard's grid to minimize selfing and mating among relatives, where the degree of genetic relatedness proportionally affects the level of separation. Complicated pedigree relationships can be easily accommodated by this design, allowing the flexibility to differentially "penalize" different relationships, thus considering closely biological properties of a given species. The outcrossing rate within this orchard is maximized. Computer program is available from ML.

Conclusion

Most seed orchards are established in outdoor conditions with trees planted to a given layout. Reproductive output differs among years, thus no design is truly optimum. Here we propose two innovative designs suitable to advanced-generation seed orchards that are easy to implement. While the first design offers simple scheme to facilitate selective seed harvest by clone, and minimizes the impact of selfing and correlated matings, the second orchard's layout uses a global assignment and leads to theoretically minimum levels of inbreeding in seed orchard's crop, while maximizing the outcrossing rate.



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2	14	18	17	1	7	4	9	12	8	15
3	8	12	5	6	15	14	10	17	11	1
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7	6	5	14	4	13	19	7	11	12	10
8	9	17	18	15	20	9	3	1	8	16
9	1	3	2	8	10	5	18	15	2	20
10	19	16	12	11	7	6	14	4	13	17

Figure 1. An example of the "Minimum Inbreeding" seed orchard design showing separation among replicated ramets of the same clone and the global separation and randomization among 20 clones each with 5 ramets.

Acknowledgements

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Problems With Seed Production Of European Larch In Seed Orchards In Poland

Piotr Markiewicz

Forest Research Institute, Department of Genetics and Forest Tree Physiology
Sękocin Stary, Braci Leśnej Street No 3, 05-090 Raszyn, POLAND
p.markiewicz@ibles.waw.pl

European larch (*Larix decidua* Mill.) is one of the most important conifers in Poland. There were planted 17 266 000 seedlings of this species in 2006. The main source of larch seeds are seed orchards, where 17 859 kg of cones, representing 62% of the total crop, were collected in 2006. The rest of larch cones collected in that year derived from economic seed stands (7 503 kg – 26%) and selected seed stands (3 437 kg – 12%). Nowadays we have got 40 clonal seed orchards of European larch with total area 252.11 ha, and 24 seedling seed orchards with total area 171.89 ha.

And what is a problem with larch in seed orchards? Larch flowers irregularly and gives large percentage of empty seeds in the yield, which may exceed even 70%. The studies concerning empty seed production of European larch made in Institute of Dendrology in Kórnik by Kosiński [1987] provided 4 reasons of that problem. The first reason was lack of pollination which might be caused by small number of male flowers, non synchronised male and female flowering, and unfavourable weather conditions (especially frosts and precipitation). Disturbances in female gametophyte development were the second reason of empty seed production. It might be caused by the nutrient competition between developing ovules and vegetative organs, and by the weather factors (mainly frosts). The next cause was lack of fertilisation due to unfavourable weather conditions during pollen development after pollination. Drought or low temperature reduces pollen viability. And the last reason of empty seed production was embryo degeneration caused by genetic reasons, which is especially visible after self-pollination.

Results reported by Kosiński show that weather conditions play a significant role in seed production of larch. Therefore the local climate must be well recognised before making choice of seed orchard localisation. Also the proper arrangement of seed orchard with as many clones as possible and maximal distances between grafts of the same clone is very important to avoid self-pollination.

The next matter is that not all clones in seed orchard flower the same good as others. The question is how to equalise participation of all clones in flowering to increase genetic diversity of obtained seeds? Some activity like pollen collection and storage, supplemental mass pollination and controlled crossings may be the solution of this problem. Several artificial treatments can also stimulate flowering, mainly those which cause some kind of stress, such as drought, girdling, root pruning and root restrictions, tying stems in a knot, training whole trees or branches horizontally, and excessive nitrogen fertilization, or occasionally nutrient deficiency [Pharis at al. 1987]. Increased flowering of conifers through the use of plant hormones, especially the gibberellins (GAs), is possible as well. For species of Pinaceae, only GAs less polar then GA_3, especially GA_4, GA_7, GA_5, GA_9 and their combination, have generally been effective [Bonnet-Masimbert 1982].

Some research works started in 2004 in European larch seed orchard in Syców (south-western Poland) confirmed positive influence of girdling on cone bud production [Markiewicz unpublished]. Girdled trees had 2-3-times higher mean grade of female flowering than control trees. The aim of girdling is to stop flow of assimilates from needles and shoots to the roots. The higher concentration of nutrients is required for buds to differentiate reproductively vs. vegetatively [Pharis at al. 1987]. But very important thing is to apply girdling at proper time of shoot development. Detectable differentiation of reproductive buds occurs in short shoots of western larch (*Larix occidentalis* Nutt.) at about the end of long shoot elongation [Eysteinsson and Greenwood 1995]. So treatment has to be applied during long shoot extension, before the end of this process. Girdling in seed orchard in Syców has been done when the long shoots have flushed and already had about 3-4 cm length.

Girdling appears to be cheap and easy method to stimulate flowering but one has to be careful with this treatment, because it may lead to the root weakness and makes trees more susceptible to blowing down by the wind. Sometimes trees break in the girdle zone as well. However, girdling may be applied on trees which are designated for cutting from seed orchard in the near future.

The influence of girdling on larch flowering in the next year after treatment was investigated in seed orchard in Syców in years 2005 and 2006. The increase of flowering caused by girdling observed in 2006 was lesser than in 2005. The reason might be that the year 2006 was good-flowering year in opposite to poor-flowering year 2005, and the positive influence of girdling might not be so evident when conditions were favourable for trees to flowering, and many trees naturally flowered well. So, this indicates that the flowering stimulation is economic only in the poor-flowering year, when almost all trees flower weakly.

However, the stimulation may enhance flowering, but not before the next year. So, there is a question, how to foresee in the year of stimulation if trees will naturally flower well or not in the next year, and make decision: stimulate or not stimulate? The answer to this question may be given by examination of correlation between the yield of seeds obtained in particular year and weather conditions occurred in the same year (influence on flowering and seed forming) and in the year before (influence on cone bud initiation). These investigations may give the knowledge which weather factors have the great impact on seed production, and how to foresee the flowering through the weather monitoring before flowering.

And the last problem: seed collection. Larches in the young seed orchards, where seed collection is easy, flower rather weakly. Larches in the oldest seed orchards flower better, but the problem is that collecting seeds from high trees is technically difficult and more expensive. So, some research projects concerning top-pruning and crown forming of larches in seed orchards are needed. These projects aim to find efficient way of trees management, which may lead to:

- limit the increase of trees height and keep crowns on the lower level,
- reduce damages caused by the wind,
- make easier seed collecting,
- reduce costs of seed production and make seed orchards more effective.

The observations made on pruned trees in a few following years may show what will be the influence of these treatments on flowering, survival and subsequent development of trees.

Taking into consideration all these problems with seed production of larch, some studies providing knowledge about enhancing flowering, the weather influence on seed forming process, and managing trees in seed orchard, are still necessary.

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A review of the seed orchard programme in Poland

Jan Matras
Forest Research Institute
Department of Genetics and Tree Physiology
Braci Leśnej Str. No 3, Sękocin Stary
05-090 Raszyn, Poland

Abstract

As results from the assumptions adopted for a long-term selective forest tree breeding programme, seed orchards in Poland constitute only a supplementary source of forest reproductive material. Following these assumptions, the share of an individual seed base used for forestry purposes cannot exceed 20 percent. 1962 hectares of seed orchards have been established so far within the State Forests National Forest Holding (State Forests NFH). Part of them established as the first conifer seed orchards in Poland today produce seeds and are used for the production of forest reproductive material, while most of the broadleaved seed orchards were established in the nineties of the past century and only entered the seed production phase. Presently, the seeds from the orchards constitute, depending on the crop, 8 – 17 percent of the reproductive material for forestry use. The estimates show that the actual area of seed orchards should fully satisfy the demand for the reproductive material from this seed base category. Recently, creation of a new category "tested" of forest reproductive material has started. The choice of tested trees will allow to establish seed orchards of the known genetic value and to select individuals with specified breeding qualities to be used for special purposes. Actions of this type have been proposed in the assumptions to the new selective forest tree breeding programme prepared for the years 2010-2035 in the State Forests NFH.

Key words: individual selection, mother trees, seed orchards, testing forest reproductive material

Introduction

The implementation of selective forest tree breeding programmes in Poland has been pursued in the State Forests NFH since the early sixties of the past century. First stands were then selected to create a selective seed base of forest tree species. Selective breeding was implemented in the State Forests NFH on the basis of the programmes developed for successive periods. The first programme was implemented in the years 1960-1975, the second one – in 1976-1990, and the implementation of the current programme 1991-2010 is coming to an end. The next stages of the selective forest tree-breeding programme assume the continuation of the key strategic goals adopted in the first stage of programme implementation taking into consideration the necessity to adjust the current goals to the changing ecological and economic conditions of forest functioning.

The strategic goals adopted for the selective forest tree-breeding programme for the years 1975-1990 were as follows:

1. Maintain the existing genetic variation in forests over a long time span.
2. Increase the productive capabilities, improvement of qualitative parameters and stability of the next generations of forests.
3. Create and maintain a seed base on the level guarantying the achievement of goals 1 and 2 (5, 6).

To achieve the strategic goals of the Programme it was assumed that population selection (seed stands) would be the main direction of the activities, while individual selection (plus trees, seed orchards, seedling seed orchards) would be complementary to these activities. The share of seeds from individual selection steadily increased in successive periods:

1960 - 1975 – 100 % of the population seed base,
1976 - 1990 – 95 % of population seeds, 5 % of seeds from the orchard,
1991 - 2010 – 85 % of population seeds, 15 % seeds from the orchard.

However, it did not reach the maximal allowable level in the State Forests NFH that is 20 percent of the total demand for seeds for restocking.

These quantities are pretty large taking into consideration the general demand for seeds in the State Forests NFH (see Table 1).

Individual seed base in Poland – the present state

In accordance with the Forest Reproductive Material Act, there are three categories of Basic Forest Material (LMP) forming the forest seed base in Poland:

Category I - stands of known origin,

Category II - selected stands,

Category III - parents of families, and the seed orchards and seedling seed orchards established on their basis (2, 3).

The first and second categories constitute a so-called population seed base, while the third "qualified" category was created on an individual selection basis. Till 2006, approx. 10,000 mother trees of the 14 main forest tree species within the State Forests NFH had been classified on the basis of phenotypic traits. A detailed specification of the qualified mother trees is given in Table 2. For indigenous trees, the amount of plus trees is strongly correlated with the share of these trees in forests. However, the share of conifers is higher, as coniferous species were qualified in the first place. Also, higher than average is the share of mother trees of admixture and introduced species, such as the common birch, European larch, black pine or Douglas fir for which seed orchards are and will be the main seed base in the nearest future.

The Table does not include individual trees selected for special purposes and used temporarily as a seed base for restocking and gene resource conservation. There are about 4,000 such trees, specifically 2,000 firs from the Sudeten region and about 1,000 common yews ready for establishing a seed base for the State Forest NFH. The majority of these trees are already in the State Forests NFH's clone archives whose aim is preservation of all mother trees, gene reserve trees and selected nature monuments growing on forest lands.

The first seed orchards were establishment primarily for conifer species which are currently in the full production phase. The selection of broadleaved mother trees was started as late as in the early nineties, so the seed orchards are relatively young and just enter the production phase. The specificity of Polish forestry is establishment of large-scale seedling seed orchards i.e. generatively produced seedlings. Their progeny shows a much higher variability and they can be maintained productive for a longer period of time, which is their unquestionable advantage. The minimal number of clones in seed orchards is 40 for pine and spruce and 30 for other forest species. The clones in seed orchards are randomly distributed at minimal distances between individuals within a given clone. Three types of cutting: schematic, genetic and combined are anticipated for seed orchards. Similar rules apply to the establishment of seedling seed orchards. The first seedling seed orchards were established on the basis of clone sets from relatively large areas. Currently, attempts are made to ensure that mother tree sets for the species embraced by a detailed seed regionalisation (geographic distribution of seed regions) represent individual regions of their origin.

Data on the established seed orchards are given in Table 2.

All the seed orchards established so far belong to so-called category I, i.e. those created through the selection of mother trees on the basis of phenotypical traits. The programme for the establishment of category I seed orchards has not yet been implemented in full. We certainly do not plan to establish this type of seed orchards for most of coniferous tree species. However, we need to establish a number of seed orchards for the broadleaves so that the quantities of seeds from the seed base of this category reach 20 percent. The quantities of seeds currently obtained from seed orchards range from 8 to 17 percent depending on the year. Table 3 shows the percentage of seed base categories meeting forestry needs (data as of 2005).

An individual seed base in the assumptions for the new Programme

In accordance with the adopted strategic assumptions regarding the functioning of the forest seed base in a long time span created in the framework of selective forest tree breeding programmes, no substantial changes are foreseen in the percentage of the population and individual seed bases. However, changes that are to appear are connected with the commencement of creating a new, category "tested" of seed base. Within this category, both the population and individual seed bases are created.

The State Forests NFH, the manager of Polish forests, started testing the seed base categorized as "selected" and "qualified" created in the framework of the previously implemented selective forest tree breeding programmes. The testing programme was introduced into forest practice by Director General's Ordinance No. 85 of 31 December 2004 on the implementation of the *Programme for testing the progeny of selected seed stands, plus trees, seed orchards and seedling seed orchards* in all the organisational units of the State Forests NFH - ref. no. ZG – 7132 – 58/2004 (1).

The aim of testing the progeny of plus trees, seed orchards and seedling seed orchards through the establishment of testing plantations is to demonstrate the high quality of the reproductive material. This is to be done by making comparative tests or by estimating its quality on the basis of genetic evaluation of core material components which, according to the Act of 7 July 2001 on forest reproductive material, include seed orchard, mother tree, clone and clone mix (Dz. U. of 18 July 2001) (7).

Moreover, progeny testing assumes the development of the principles of rational use of a seed base delimited by seed regions and identification of the area of possible transfer in accordance with the adopted areal regionalisation rules and, additionally in the mountains – the altitudinal zonation rules (4). These rules should be established on a genetic basis. Also, the testing programme aims at the optimization of tasks presently pursued in the State Forest NFH under sustainable forest management in Poland, i.e. improvement of forest management on an ecological basis (6).

The choice of the best progeny for registration in category IV "tested" of seed base will be the effect of its genetic evaluation. The testing region comprising the territory of the Regional Directorates of State Forests is the basic unit for which seed tree and orchard progeny testing programmes are being developed. In this region, forest core material components are located i.e. plus trees, seed orchards, seedling seed orchards in the amount facilitating testing of representative progeny collections. In the case of mother trees, their number will be close to 100. The progeny of such a set will be tested in four different locations. The test sites will be established in a single-tree plot design with 64 replications (table 4).

Seed orchards and seedling seed orchards will be tested as artificial breeding populations in the sets of 30 items per set. Test sites will be established in a randomised block design in four replications with 100 seedlings in each replication (table 5).

The main actions in the part related to selective forest tree breeding planned for the implementation in the new Programme as regards individual selection will include:

a) continuation of the tasks implemented recently:

- choice and use of mother trees (DD),
- establishment of the first generation seed orchards and seedling seed orchards,
- setting progeny plantations,
- evaluation of the breeding qualities of progeny plantations entering the seed production phase.

b) New tasks related to the genetic evaluation of LMP:

- establishment, management and evaluation of LMP for the production of category III material – LMR (mother trees, seed orchards, seedling seed orchards), in progeny tests,
- choice of LMP stands for the production of LMR – category "tested",
- development of the rules for the management and use of LMP registered in Part IV of the National Register in forestry,
- establishment of the second generation seed orchards and seedling seed orchards,
- establishment of orchard blocks with LMP of known genetic value.

The selection tasks included in the new Programme for the years 2010-2035 will cover:

- improvement of qualitative and quantitative traits of populations (stands) – population selection (choice of stands from the categories "selected" and "tested"),

- choice of populations and genotypes of high plasticity for breeding under the changing climate conditions (population and individual selection),
- creation of artificial breeding populations on a seed orchard basis with specified (relatively high) genetic variation (individual selection – the choice of different genotypes for tree sets for seed orchards and seedling seed orchards based on DNA analyses),
- improvement of the qualitative traits of genotypes – individual selection – the choice of genotypes of specified traits from the category "tested" – creating artificial breeding populations for forestry needs on a seed orchard basis,
- improvement of resistance traits of genotypes to biotic and abiotic stresses – individual selection – the choice of genotypes of specified traits from the category "tested" – creating artificial breeding populations for forestry needs on a seed orchard basis,
- improvement of the quantitative traits of genotypes – wood mass production in short and long rotation – individual selection – the choice of genotypes of specified traits from the category "tested" – creating an LMP base for clone and clone mix production and the establishment of seed orchards.

Anticipated effects of Programme implementation:

- creation of a "tested" category seed base representing 10 percent of the national demand for seeds (anticipated selection gain for growth traits at population level – 15%, at family level – 25 % in relation to growth traits for the material from outside the base),
- maintenance of the selected seed base representing 30 percent the national demand for seeds (anticipated selection gain for growth traits at population level – 10 %, at family level – 15%),
- maintenance of a permanent seed base from the identified source representing 60 percent the national demand for seeds (anticipated selection gain for growth traits at population level – 2-5%).

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Table 1. Seeds demand of the main forest tree species for the years (1991-2005) (KG)

Year	Pine	Spruce	Larch	Fir	Oak	Beech
1991	13 080	2750	2990	17 760	871 200	66 210
1992	13 510	2990	2663	19 190	1 153 700	105 220
1993	14 230	2620	3480	15 190	1 242 300	78 480
1994	11 570	2330	2450	13 220	1 107 100	71 780
1995	10 680	2050	2400	12 500	1 307 900	123 370
1996	11 460	1720	2500	10 500	1 245 400	72 040
1997	8090	1370	1450	16 120	1 281 100	75 170
1998	7560	1250	1310	9040	1 244 400	108 480
1999	7470	1140	1680	16 790	1 255 500	82 460
2000	8090	1045	1301	9 269	1 291 368	116 842
Mean (91 -00)	**10 574**	**1 926**	**2 222**	**13 958**	**1 199 997**	**90 005**
2001	7348	1249	1427	9 776	1 150 311	79 373
2002	7593	830	996	6 537	869 490	83 963
2003	6944	840	1102	5 974	909 805	77 861
2004	5996	795	908	6 427	890 081	87 003
2005	6479	788	800	8 785	1 125 543	74 857
Mean (01-05)	**6 872**	**900,4**	**1 046,6**	**7 500,2**	**989 046**	**80 611,4**

Table 2. Present state of forest seed base in Poland [ha]

Species	Populations of known origin	Selected stand	Plus trees	Seed orchards	Seedling seed orchards
Pine	154 500	6957	3670	425,78	279,30
Spruce	14 050	2277	950	75,60	10,89
Larch	1020	445	1016	252,11	171,89
Fir	5580	1370	484	80,15	15,35
Black Pine	100	72	232	25,00	110,64
Other coniferous	180	162	583	52,63	47,30
Birch	2 600	195	267	47,62	13,39
Beech	17 300	2076	553	50,47	11,22
Pedunculate Oak	13,325	1370	557	31,95	23,40
Sessile Oak	2 075	1452	332	53,38	11,00
Black Alder	6 700	563	517	50,23	0
Small-leaved Lime	300	142	135	96,56	0
Other deciduous	740	121	499	21,92	4,55
Total	219 100	17203	9795	1263,40	698,93

Table 3. Proportion (in %) of different category of FRM in seed base (2004)

Species	Populations of known origin	Selected seed stand	Seed orchards seedling seed orchards	Other
Pine	82,0	8,0	10,0	0,0
Spruce	58,0	30,0	2,0	10,0
Larch	35,0	10,0	47,0	8,0
Fir	58,0	37,0	3,0	2,0
Other coniferous	11,0	40,0	27,0	22,0
Mean	**73,0**	**13,0**	**12,0**	**2,0**
Birch	79,9	12,3	7,8	0,0
Beech	74,6	25,4	0,0	0,0
Pedunculate Oak	100,0	0,0	0,0	0,0
Sessile Oak	95,1	3,9	0,0	0,0
Black Alder	91,9	5,9	2,2	0,0
Small-leaved Lime	88,2	1,8	10,0	0,0
Other deciduous	100,0	0,0	0,0	0,0
Mean	**90,1**	**7,0**	**2,9**	**0,0**

Table 4. Number and localization the testing plots for mother trees

REGIONAL DIRECTORATE OF STATE FORESTS	SPECIES									
	PINE	SPRUCE	LARCH	FIR	D.FIR	BEECH	P. OAK	S. OAK	BIRCH	ALDER	TOTAL
Białystok	24	12					8		2	4	**50**
Gdańsk	8		4		2	4	1	2			**21**
Katowice	12	8	4	2	2	2	2	2			**34**
Kraków	8	2	8	8	2	4	1		2	2	**37**
Krosno	4	2	4	8	2	4	1			2	**27**
Lublin	8	2	4	2		2	1		2	4	**25**
Łódź	8								1	1	**10**
Olsztyn	16					1	1		2	2	**22**
Piła	8	2	1			1	1	2	1		**16**
Poznań	8				2		2	2		1	**15**
Radom	4	2	4	2		2	1			4	**19**
Szczecin	16	2	4			4		4	1		**31**
Szczecinek	8		3			1		2			**16**
Toruń	16					1	1	2	1	2	**25**
Warszawa	4									2	**6**
Wrocław	4	4	8	2	2	2					**22**
Zielona Góra	4										**4**
TOTAL	**160**	**36**	**44**	**24**	**16**	**28**	**20**	**16**	**12**	**24**	**380**

Table 5. Number and localization the testing plots for seed orchards and seedling seed orchards

REGIONAL DIRECORATE OF STATE FORESTS	SPECIES												
	PINE	SPRUCE	LARCH	FIR	D.FIR	B.PINE	BEECH	P.OAK	S.OAK	BIRCH	LINDEN	ALDER	TOTAL
Białvstok	2	2						1		1		1	**7**
Gdańsk	2		2		1		1			1	1		**8**
Katowice		2	2	1	1	2	1	1	2				**12**
Kraków				2		2	1						**5**
Krosno	2	1	2	1	1		1	1					**9**
Lublin	2			1				1		1		1	**6**
Łódź	2						1			1		1	**5**
Olsztvn	2	1						1			1	1	**6**
Piła	2								1				**3**
Poznań	2				1	2		1	2	1		1	**10**
Radom			2	2								1	**5**
Szczecin	2				1		1	1	1				**6**
Szczecinek					1		1		1		1	1	**5**
Toruń	2				1	2			1	1			**7**
Warszawa	1									1		1	**3**
Wrocław	2	2	2	1	1			1			1		**10**
Zielona	1						1			1			**3**
TOTAL	**24**	**8**	**10**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**8**	**110**

Seed Orchard Management Strategies for Deployment of Intensively Selected Loblolly Pine Families in the Southern US

Steven E. McKeand[1], Davis M. Gerwig[2], W. Patrick Cumbie[1], and J.B. Jett[1]

Operational family block planting with loblolly pine (*Pinus taeda* L.) was begun by Weyerhaeuser Company in the mid-1970's (Gladstone 1981) and has been one of the most significant forest management decisions made with southern pines. After more than 30 years, planting of individual open-pollinated families, and more recently full-sib families and clones, has become standard practice in the southern United States. In the early 2000's, 59% of the loblolly pine plantations were established as single open-pollinated (OP) family blocks (McKeand et al. 2003). About 80% of the regeneration on company lands was with OP families, and 48% of seedlings used for market sales were as OP families. Fourteen companies used family blocks for deployment of loblolly pine. On average, a company deployed 47 different families on its own land, but there was tremendous variation in the number of families deployed in a region, ranging from as few as 4 to as many as 90.

Although these survey results have not been updated since 2002, the percentage of plantations established with individual families as opposed to seed orchard mixes has increased. Companies sell very few mixed seedlots, and state agencies have begun to sell individual OP families. We believe that about 75% of the loblolly pine plantations are now established as single open-pollinated (OP) family blocks.

In our opinion, nothing has had a greater impact on operational genetic gain than deployment of individual families of loblolly pine to specific sites. With loblolly pine, deployment is fairly straightforward; plant the best families on the best sites to realize the most genetic gain (Duzan and Williams 1988). The best families tend to be the best on all sites within broad adaptability zones, so more wood production is realized from the combination of the best families, sites, and silvicultural practices (McKeand et al. 1997, 2006; Li and McKeand, 1989). Even with the availability of many tonnes of loblolly pine seed available each year (Figure 1), foresters don't plant the best family everywhere, since seedlings of best genotypes are always in short supply and because of diversity concerns.

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[1] NC State University, Raleigh, NC USA [2]ArborGen, Summerville, SC USA
Emails: Steve_McKeand@ncsu.edu, dmgerwi@arborgen.com, WPCumbie@ncsu.edu, JB_Jett@ncsu.edu

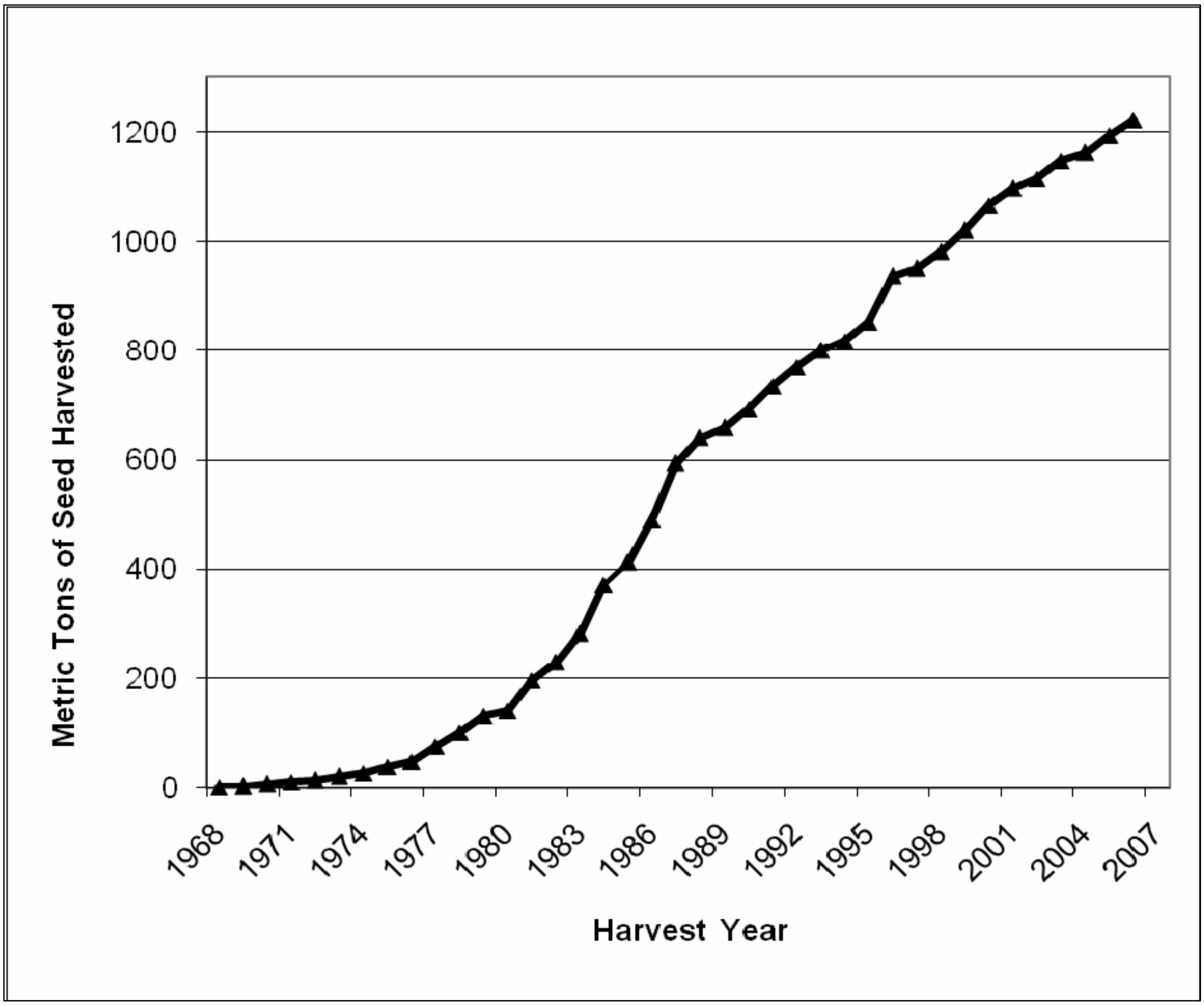

Figure 1. Cumulative production of loblolly pine seed by members of the NC State Tree Improvement Cooperative. Enough seeds have been produced to plant over 30 billion seedlings in the last 40 years.

Over the last 10 years, seed orchard managers have had great success in developing methods to mass produce full-sib families for operational planting. The gains from improved quality and yield are very impressive when both the female and male parents are selected (e.g. Bramlett 1997, Bridgwater et al. 1998, Jansson and Li 2004). Figures 2 and 3 show gain estimates in the MeadWestvaco / ArborGen tree improvement program due to different levels of selection. The gains in productivity, fusiform rust resistance (caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*), and stem straightness are most impressive when both the male and female parents are controlled. As forest managers in the southern US move almost exclusively to silvicultural regimes to promote sawtimber production (Figure 3), the improvement in stem quality from lower levels of rust and better stem straightness have exceptionally high economic value.

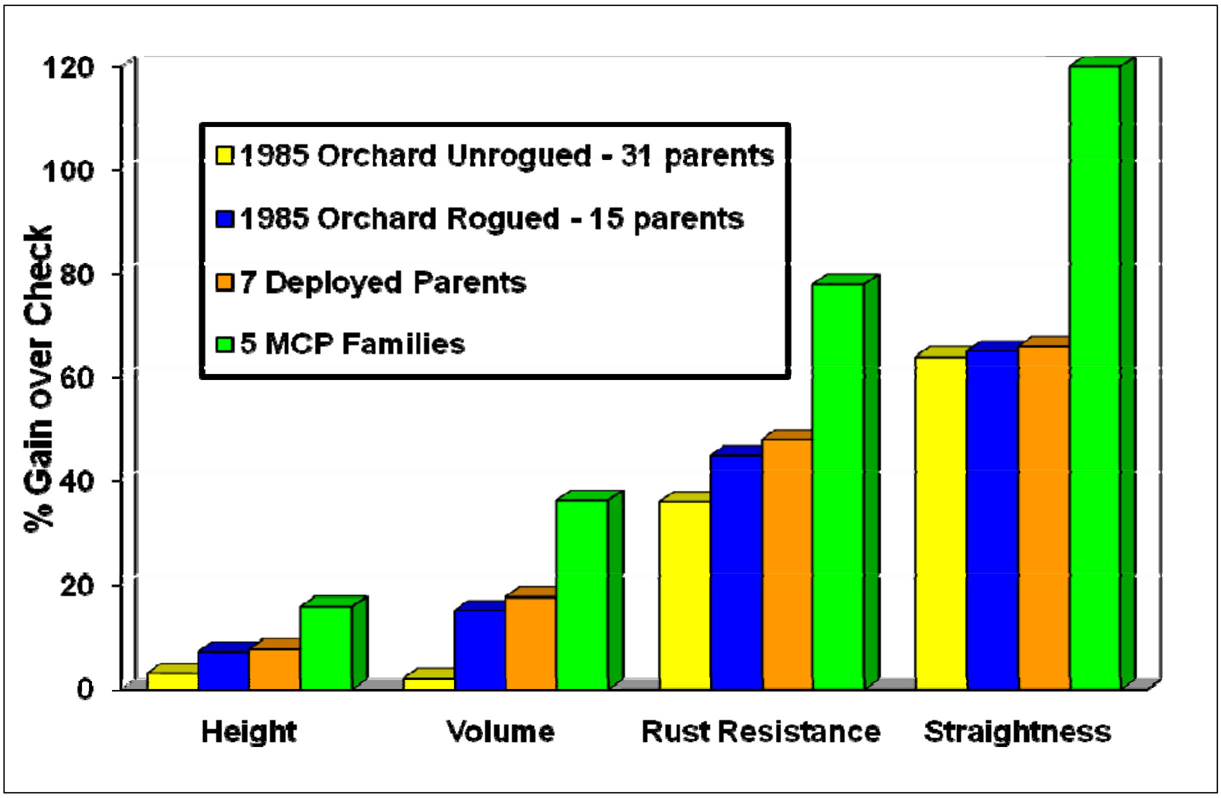

Figure 2. Estimates of genetic gain over unimproved checklots for different levels of genetic entries from the MeadWestvaco / ArborGen tree improvement program in the Atlantic Coastal Plain in South Carolina, USA. The yellow bar is for the average of all 31 parents in the unrogued orchard. The blue bar is for the average of the 15 parents in the rogued orchard. The orange is the mean of 7 OP families that were operationally deployed in the 1990's by MeadWestvaco. The green bar represents the mean of the 5 MCP families now planted.

Since production costs for mass control pollination (MCP or CMP) are large, production characteristics of the parent selections can be overwhelmingly important. When orchard managers are pollinating 40,000 to 80,000 bags each spring, production efficiency is critical. While the genetic quality of the parent trees used for MCP is critical, the morphological, physiological, phenological, and yield characteristics of the trees are extremely important. Virtually everything in a seed orchard is clonal; flowering, susceptibility to insects, drought, diseases, animal predation, and even hurricane~~s~~ damage varies tremendously among parental selections. For an orchard manager to make MCP cost-effective, he or she must understand these differences and manage the orchard and MCP production efficiently.

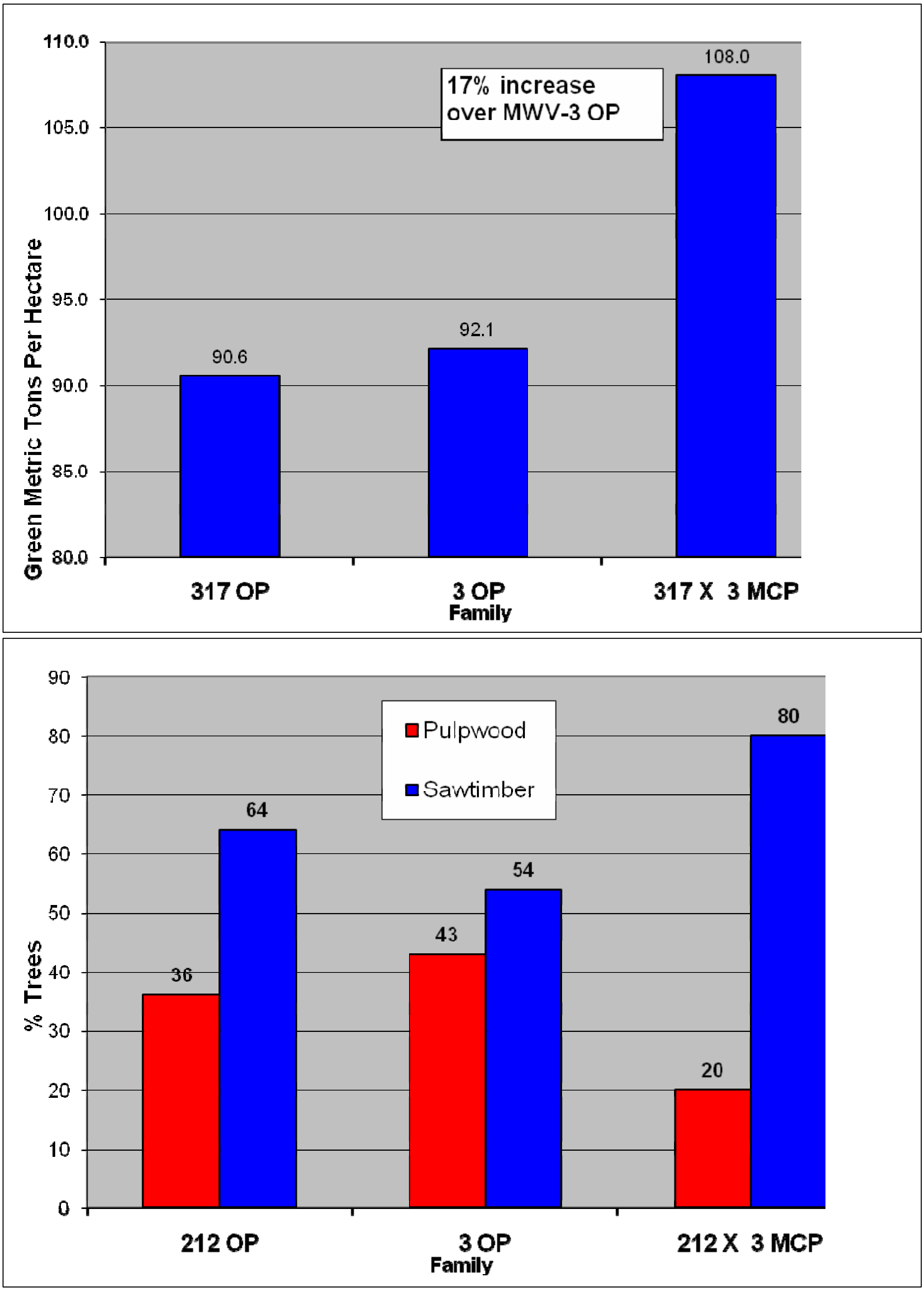

Figure 3. Comparison of yield per hectare (top) and percentage of trees producing sawtimber (bottom) of the full-sib cross compared to open-pollinated offspring of both parents. The gains from controlling both parents are most impressive in MCP families through age 8 years.

In 2007, the companies that have been mass producing full-sib seedlings for operational planting were surveyed to determine how many Mass Control Pollinated (MCP or CMP) seedlings have been produced. Since 2000, over 94 million full-sib family seedlings have been planted in the South (Figure 4). While the current annual production of full-sib seedlings is only about 3% of the total seedling production of the 800,000,000 to 1 billion loblolly pine seedlings, we anticipate that full-sib seedling production will become a much more significant component of the seedling market in coming years.

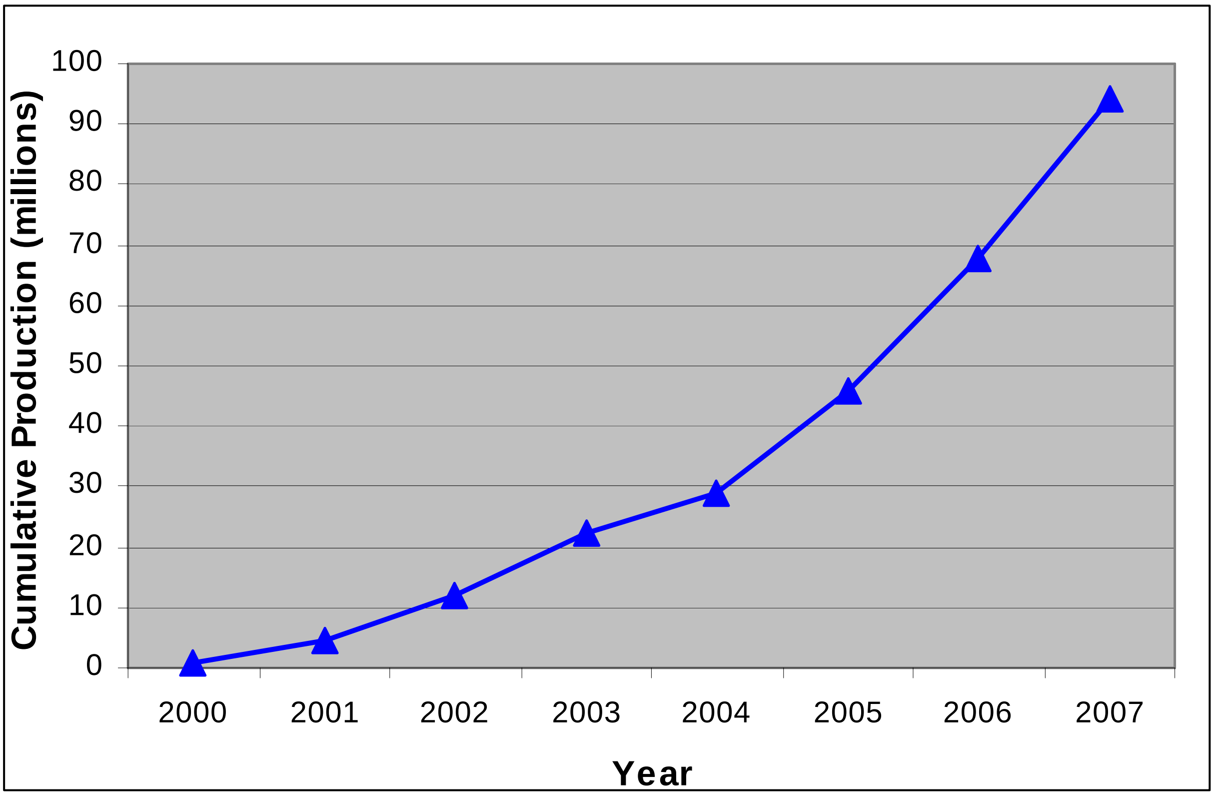

Figure 4. Operational planting of full-sib families of loblolly pine has become a reality. Since 2000, over 94 million full-sib family seedlings have been planted by landowners in the southern United States.

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Paternal gene flow in *Cryptomeria japonica* seed orchards as revealed by analysis of microsatellite markers

Yoshinari Moriguchi[1], Hideaki Taira[2], Yoshihiko Tsumura[3]

[1] Department of Forest Genetic Resources, Korea Forest Research Institute, 44-3 Omokchon-dong, Kwonsun-ku, Suwon, Gyeonggi-do, 441-350, Republic of Korea.
E- mail : ym-chi.mori@hotmail.com
[2] Graduate School of Science and Technology, Niigata University, Igarashi 2-nocho, Niigata, 950-2101, Japan
[3] Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

Seed orchards, which are stocked with superior tree clones, are important sources of materials for afforestation. Ideally, such materials should have high genetic value and diversity. In order to maintain high genetic value of their seeds, rates of contamination by external pollen and self-fertilization within the orchards should be minimal, while the paternal contribution of the constituent clones should be roughly equal to ensure the seeds have high diversity.

Sugi (*Cryptomeria japonica* D. Don) is an allogamous, wind-pollinated conifer species that is frequently used for commercial afforestation in Japan. To ensure a supply of high-quality reforestation materials, wind-pollinated clonal seed orchards of *C. japonica* have been established in various parts of Japan by propagating superior clones. In the present study, we estimated the levels of pollen contamination, self-fertilization and paternal contribution, using microsatellite markers, in three common and two miniature clonal seed orchards of *C. japonica*. To obtain basic data that could be used to further improve seed orchards, we attempted the comparison of gene flow data among seed orchards. We analyzed gene flow in each of the seed orchards and investigated reasons for observed differences in pollen contamination, self-fertilization and paternal contributions to elucidate the scale of these potential problems and to help identify possible ways to counter them.

Two types of seed orchards have been established in Japan: common clonal orchards and miniature clonal orchards (Longman and Dick 1981, Itoo and Katsuta 1986). The latter type has been used since 1982. Miniature clonal seed orchards are more efficient, owing to their smaller labor requirements and shorter establishment times. Five clonal seed orchards of *C. japonica* (three common types and two miniature types) were investigated (Moriguchi et al. 2005b). The common seed orchards had average tree heights for each clone of about 5 m; the spacing was 5 × 5 m. The miniature clonal seed orchards had average tree heights for each clone of about 2 m; spacing was 1 × 1 m. In each seed orchard, crude DNAs were extracted from needle tissues of all the constituent parental clones and from germinated seedlings collected from 12 randomly selected ramets. DNA extraction was carried out using the CTAB method (Murray and Thompson 1980) and the modified CTAB method (Tsumura et al. 1995). Thirty seeds per mother tree were analyzed using microsatellite markers in five seed orchards. We selected microsatellite markers that show high stability and polymorphism (Moriguchi et al. 2003, Tani et al. 2004). The multi-paternity exclusion probability (Weir 1996) for the selected markers was high (more than 0.999) in all seed orchards. PCR amplifications were carried out using the method of Moriguchi et al. (2005b). Paternity was determined by a simple exclusion method (Moriguchi et al. 2004).

The average proportions of the seeds originating from contaminating pollen in the seed orchards varied between 35.0 (±3.47)% and 65.8 (±4.11)%. The high contamination rate detected in this study was similar to rates reported for other conifer seed orchards of dominant species (El-Kassaby et al. 1989, Yazdani and Lindgren 1991, Wang et al. 1991, Adams et al. 1997, Burczyk and Part 1997, Stoehr et al. 1998, Pakkanen et al. 2000). The area of *C. japonica* forests in the vicinity of the seed orchard (ha) was correlated with the pollen contamination rate (Table 1). The contamination rates seem to be affected by the rate of pollens from outside the seed orchard in the cloud of pollen. Because even the seed orchard located in an excellent environment had pollen contamination levels of about 30%, it may be impossible to avoid pollen contamination levels below 30% in outdoor seed orchards.

Table 1 Average pollen contamination rates and the area (in ha, and as a percentage of the total area) of artificial *C. japonica* forests within 3, 5, 8, 10 and 15 km of the five seed orchards. (Moriguchi et al. 2005b)

	radius of 3km (ha (%))	radius of 5km (ha (%))	radius of 8km (ha (%))	radius of 10km (ha (%))	radius of 15km (ha (%))	Contamination (%)
C - 1	612.8 (21.7)	1796.4 (22.9)	3373.5 (16.8)	4151.6 (13.2)	6430.7 (9.1)	47.8
C - 2	782.8 (27.7)	1582.1 (20.2)	5000.4 (24.9)	7970.0 (25.4)	19770.3 (28.0)	65.8
C - 3	8.2 (0.3)	197.5 (2.5)	326.9 (1.6)	476.6 (1.5)	2702.4 (3.8)	35.0
M - 1	0.0 (-)	0.0 (-)	851.7 (4.2)	1521.0 (4.8)	5815.4 (8.2)	40.8
M - 2	585.6 (20.7)	1755.8 (22.4)	3918.8 (19.5)	5616.9 (17.9)	15169.2 (21.5)	50.0
Spearman's ρ (*p* value)	0.472 (<0.001)	0.217 (0.096)	0.562 (<0.001)	0.562 (<0.001)	0.562 (<0.001)	

The average self-fertilization rates in the seed orchards varied between 1.4 (±0.64)% and 4.4 (±2.02)%. The number of ramets per clone was correlated with the self-fertilization rates (Table 2, Fig. 1, Moriguchi et al. 2005b). The self-fertilization rates seem to be affected by the rate of self pollen in the cloud of orchard pollen. The self-fertilization rate in conifer seed orchards is thought to be generally less than 5 % (Ritland and El-Kassaby 1985; Rudin et al. 1986; Goto et al. 2002). However, a much lower self-fertilization rate (0.426%) was observed in the progeny test (Moriguchi et al. submitted). Therefore, self-fertilization does not appear to present a major threat in conifer seed orchards, despite its potentially adverse effects, as reported by Goto et al. (2005).

Table 2 Average self-fertilization rate, the average rate of ramets derived from one clone in all ramets and the average rate of self-fertilization in the fertilization with orchard clone.

	Self-fertilization rate (%)	The average rate of self-fertilization in the fertilization with	The average rate of ramets derived from one clone in all ramets
C - 1	2.2	4.2	2.4
C - 2	1.4	4.1	2.9
C - 3	4.4	6.8	4.2
M - 1	1.7	2.9	1.9
M - 2	3.6	7.2	4.1

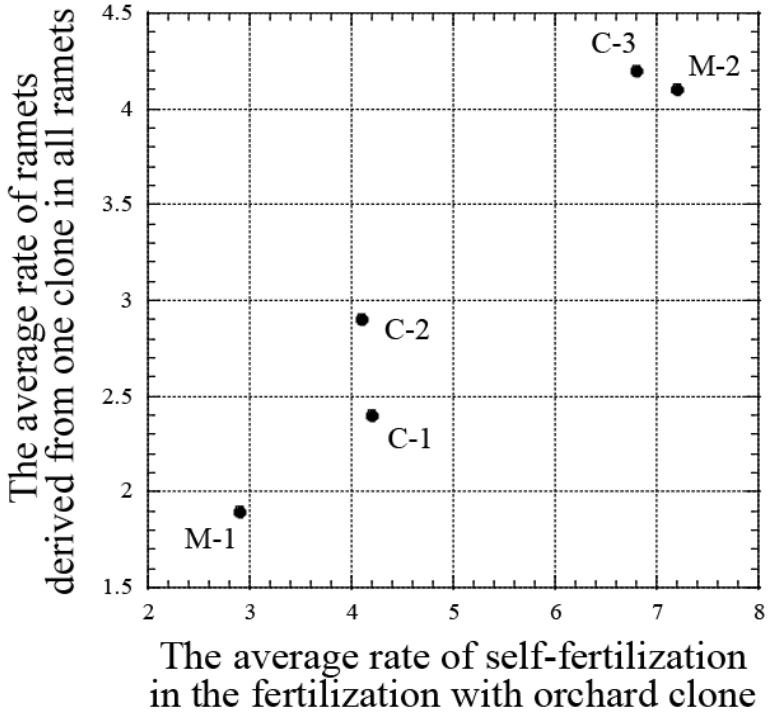

Fig.1 Relationship between the average rate of ramets derived from one clone in all ramets and the average rate of self-fertilization in the fertilization with orchard clone.

In the result of χ^2 test, paternal contributions to seed production by the constituent clones differed significantly in all seed orchards ($p < 0.001$). In spite of the differences in the types of seed orchard and their locations, the same tendency was revealed for all of the seed orchards, i.e. about 20% of the clones accounted for about 60% of the total gene flow and about 30% of total clone made no contribution (Moriguchi et al. 2005b). Similar results have been found in seed orchards of other conifer species, such as *Pinus contorta* Dougl., *Pinus thunbergii* Parl. and *Pseudotsuga menzeisii* Franco (Fig. 2, Moriguchi et al. 2005a). Paternal contribution is affected by the male flower production, floral synchrony, distance between parents, wind direction and pollen competition (Shen et al. 1981, Schoen and Stewart 1986, Erickson and Adams 1989, Burczyk and Prat 1997, Stoehr et al. 1999, Nikkanen et al. 2000, Aronen et al. 2002, Goto et al. 2002). In *C. japonica*, total male flower production strongly affects male reproductive success and the inter-tree distance also has some effect (Moriguchi et al. 2007).

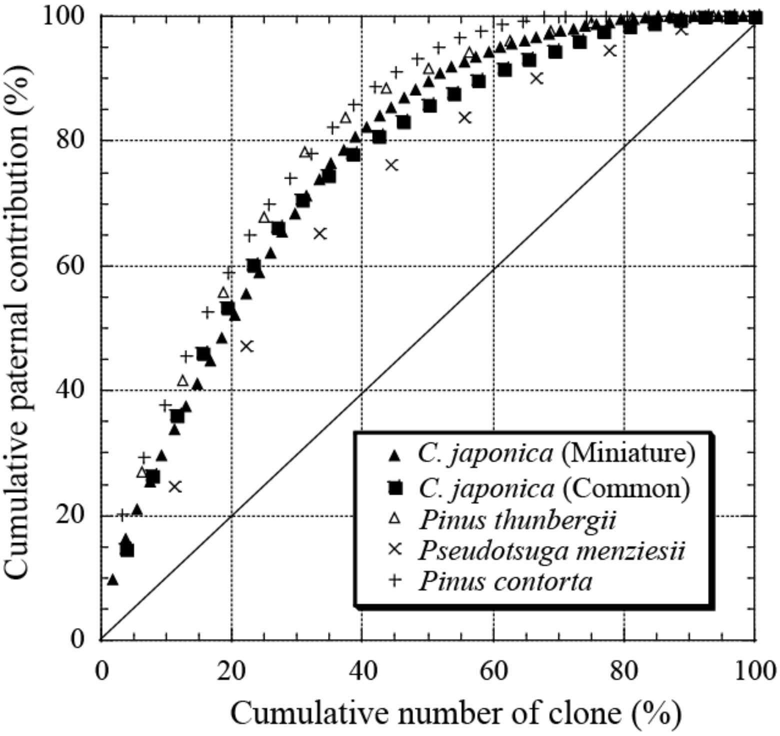

Fig.2 Relationship between cumulative number of clones (%) and cumulative paternal contribution (%) (Moriguchi et al. 2005a, in Japanese). The numbers of clones were cumulated sequentially from the clones with the highest contributions. The data of *Pinus thunbergii*, *Pseudotsuga menziesii* and *Pinus contorta* obtained from Goto et al. (2002), Stoehr et al. (1998) and Stoehr and Newton (2002), respectively.

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Fertility Variation across Years in Two Clonal Seed Orchards of Teak and its Impact on Seed Crop.

A. Nicodemus[1*], M. Varghese[1], B. Nagarajan[1] and D. Lindgren[2],
[1]Institute of Forest Genetics and Tree Breeding,
PB 1061, Coimbatore 641 002, Tamil Nadu. India
[2]Department of Forest Genetics and Plant Physiology,
Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.
*email:nicodemus_a@rediffmail.com

Introduction

Teak (*Tectona grandis* L.f.) is a highly valued timber species raised in plantations throughout the tropics. Clonal Seed Orchards (CSO), established through grafts of selected trees are considered to be channels of genetically improved seed and starting point for domestication. India has over 1000 ha of CSOs but seed production from them has been too low to make any impact on the new plantations or advancing the breeding cycle. Genetic improvement of teak has not moved beyond the first generation orchards during the last 50 years.

Reproductive biology of teak and in particular the causes for low seed production in orchards has been intensively studied during the past decade. Teak is a predominantly outcrossing species and insects are the major vectors of pollination. Absence of flowering, clonal variation in flowering phenology and pollinator limitation are reported to be the major reasons for low seed output (Nagarajan *et al.* 1996; Palupi and Owens, 1998; Gunaga and Vasudeva, 2002). The objectives of the present study were to quantify flower and fruit production in two orchards during consecutive years, to estimate fertility variation and its impact on the seed crop and to determine the factors that influence fertility status of orchards.

Orchard Details and Study Methods

Flower and fruit production was estimated during four consecutive years (2003-06) in two orchards located at Topslip (CSO I: 10˚ 25’ N, 76˚ 50’ E; rainfall: 2080 mm) and Walayar (CSO II: 17˚ 40’ N; 81˚ 00’ E; rainfall: 1000 mm). CSO I has 15 clones and CSO II 20 clones and 13 clones are common between them. Both the orchards were established in 1976 in a completely randomized design at a spacing of 5 m. Two thinnings were undertaken in the orchards which resulted in an average spacing of 10 m between trees. During the study period CSO I had 175 trees comprising 6 to 9 ramets each of 15 clones and CSO II had 454 trees represented by 13 to 30 ramets each of 20 clones.

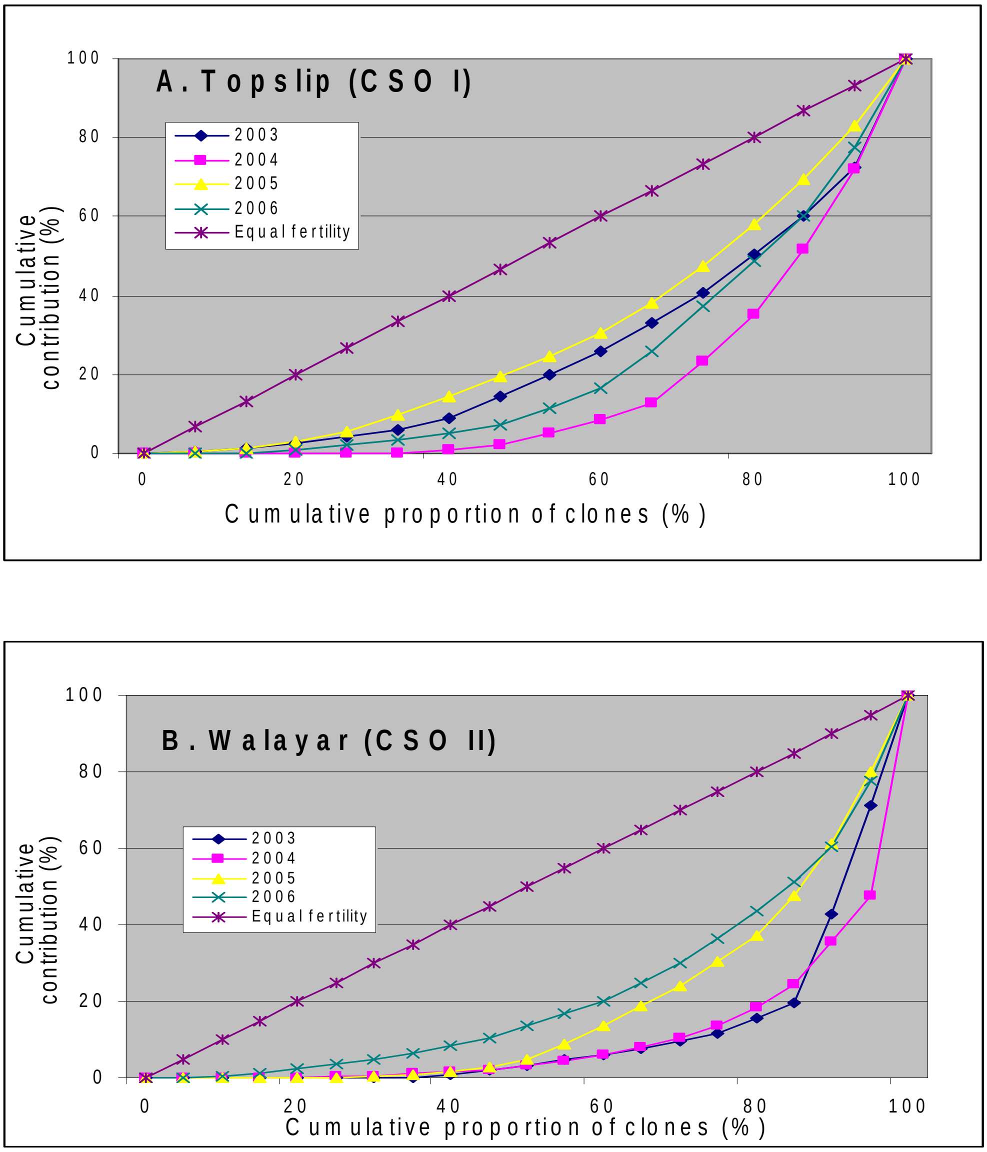
All the flowering ramets were assessed for flower and fruit production following the methods of Bila *et al*. (1999). Diameter was measured for all trees every year while total height and clear bole height were measured only in the last year i.e. 2006. The significance of clonal variation for different traits was determined through analysis of variance. The orchards were divided into four blocks and one random flowering tree in each block was chosen for the analysis. Broad sense heritabilities on individual ramet basis and simple correlations were calculated for all traits studied. Sibling coefficient (Ψ), group coancestry (Θ), status number (*Ns*), relative status number (*Nr*) and gene diversity (GD) were calculated using the methods of Lindgren and Mullin (1998) and Kang and Lindgren (1999).

Results and Discussion

Fertility was generally low in both the orchards with the proportion of flowering ramets ranging from 16 to 53%. Each orchard had one abundant flowering year in which CSO I had 53% of ramets flowering while 39% flowered in CSO II. Fruit production per hectare of orchard ranged from 1 to 18 kg in CSO I and 9 to 17 kg in CSO II. Clones and ramets of a clone differed in fertility across years and orchards. Only 60% of the clones flowered in all four years in both the orchards. At individual tree level, only 11% flowered in all years in CSO I and 19.8% in CSO II while 35% and 52% of trees respectively did not flower any of the four years.

Clones significantly differed in flower and fruit production per tree. A few clones contributed more than others and this imbalance was more pronounced in CSO II than CSO I and during low flowering years than abundant years. About 80% of flowers were produced by 50% of clones in CSO I in three of the four years whereas 40% of clones produced 80% of flowers in CSO II even during abundant flowering year (Fig. 1). Broad sense heritability was moderate to high (0.31 to 0.76) for flower production but low (0.07 to 0.35) for fruit production. In general heritability values were higher in low flowering years in both the orchards. Correlation between flower and fruit production was strong and positive in each year and also for the same trait between successive years. Fertility traits showed low and positive correlation with tree diameter but weakly negative relationship with height and clear bole height.

Fig. 1. Cumulative contribution of gametes by teak clones in two orchards during four years



Sibling coefficient (*Ψ*) and group coancestry (Θ) were higher in low flowering years compared to good years (Table 1). Between the two orchards CSO II showed up to 3 times more fertility variation than CSO I. As a result status number (*Ns*), relative status number (*Nr*) and gene diversity (GD) were generally higher in CSO I than CSO II. However in abundant flowering years the differences in fertility status between the two orchards were greatly reduced and showed comparable sibling coefficient and group coancestry values.

Table 1. Fertility variation (Ψ), group coancestry (Θ), status number (*Ns*), relative status number (*Nr*) and genetic diversity (GD) for four years in two teak CSOs.

	Topslip (CSO I)				Walayar (CSOII)			
	2003	2004	2005	2006	2003	2004	2005	2006
Ψ	1.97	2.64	1.49	2.01	4.45	6.22	2.47	2.26
Θ	0.066	0.088	0.050	0.067	0.111	0.155	0.062	0.057
Ns	7.60	5.68	10.05	7.48	4.50	3.22	8.11	8.84
Nr	0.51	0.38	0.67	0.50	0.22	0.16	0.41	0.44
GD	0.93	0.91	0.95	0.93	0.89	0.84	0.94	0.94

The highest fruit production observed in the present study, 18 kg per ha of orchard would be sufficient to raise only 5 ha of plantations assuming a 30% germination. It could be lower than that if orchard seeds germinate poorly as reported earlier (Indira and Basha, 1999; Mathew and Vasudeva, 2003). The major reason for low fruit production in orchards is a general lack of flowering. Seed production areas of similar age in India have better proportion of fertile trees (58 to 97%) (Varghese *et al*. 2007). Locating orchards in sites suitable for flowering and fruiting like Topslip (CSO I) with high rain fall and deploying clones known to have high fertility in similar sites are expected to increase orchard output.

Moderate to high heritability for flowers and fruits per tree indicate that reproductive traits are under fairly strong genetic control in teak as reported for dimensions of floral parts and flowering phenology (Vasudeva *et al*. 2004). Tree size (diameter) has low but positive correlation with fertility traits indicating that selecting trees based on size will not result in reduction of reproductive output. But total and clear bole height showed weakly negative correlation with flower and fruit production. It is reported that in teak the first flowering is terminal which results in forking of the main stem. The early flowering trees have shorter clear bole length and are usually ignored while selecting plus trees. Forking of the main stem results in a wide crown with many positions for floral development and thus making the tree more fertile than others. The most fertile clone in CSO II (SBL 1) had the shortest clear bole height.

Since fertility variation and group coancestry are more in low flowering years compared to abundant years, seed collection may be restricted to abundant years only especially if seeds are collected for progeny testing and other breeding purposes. Intentional adjusting of ramet number to balance contribution of clones and mixing of seeds from successive years may also reduce relatedness among orchard progeny. Existing orchards typically with 15 to 30 clones may not achieve the production levels assumed at the time of establishing them (250 kg ha^{-1}; Hedegart, 1976). Seed production areas (SPA) which are rigorously thinned plantations can be regarded as low input breeding options for teak (Lindgren and Wei, 2007). Flowering, seed production and germination are generally reported to be better in SPAs compared to orchards (Indira and Basha, 1999; Varghese *et al*. 2005). The large number of parent trees in SPAs ensures that high level of diversity is maintained in the progeny even if the gains are modest. They can also be a source of seed for developing next generation seedling seed orchards.

Acknowledgements

This study was supported by International Foundation for Science (IFS), Sweden through a research grant to A. Nicodemus (Grant No.D/3384-1) and Swedish Research Council and SIDA by a research grant to Mohan Varghese and Dag Lindgren. Assistance and field support was provided by research wing of Tamil Nadu forest department.

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A review of Scots pine and Norway spruce seed orchards in Finland

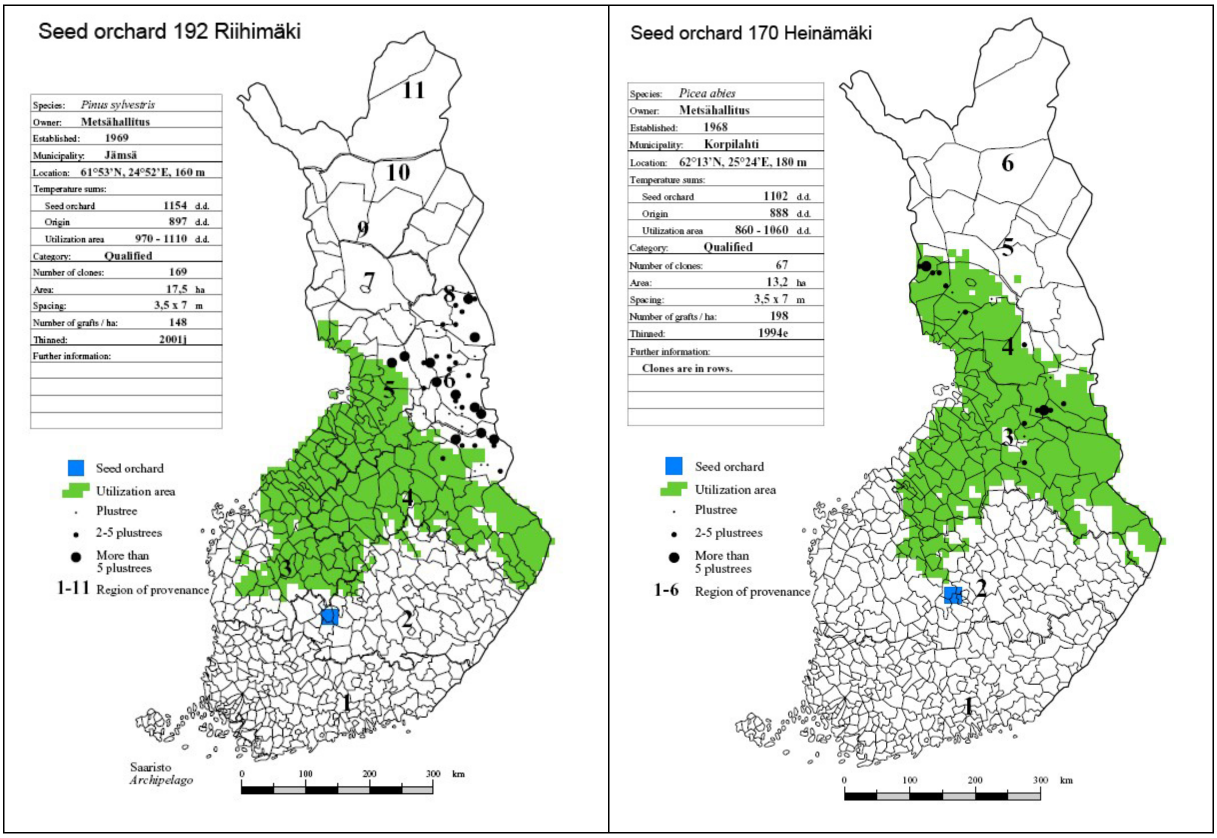
Teijo Nikkanen
Finnish Forest Research Institute, Punkaharju Research Unit
Finlandiantie 18, FIN-58450 Punkaharju, Finland
E-mail: teijo.nikkanen@metla.fi

The first seed orchards were established in Finland in the early 1950's. However, the large-scale establishment of seed orchards started in the late 1960's, and was completed by the mid 1970's (Fig. 1). The goal was to produce in seed orchards all the seed needed in nurseries and for direct seeding. In all about 3000 ha of Scots pine (*Pinus sylvestris* L.), and 300 ha of Norway spruce (*Picea abies* (L.) Karst.) seed orchards were established.

Figure 1. First generation seed orchards were established with phenotypically selected plus tree clones. Scots pine seed orchard in Central Finland photographed in the early 1980'.

Nowadays part of the early seed orchards have already been abandoned and there are 141 (2202 ha) first generation seed orchards registered for Scots pine and 25 (282 ha) for Norway spruce in Finland. The total number of plus tree clones grafted in orchards is 5903 for pine and 601 for spruce. The average number of clones (and the average area) in an orchard is 139 (15.5 ha) for pine and 75 (10.9 ha) for spruce.

In Finland almost all of the seed orchards of northern Finnish origin were located in the southern parts of the country. This was done in order to enhance flowering and to achieve better seed maturation. When clones originating from geographically and climatically limited areas, and from northern Finland were used in seed orchards established in southern Finland, it was assumed to achieve phonological isolation between the seed orchard clones and surrounding forests. However, no phenological isolation has been found, and background pollination has become a serious problem. The proportion of background pollination both in pine and spruce is often more than 50 %. This reduces the genetic gain, and adaptability of seedlings when used in areas where the mother clones originated. That is why the seed from the orchards of the northern origin is not utilised as far north as the mother clones were located (Fig. 2).


Seed orchard 192 Riihimäki

Species: *Pinus sylvestris*
Owner: **Metsähallitus**
Established: **1969**
Municipality: **Jämsä**
Location: **61°53'N, 24°52'E, 160 m**
Temperature sums:
Seed orchard **1154** d.d.
Origin **897** d.d.
Utilization area **970 - 1110** d.d.
Category: **Qualified**
Number of clones: **169**
Area: **17,5** ha
Spacing: **3,5 x 7** m
Number of grafts / ha: **148**
Thinned: **2001j**
Further information:

Seed orchard 170 Heinämäki

Species: *Picea abies*
Owner: **Metsähallitus**
Established: **1968**
Municipality: **Korpilahti**
Location: **62°13'N, 25°24'E, 180 m**
Temperature sums:
Seed orchard **1102** d.d.
Origin **888** d.d.
Utilization area **860 - 1060** d.d.
Category: **Qualified**
Number of clones: **67**
Area: **13,2** ha
Spacing: **3,5 x 7** m
Number of grafts / ha: **198**
Thinned: **1994e**
Further information:
Clones are in rows.

Figure 2. Examples of Scots pine and Norway spruce seed orchards originating from northern Finland.

All the first generation seed orchards are still in the production phase. Scots pine seed orchards produce enough seed for all the nursery use in southern and central Finland, and part of the seed needed for direct seeding. Because of background pollination the seed produced by the orchards of northern Finnish origin cannot be used in the areas of mother clones. That is the reason, why in Scots pine the proportion of orchard seed used in nurseries has been only from 50 to 60 percent for the last 15 years (Fig. 3), although there is a plenty of pine seed orchards and the amount of seedlings used for planting has been reduced substantially. Situation in Norway spruce is quite different. Spruce seed orchards started to produce seed in older age than pine orchards, but after seed production had started the proportion of spruce orchard seed used in nurseries increased from 10 to almost 80 percent during the 1990' (Fig. 3). Insufficient flowering together with cone and seed damages have been the reason for the decrease in seed production during the last years.

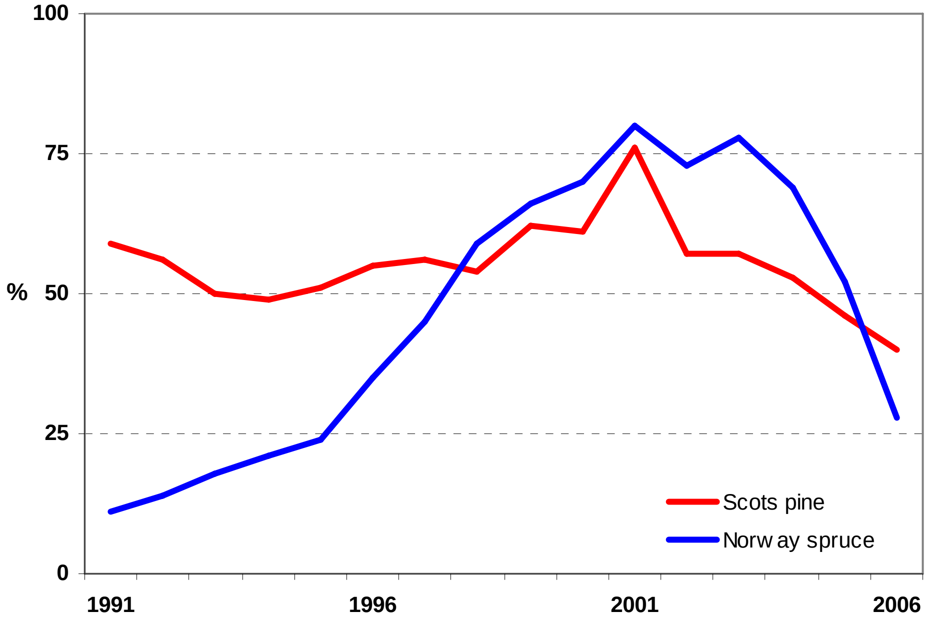


Figure 3. Proportion of orchard seed used in nurseries 1991 - 2006.

New generation of seed orchards, established with tested clones, started in the late 1990'. For the new seed orchards, the target to produce all the seed needed in the whole country in seed orchards was revised, especially concerning seed production for the northernmost part of the country and for direct seeding. The main target of the so-called 1.5 -generation seed orchards is to produce seed with as good genetic quality as possible. The plan is to achieve this by using the best 20 % (based on progeny tests) of the phenotypically selected plus tree clones. The aim is to produce seed with genetic gain about 10 % higher than in the first generation seed orchards. Only 20 – 30 clones are used in one seed orchard. The seed orchards for northern Finland are going to be established at more northern locations than the corresponding first generation seed orchards.

Figure 4. Scots pine seed orchard 406 (Seppälä) established in 1998 with tested clones.

A new master plan for forest tree seed production, made in 2003-2004 by a working group appointed by the Ministry of Agriculture and Forestry, was based on earlier programmes carried out in 1989 and 1997. The task of the new, broad-based working group was to evaluate the need for forest tree seed in 2005-2030, and based on this evaluation to revise the existing seed orchard programme. Annual need for Norway spruce seed orchard seed for nursery use was estimated to be 1827 kg, and that for Scots pine 542 kg. In addition, 3416 kg of Scots pine orchard seed is needed for direct seeding. The total seed orchard area needed for Norway spruce was thus 290 ha from which still 150 ha has to be established, and the total area for Scots pine was 610 ha from which 160 ha has to be established.

Because of the high establishment costs and long waiting time for the first crops, seed production in seed orchard is not a tempting business investment. That is why the working group recommended that the state should subsidy 85 % of the costs caused by establishment of seed orchards (excluding land costs) and tending of young orchards. Also decentralized seed production strategy, i.e. several independent seed producers, was recommended.

Finnish Birch Seed Production 1970-2007

Sirkku Pöykkö
Forestry Engineer
Finnish Forest Research Institute (Metla)
Haapastensyrjä Tree Breeding Station
Haapastensyrjäntie 34, 12600 Läyliäinen, Finland
E-mail: sirkku.poykko@metla.fi

History

Forest tree breeding started in Finland by the plus tree selection in 1947 when the Foundation for Forest Tree Breeding was founded. Intensive birch breeding was initiated in a large scale in the early 1960's with crossings and progeny testing. Breeding work was supported by the plywood industry.

At the beginning of the 1970's the seed production of birch was revolutionized when the Foundation for Forest Tree Breeding developed the plastic greenhouse and the related growing technique. The first birch seed orchards were established already at the beginning of the 1970's. The Finnish plywood industry supported also this work. All seed orchard seed is nowadays produced in plastic greenhouses.

Usage of birch

During the 1980's the use of birch seedlings increased manifold in artificial regeneration. At the same time wood consumption of birch increased in paper industry. In the 1990's the afforestation of arable land was financially supported by state and nearly 100 000 ha of agricultural land were planted into forest. The use of birch was at its height, about 28 million seedlings in 1992. Also direct seeding was introduced as an artificial regeneration method which needs much more seed than plant production. (Figure 1.)

In the 2000's the use of birch seedlings has diminished to about 5 million seedlings. The support for afforestation of arable land has considerably decreased and is finishing at the end of this year. During the 2000's the moose population has been very high. Moose is the biggest obstacle in birch planting at the moment.

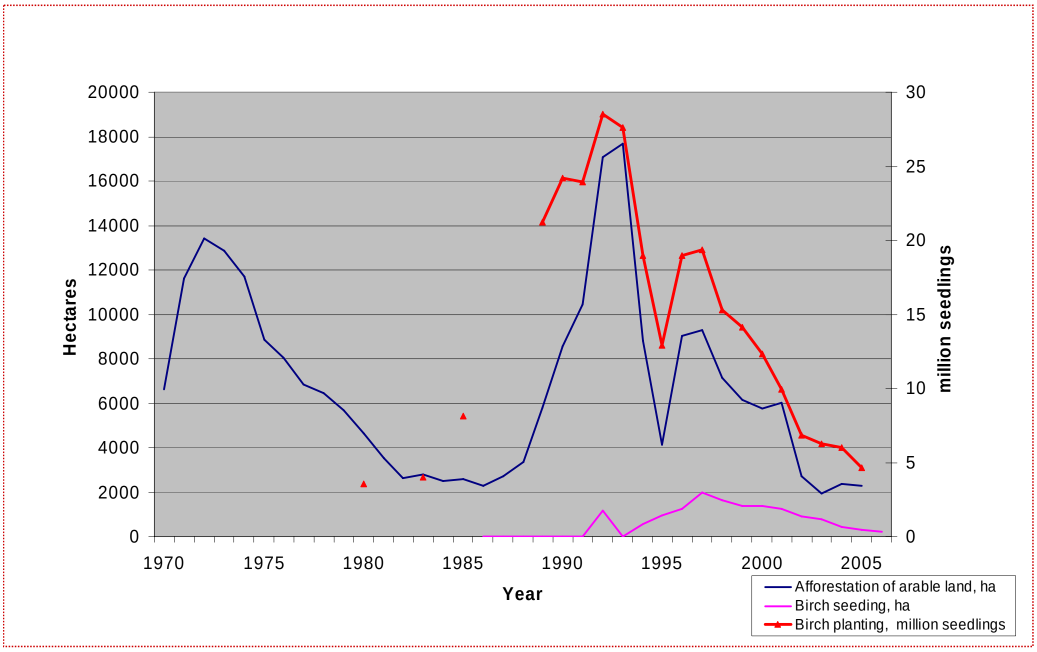


Figure 1. Artificial regeneration of birch.

Birch seed production

In the 1970's there were the first three orchards for testing and developing the production method. In the 1980's, when the artificial regeneration of birch begun to increase, several new orchards were established. And in the 1990's during the golden days of birch planting, there were 18 birch seed orchards in Finland in the middle of the decade. Record amount is from 1998; over 300 kg of birch seed was produced in Finland.

In the 2000's production area has been decreased according to the decreasing regeneration area. At the moment there are seven orchards and five of them will be cut down in a year or two. The seed stock in storage is quite big and new seed can be produced in 5 years if the need for birch seedlings gets higher again. (Figure 2.)

In total we have had 34 different seed orchards in Finland so far: 26 silver birches, 6 downy birches and 2 curly birches. Total amount of produced seed is 3100 kg! There are about 1 million seeds in one kilo and we can get about half a million seedlings per kilo.

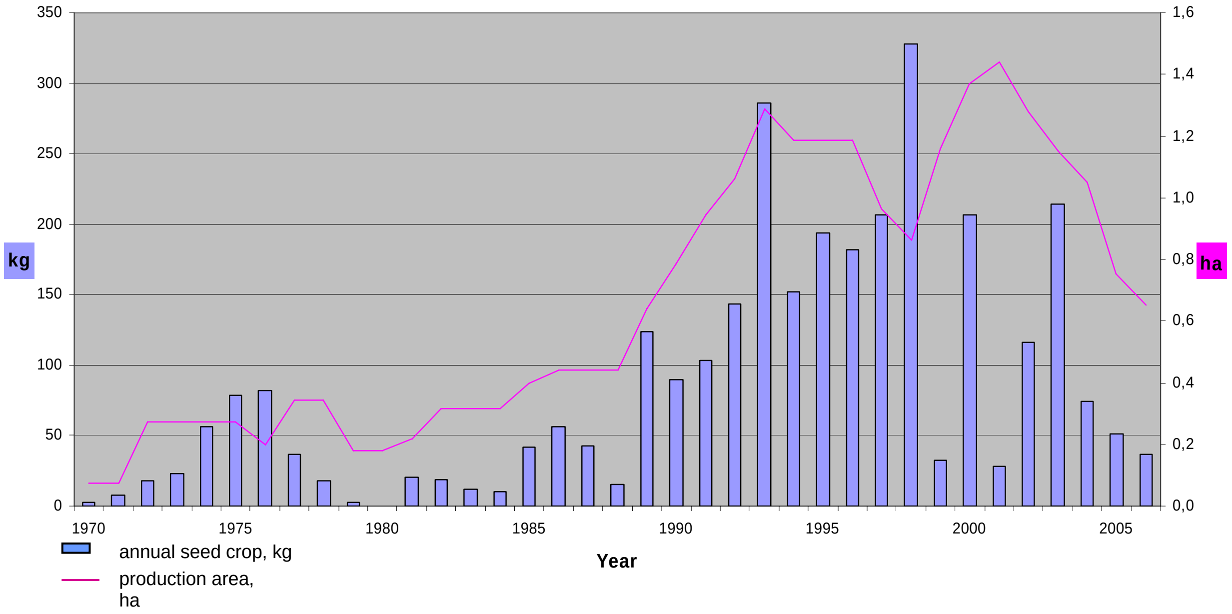


Figure 2. Annual seed crop and production area of birch.

Birch seed usage in nurseries

Amounts of seed used in nurseries have decreased considerably. Even when the artificial regeneration was in a high level at the beginning of the 1990's, the seed use in nurseries has decreased because the growing methods have improved. At this moment nurseries use only about 20 kg birch seed per year. (Figure 3.)

The proportion of qualified birch seed used in nurseries is very high; in 2005 95% of Betula pendula and all Betula pubescens seed was qualified. (Figure 4.)

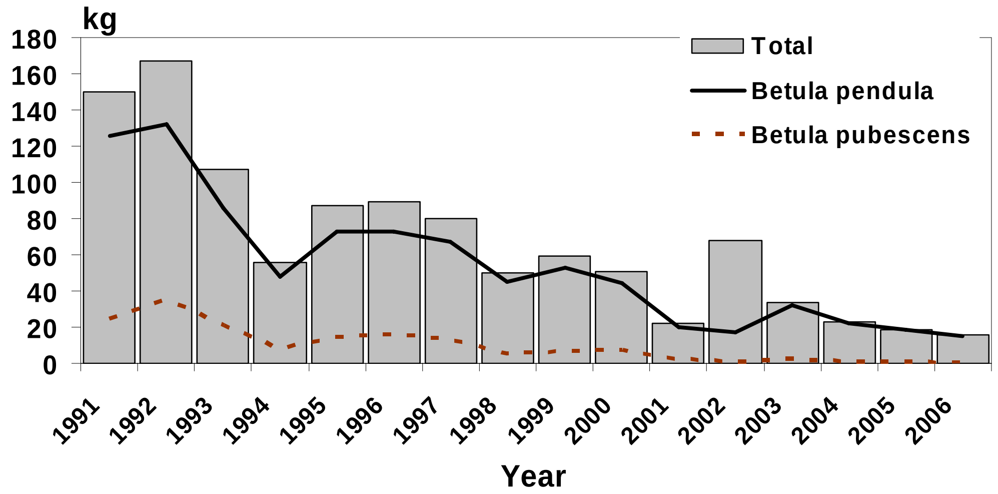


Figure 3. Amounts of seed used in nurseries 1991-2005.

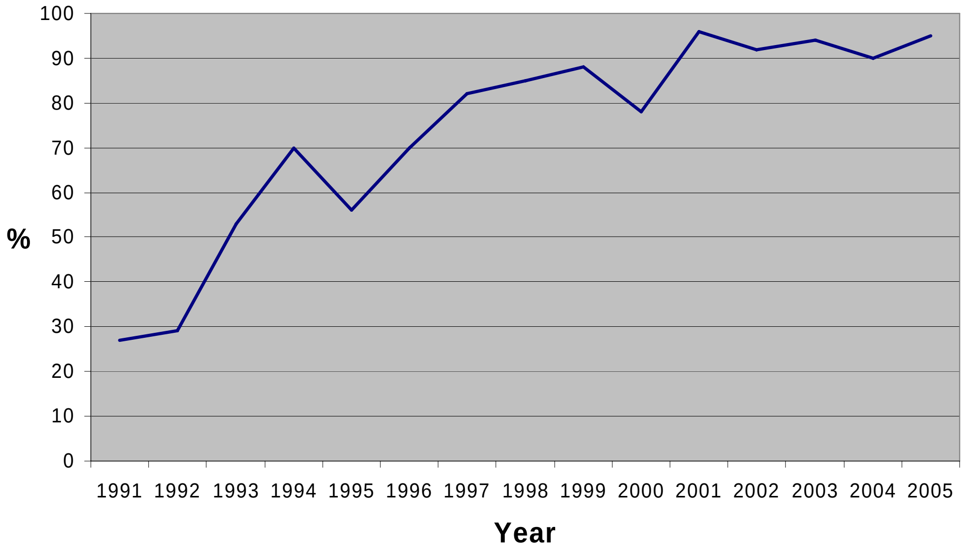


Figure 4. The proportion of qualified *Betula pendula* seed used in nurseries.

Breeding generations and gains

At the moment seed orchard clones of Betula pendula come mainly from the 2nd and 3rd breeding generations. The clones of Betula pubescens are from the 1st generation.

Tree breeders Risto Hagqvist and Juhani Hahl studied the genetic gains of the three first seed orchards of Betula pendula. The stem volume of the orchard material from Southern Finland increased significantly by 29% compared to control stock. Improvement in stem form also took place: relative stem taper decreased significantly in the seed-orchard material by 13%. Relative branch thickness was significantly 10% smaller in the seed-orchard entries than in the control stock. In new seed orchards quality will be even better, because there is more attention concentrated on quality in clone selections.

Export of the method

Finnish birch breeding and seed production have aroused interest also abroad. Seed production method has been exported in several countries like Sweden, Baltic countries, Iceland, Germany and China.

British Columbia's Seed Orchard Program: Multi Species Management With Integration To The End User

By

David J.S. Reid, R.P.F.
Manager, Seed Production,
Tree Improvement Branch,
British Columbia Ministry of Forests and Range
Victoria, British Columbia, Canada
David.Reid@gov.bc.ca
http://www.for.gov.bc.ca/hti/index.htm

British Columbia is Canada's most western and third largest province with an area of 950,000 square kilometres, ranges in latitude from 49 to 60 degrees, and 95% of the land is owned by the province. It is also Canada most ecologically diverse province. B.C. is divided into 14 unique ecosystem zones. It uses what is called a Biogeoclimatic Ecosystem Classification system to provide resource professionals with detailed information about the climate, soils, growing conditions and characteristic vegetation in each zone so they can be sure management decisions about forests, rangeland and wildlife reflect the special needs of each area.

The province has 49 tree species (25 conifer and 24 deciduous) of which 20 are considered commercially useable. Seed is very important in British Columbia. In the last 14 years, over 200 million seedlings were planted annually. In the last three years over 250 million seedlings were planted while 2007 saw 275 million seedlings of 23 different species planted on over 150,000 hectares. The 2007 sowing was comprised of 3,300 sowing requests from 1,341 seedlots that used 2,493 kilograms of seed.

British Columbia's tree improvement program started in 1951 with Dr Alan Orr-Ewing conducting studies with "in-breeding". Plus tree selection began in the late 1950's in coastal Douglas-fir. The first seed orchard was built in 1963 while other species were started in the early 1970's. There are two Branches of the Ministry of Forests and Range (MFR) that are involved with tree improvement in B.C. Research Branch has nine scientists and many technicians responsible for breeding, testing, and selection, while Tree Improvement Branch is responsible for seed production on six production sites, extraction & storage at a centralized Tree Seed Centre, and policy and planning. Today there are 11 species of conifers and 1 deciduous species that are in BC's tree improvement program (see Table 1)

Table 1: Tree Species of the British Columbia Tree Improvement Program

Species	Common Name	# ramets	# orchards
1. Pinus contorta var. latifolia	Lodgepole Pine	63,260	27
2. Picea glauca	White Spruce	26,280	26
3. Pseudotsuga menziesii var menziesii	Coastal Douglas-fir	12,775	14
4. Pseudotsuga menziesii var glauca	Rocky Mountain Douglas-fir	10,760	9
5. Larix occidentalis	Western Larch	4,080	4
6. Pinus monticola	Western White Pine	5,440	6
7. Pinus ponderosa	Ponderosa pine	1,775	2
8. Tsuga heterophylla	Western Hemlock	3,145	9
9. Thuja plicata	Western Redcedar	1,405	6
10. Picea sitchensis	Sitka spruce	1,440	4
11. Chamaecyparis nootkatensis	Yellow cedar (hedges)	22,090	4
12. Betula papyrifera	Paper birch	150	2
		152,600	113

Every parent in every seed orchard has a breeding value calculated from progeny tests and BC's seed orchards have an average of 57 parents in them. Additional progeny test results will reduce this number in the future; however seedlots must have an effective population size of 10 before they can be used in reforesting BC's public land base, so most orchards will probably maintain an average of 25 to 30 parents.

The contribution of the parental breeding values to a seedlot results in the calculation of a genetic worth for that seedlot. Genetic worth (GW) can be calculated for volume gain, wood quality, or pest resistance. GW's for volume gain range from 5 to 34.

While most of the seed orchards listed in Table 1 have been developed for volume gain, some orchards have also been selected for pest resistance against pests such as the White Pine Blister rust, Sitka Spruce leader weevil, and various rust diseases. Research is finding resistance against other pests such as the Mountain Pine Beetle, deer browsing, and root rot all of which will one day be incorporated into new seed orchards.

BC has 14 major seed orchard sites, six are on the coast and eight are in the interior. Seed production is also a shared responsibility among the private sector and the MFR. The MFR has six sites and the private sector has eight. These sites are located in three main areas of B.C.; on the south-east coast of Vancouver Island, the north Okanagan area of the southern interior, and near Prince George in the central interior of the province. The coast and southern interior sites get over 2000 hours of sunshine per year; the coastal orchards receive around 85 cm of precipitation per year, while the interior orchards receive around 45 cm per year. The most northerly orchard is located at 54 degrees latitude near Prince George.

The Forest Genetics' Council is an advisory body that provides advice to the Province's Chief Forester and has set two major goals:

1. That 75% of all seed used for reforestation in the province is to come from seed orchards by the year 2105. Currently the province plants over 270 million seedlings per year and over 50% comes from orchards, so that means around 135 million seedlings per year are currently produced from orchards and this figure will rise to around 200 million to meet the goal.
2. The average genetic worth (in volume gain) of the seed is to be 20% by 2020. Currently, the average gain of the seed orchard production is 14%.

All of the productive capacity needed to reach these goals has now been established in seed orchards and just need time to mature to the seed production age.

The key to making this program work is a very structured business and strategic plan.
The province is divided into multiple seed planning zones for each species. These zones, when classified elevationally are called seed planning units (SPU). Seed planning units are geographically distinct areas that form the basis for gene resource management, including: tree improvement (breeding, orchard crop production), seed transfer (areas of use), monitoring and gene conservation. SPUs are based on species (see figure 1), ecological zone, elevation, and, in some cases, latitude band. B.C. has 90 SPU's of which 50 have been selected for inclusion into the business plan. There are seed orchards for 37 SPU's while the balance of the zones are included in the plans for genecological or conservation purposes. For some species in some SPU's there are multiple orchards on different sites due to historical development or ownership patterns.

The sites are generally multi- species sites - the largest Ministry site is about 35 hectares and 20,000 ramets (see figure 2) while the largest private site is 85 hectares and 40,000 ramets. In total, the fourteen seed orchard sites cover just over 400 hectares of land.

Figure 1: Interior Spruce Seed Planning Zones

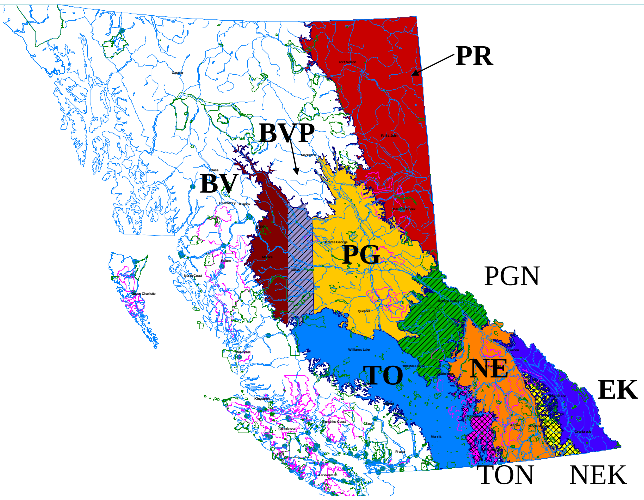
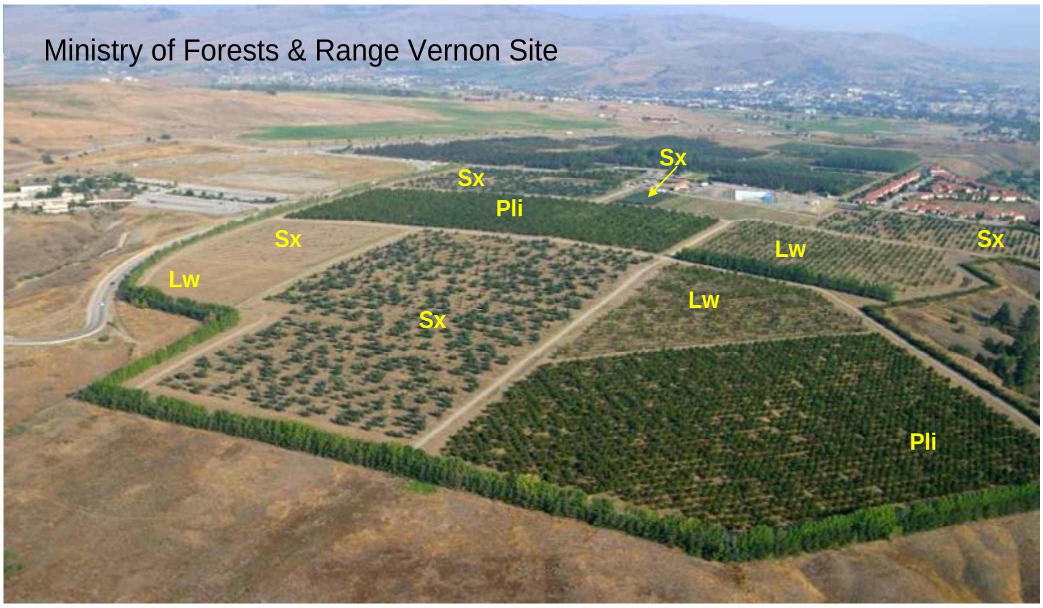


Figure 2: A 35 hectare site that has ten seed orchards of three species. Each seed orchard produces seed for a different Seed Planning Unit. This site and three others were developed on grasslands while most other sites were derived by clearing forested areas. Tree spacing varies by species: Lw: 5m x 2m, Sx: 5m x 5m, Pli: 5m x 4m and 7m x 3.5m, Fdi: 6m x 3m, Pw: 6m x 4m.



Managing multiple species means that pollination season lasts for 5 months as the individual species have different physiological developmental times. Red cedar pollen season starts in February and White Pine in mid June with the other species in between (Figure 3). Supplemental mass pollination (SMP) is used frequently in young or old orchards where there is an insufficient internal pollen cloud to ensure cones maximize their filled seed potential. SMP is also used to help boost the genetic worth of seedlots by applying high breeding value pollen. Controlled pollination is done operationally in B.C. seed orchards but not on a large scale due to the high labour cost of this technique.

Figure 3: Multi-Species Pollen Management runs from February to June

British Columbia MFR employs three cone and seed entomologists to help control insect problems at the seed orchards and conduct research into new pests that constantly appear and develop integrated pest management programs to help control populations as the ability to use and availability of registered pesticides shrinks. Entire seed crops can be lost if staff are not vigilant.

Cone harvest can start in July for Interior spruce and go into December for Lodgepole pine. Since the first seed orchard cone crop in 1976, and not including the 2007 crop, over 15,240 kilograms of seed have been produced from BC seed orchards. This is enough seed for over two billion seedlings.

Most cones are sent to the Provincial Tree Seed Centre (TSC) (celebrating its 50th anniversary in 2008) for seed extraction, testing and storage; although there are three private extractories in B.C. It is a provincial requirement that all seed to be used in public land reforestation must be tested and stored at the TSC. Approximately 70,000 kilograms of orchard and wild seed are stored at the facility. Selection of seed by the forestry companies for their sowing requests is done through an online computerized catalogue ordering system called the " Seed Planning and Registry" system (SPAR) maintained by the MFR. It is provincial policy that all public land must use seed orchard seed of GW 5 or better first if it is available on SPAR before wild stand seed can be used. The cost of seed orchard seed varies by species. The provincial or domestic price list appears in Table 2 below:

Table 2: British Columbia MFR Domestic Seed Price List

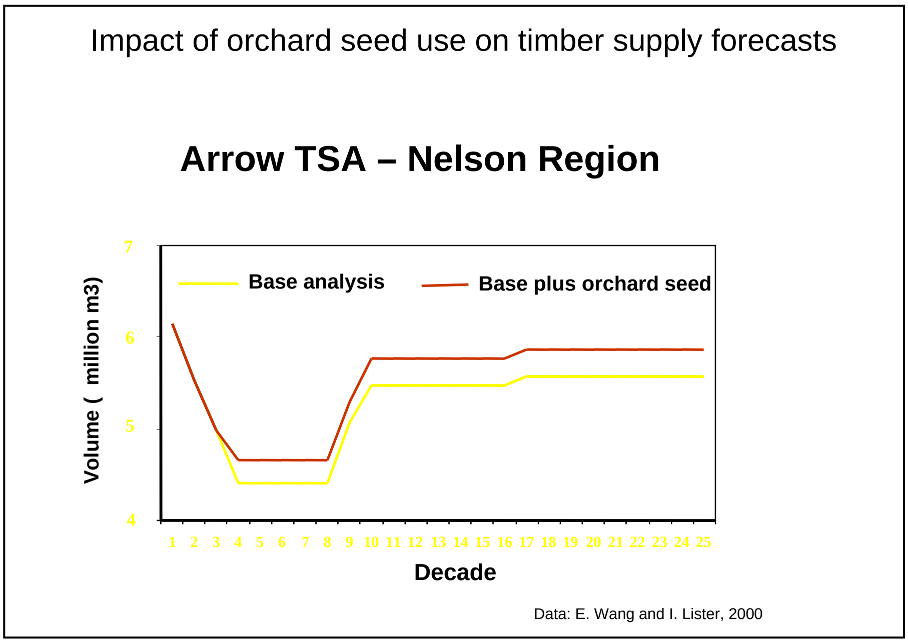
	Species	Cost / Kg ($CAN)
1	Douglas fir	$3,850
2	Red Cedar	$6,500
3	Lodgepole pine	$6,300
4	Spruce	$4,400
5	Western Hemlock	$5,500
6	Western Larch	$3,850
7	Western White Pine	$2,500

While the above prices may seem high to some, the incremental cost of using orchard seed over wild seed translates to approximately $40 per hectare when planting 1,600 seedlings per hectare. There are also many other benefits to using seed orchard seed. In the early years of plantation survival, orchard seed grows faster and thus may eliminate the need for costly brushing and weeding treatments that can cost over $1,000 per hectare per year. Seed orchard plantations will also reach "free growing" sooner which is a regulatory designation that will permit adjacent timber to be harvested. Then, there is the benefit of producing more timber volume faster and reducing rotation ages. This last benefit is where the use of seed orchard seed is a major benefit to the end users in B.C. which are the forest company licensees that operate on the public Timber Harvest Land Base (THLB).

Timber supply in BC is calculated on 71 management units called timber supply areas. The Chief Forester sets the Allowable Annual Cut (AAC) and is reviewed every 5 years. As the source of the seed planted out is tracked in a database, it is a matter of tracking the genetic gain of the seed used and inputting this data into a managed stand growth model.

Data is then run through forest estate models and other considerations to arrive at an AAC determination. The volume gain from using seed orchard seed is represented in Figure 4. A base case is calculated without using improved seed and then run using improved seed. The resulting difference can be seen in the higher line. This is volume that is available for use to the licensees.

Figure 4:



Impact of orchard seed use on timber supply forecasts

Arrow TSA – Nelson Region

Data: E. Wang and I. Lister, 2000

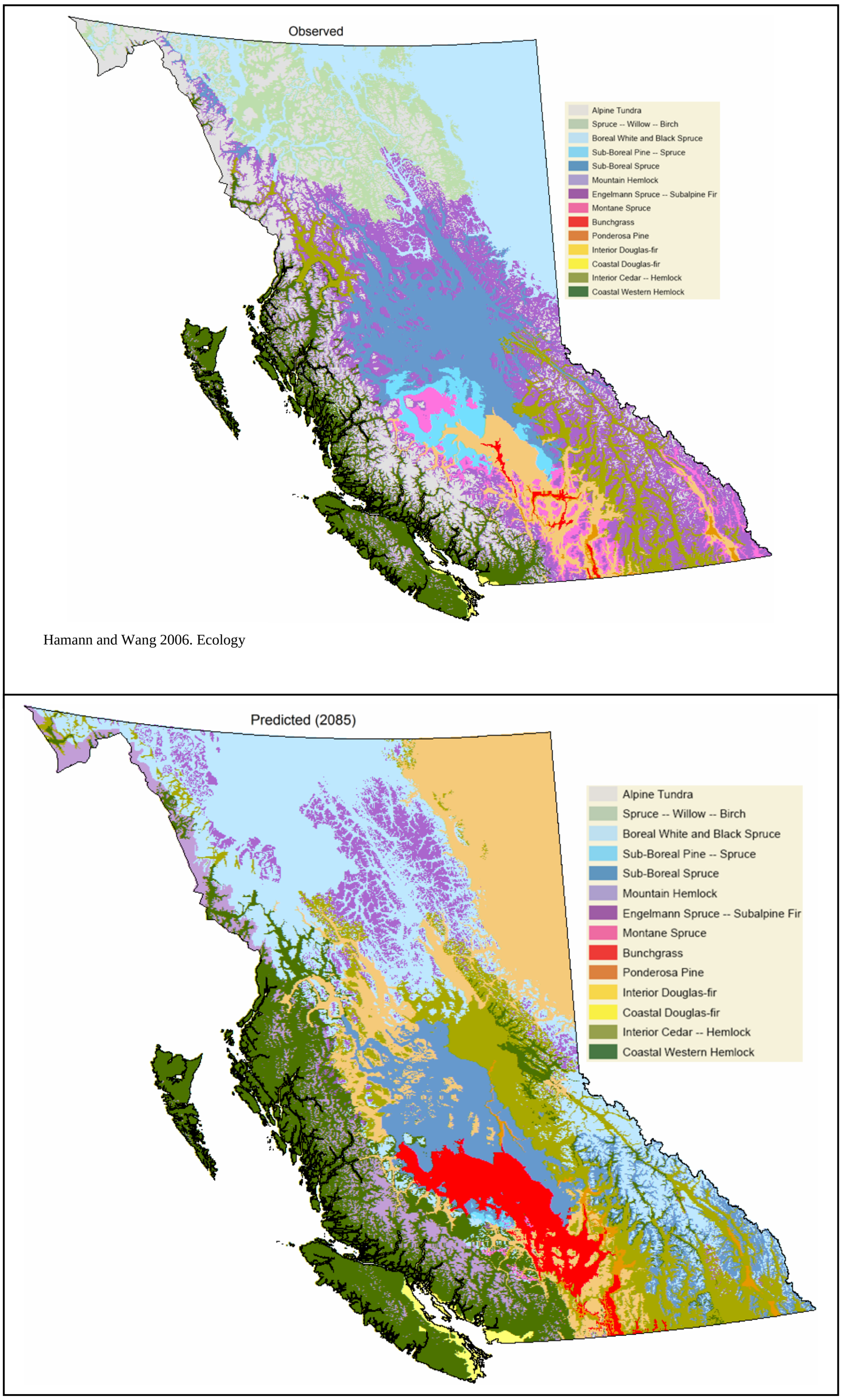
The use of seed orchard seed in this TSA has an impact on the timber flow as early as decade three.

Calculating AAC's in B.C. has been challenging since the Mountain Pine Beetle (MPB) insect infestation has been attacking the Lodgepole pine forest of BC since the mid 1990's. The normal cold climatic conditions that normally control such outbreaks have not occurred. Thus, as of 2007, the outbreak has killed over 50% of the Pli on 10 million hectares (Figure 5) and when the outbreak is expected to finish around 2013, it will have killed over 80% of the Pli over 13 million hectares or an estimated one billion cubic meters of wood. The insect has not behaved according to its normal pattern which is to normally attack mature and over mature timber. Due to its outbreak size it is attacking trees as young as 15 years old. This has serious long term implications for timber supply in BC. BC's seed orchards were never sized to meet this extra need, so reforestation of beetle killed areas is being done with wild stand seed. Thus volume gains will not be expected in these areas.

Figure 5: An example of the widespread Mountain Pine Beetle infestation killing Lodgepole Pine in British Columbia's forests.

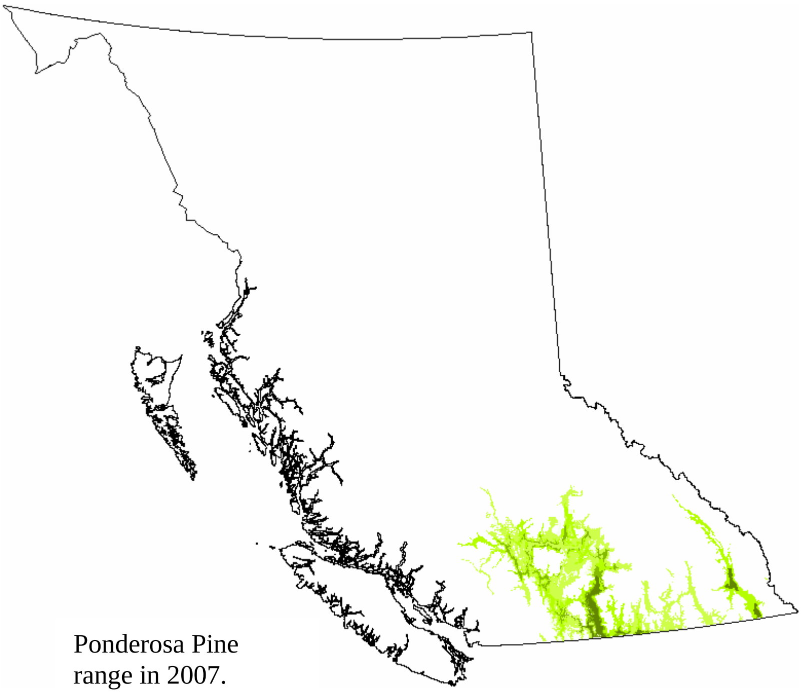
Climate change is a global concern. Studies conducted at the University of British Columbia Centre for Gene Conservation have outlined how temperatures in B.C. will shift over the next 70 years and the impact this may have on the ecosystem zones (see Figure 6). Species will shift also and where some species may increase their potential range, other species range will decrease (see Figures 7 & 8). This presents dilemmas to forest managers who must decide what seed source to plant now that will survive the anticipated upcoming climate change. SPU boundaries will change and seed orchards will have to change as well. B.C. is implementing a new range of multi-species test plantations over 18 locations around the province that will help guide decision makers and indicate a course of direction. However, we may not be able to wait for the data to be analyzed as the trees we plant today must survive for the next 60 to 100 years depending upon location. One course of action that is being considered is what is called "facilitated migration". This is where, for example, instead of using 100% of the seed for a defined SPU, a manager will start to include seed from either lower elevations or more southerly latitudes from other SPU's. As the first ten years of a plantations survival is the most fragile, this course of action is not without its risks. To do nothing also appears to be risky, so the key question will be “when to implement new strategies and actions?”

Figure 6: Prediction on how climate change may impact the ecological zones of B.C. from the current zones to a prediction for the year 2085.



Hamann and Wang 2006. Ecology

Figure 7: The top diagram indicates the current range of Ponderosa Pine in 2007 while the bottom diagram shows the potential range in 2085.



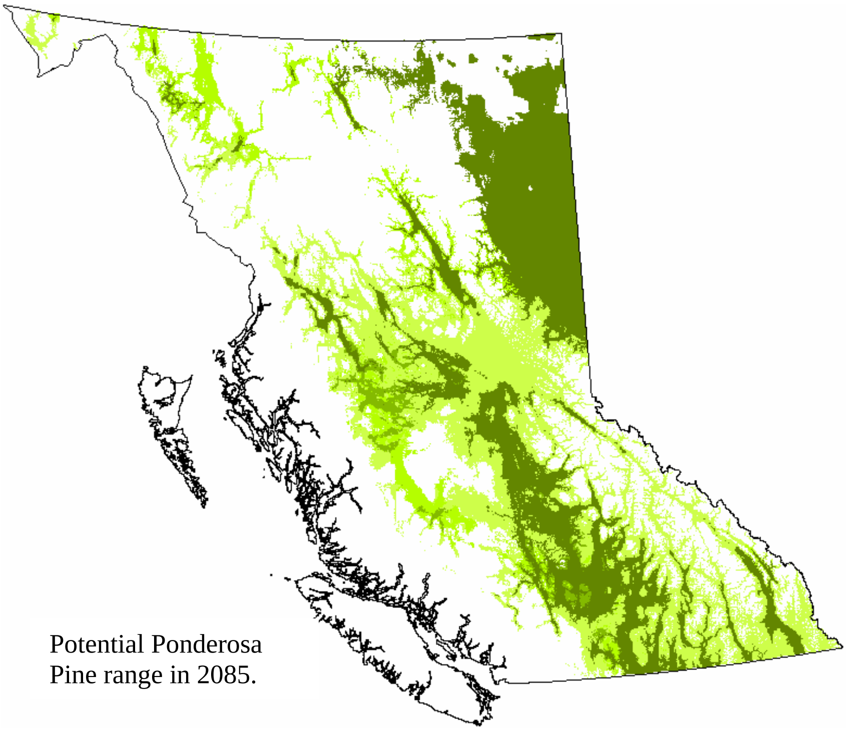
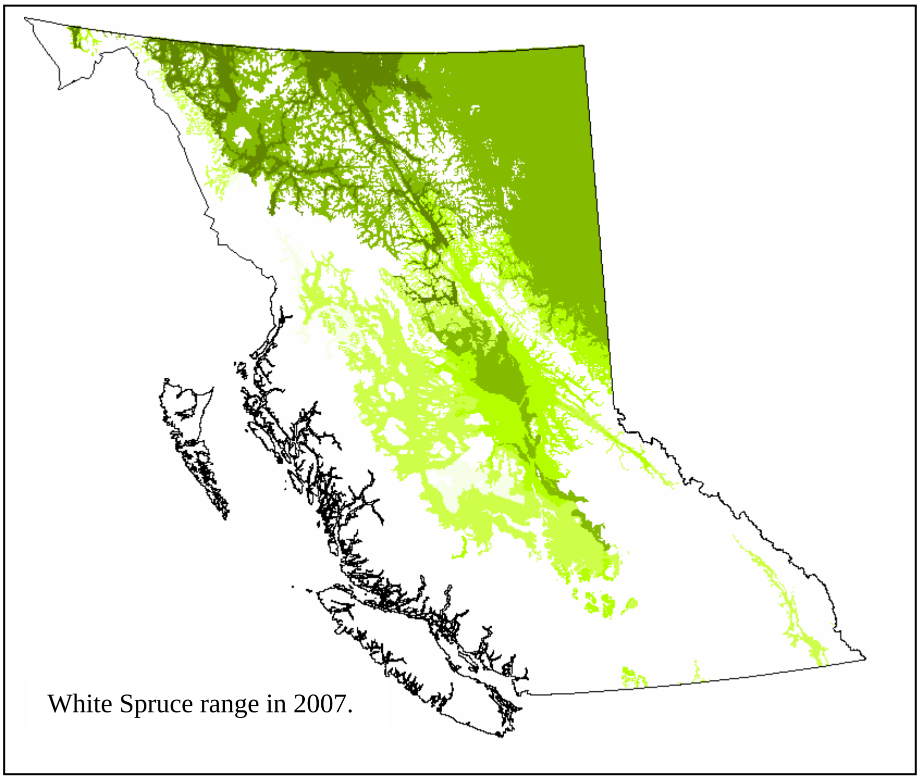
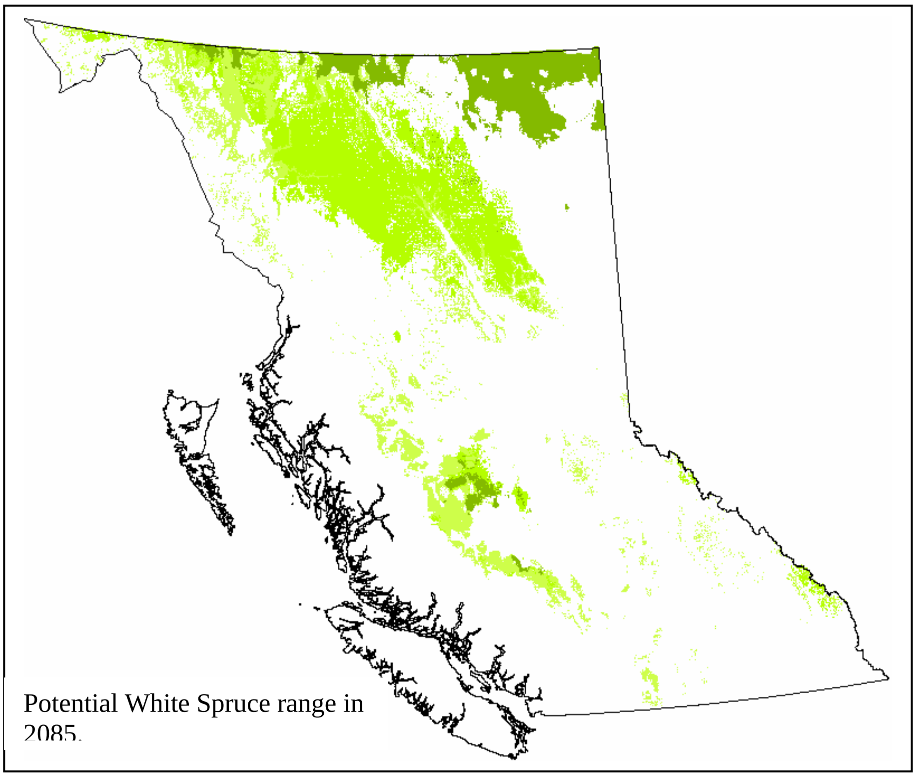


Figure 8: The lower figures are the current and predicted ranges for White Spruce. (from Hamann and Wang 2006, Ecology).



White Spruce range in 2007.



Potential White Spruce range in 2085.

Pest insects and pest management in Swedish spruce seed orchards

Rosenberg, O. & Weslien, J.
The Forestry Research Institute of Sweden (Skogforsk)
Uppsala Science Park
SE-751 83 UPPSALA

Extended abstract

In Sweden, seed orchards are established to produce high quality seeds for reforestation. These seeds not only have a high market value (currently c 1100 Euro/ kg), but trees derived from these seeds ensure higher increment and better wood quality than do trees that originate from seeds collected in the forest. Seed production in Norway spruce seed orchards is far below the demand. One reason for the low and unpredictable seed production is damage caused by insects. Among the most serious pests are three lepidopteran species, *Cydia strobilella* L., (Tortricidae), *Dioryctria abietella* Den. et. Schiff.(Pyralidae), *Eupithecia abietaria,* Goetze (Geometridae) and one dipteran species, *Strobilomyia anthracina* Czerny (Anthomyiidae). Since 1996 we have been working with pest management in seed orchards. In trials 1996, 2000, 2002 and 2003 we used *Bacillus thuringiensis* var. aizawai x kurstaki, active against lepidopteran species. We found that this biological insecticide reduced damages of *D. abietella* and *E. abietaria* from about 60 % to 20 %, but did not affect *C. strobilella*. In order to reduce damage of all insects we started trials with injections of systemic insecticides (mainly imidacloprid and abamectin). Due to low densities we could not evaluate the efficacy of the insecticides against *C. strobilella* and *S. anthracina*, but according to preliminary results, *D. abietella* and *E. abietaria* were reduced from about 25 % to 10 %. In 2007 we have injected higher volumes in order to achieve greater damage reductions. During 2007, we have also monitored *D. abietella* using a new pheromone formulation. In the traps we found almost exclusively this species which indicates that it could be possible for the seed orchard keeper to use this pheromone in order to decide if and when pest management should be used.

New Swedish Seed Orchard Program

Ola Rosvall[1] and Per Ståhl[2]

[1]Research Director [2]Senior Researcher,
Skogforsk, The Forestry Research Institute of Sweden, Umeå[1] and Uppsala[2], Sweden

Swedish forestry is now gradually switching their seed source of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L Karst.) from the first to the second round of seed orchards, and at the same time in cooperation establishes a third set of orchards. Tree breeding enhances genetic gain in wood production by about 10% every 20 years, which fits well with the planting of new seed orchards at the same intervals. Swedish orchards reach full seed production after 15 years and have a life span of approximately 40 years.

Generally, at present, 25% increase in long-term wood production per unit area can be reached with new seed orchards. By 2010–2020 a gain of 35% can be achieved. Norway spruce will proceed more rapidly than Scots pine since clonal testing is applied in the spruce breeding program. About 40% pollen contamination in production seed orchards and up to 20% natural regeneration in planted stands causes realized gain to vary between 16 and 28%.

There are 14 seed zones for each of Scots pine and Norway spruce. An ordinary seed orchard is made up from 25 clones, which are deployed in higher frequency the greater the breeding value. The genetic diversity corresponding to at least status number = 15. Scots pine orchards are planted at 3 m spacing within rows at a distance of 7 meters. The tree rows are regularly pruned into hedges where the first cut is carried out when they have reached 3 m height. Spruce orchards are preferably planted at 2.5 by 7 m spacing in order to enhance early seed crops. They are later thinned to 5 m within-row spacing. The tree tops are cut off after mast years, whereupon new leading shoots will regenerate.

The third round of orchards is estimated to cost $12.5 millions including establishment and management until the first seed crop. This corresponds to $0.0025–0.005 per seedling or $0.08 per additional cubic meter of wood produced. The internal rate of return is estimated to 7%.

There is a tradition in Sweden to run seed orchards in cooperation. In the 3:d round of orchards the forest owners' associations in Sweden are new partners among the large forest companies, the national forest enterprise, and independent forest nurseries. Each collaborator owns his share of an orchard, which is managed by an entrepreneur, generally a forest nursery. To facilitate efficient administration cooperation is carried out in three regional groups. Skogforsk initiated the national program, produced the basis for decision making, and is coordinating the program under supervision of a board of representatives from the three cooperative groups.

Comparison of seed orchard and stand seed of Scots pine in direct seeding

Seppo Ruotsalainen
Finnish Forest Research Institute, Punkaharju Research Unit
Finlandiantie 18, FI-58450 Punkaharju, Finland

Background

Direct seeding of Scots pine is an important forest regeneration method in Finland. Annually some 30 000 ha of forest is regenerated by sowing. This comprises more than half of Scots pine cultivation and about 1/4 of all artificial regeneration. In total Scots pine has been sown on an area of 2.1 million hectares.

Direct seeding is mechanised to a high degree. In 2005 sowing by machine in connection to soil scarification was done on 69 % of all sowing area. The easiness in mechanisation and lower costs (< 50 %) compared to planting have helped to maintain the amount of direct seeding at a rather constant level.

Sowing consumes great amounts of seed. In 2005 about 8500 kg Scots pine seed was used for direct seeding, when at the same time the nurseries used only 475 kg’s. About 1/3 of the Scots pine seed used in direct seeding is produced in seed orchards. There has been discussion whether this proportion should be increased. In order to find out the usability of the seed orchard seed in direct seeding a research project was initiated at Finnish Forest Research Institute in 2002.

Study methods

The study was accomplished by establishing field experiments where seed lots from several seed orchards were compared to stand seed lots in three years (2002 – 2004) at four localities in southern and central Finland (in the first year only at two localities).

The experimental material consisted of 4 stand and 7 seed orchard seed lots. However, only 2 (stand) and 5 (seed orchard) of them were representative to their respective groups and were therefore used for the comparison between the types. The other seed lots were used to study the relationship between seed characteristics and seedling establishment and growth. Sowing was done on scarified mineral soil on shallow holes made by a special tool. 16 seeds were sown on each sowing spot and covered with a thin layer of mineral soil. The experimental design was randomised blocks with one sowing spot of each entry in each of the 30 blocks.

The seedling establishment was monitored and the height of the tallest seedling in each sowing spot was measured at the end of each growing season. As the germination rate was high (55 % on the average in the first autumn) it was difficult to count the exact number of the closely spaced seedlings, when they grew bigger. Therefore the success of seedling establishment was expressed as the proportion of sowing spots with at least 4 seedlings. Here the results of inventories after three growing seasons are reported.

Three year results

There was great variability between seed lots within each seed type as regards seedling establishment rate, so no general conclusions can be drawn from single seed lots. Due to mortality the proportion of sowing spots with at least four seedlings decreased between the years, especially from first to second autumn.

Seedling establishment did not differ statistically significantly between seed orchard and stand seed lots, although the proportion of sowing spots with at least four seedlings was slightly higher with seed orchard than stand seeds (75 and 72 %, respectively). However, when comparison was done on the basis of germinable seeds or equal weight of seeds, the stand seed was better than orchard seed (neither these differences were statistically significant). In two experiments there was a pronounced tendency for seed orchard seed lots to improve their relative seedling establishment from year to year. It is possible, that this development was caused by frost heaving, as the climatic and/or soil conditions in these experiments were favourable for it.

The seed orchard seed lots had on the average 20 % greater height (height of the tallest seedling in the sowing spot) than the stand seed lots (the difference was statistically significant) (Fig. 1). This relative difference remained rather stable from year to year. In one experiment there was a deviating development, which led in third autumn to inferior height of seed orchard seed lots. This experiment suffered from heavy early summer frost in the second growing season.

There was a clear tendency for those seed lots with smallest seedlings to have greatest decrease in number of seedlings between 2nd and 3rd autumn.

Conclusions

No general superiority of seed orchard seed lots in seedling establishment could be observed in these experiments, but the seed types were approximately equal. It is possible, that the conditions for germination and early development were rather favourable in these experiments, and therefore differences in seedling establishment were not present, as in many earlier studies in Sweden. However, the observed dependence between seedling size and decrease in seedling number can be an indication of selection favouring bigger seedlings, which can lead to better plant establishment of seed orchard seed lots at a higher age.

In height growth the seed orchard seed lots were superior to the stand seed lots as has been shown also in earlier studies. This height superiority was greater than commonly obtained with planted material.

The results show that seed orchard seed can be used in direct seeding and its genetic and physiological superiority in height growth is visible in the field. Measurements in the coming years are needed to confirm the long term constancy of the obtained results.

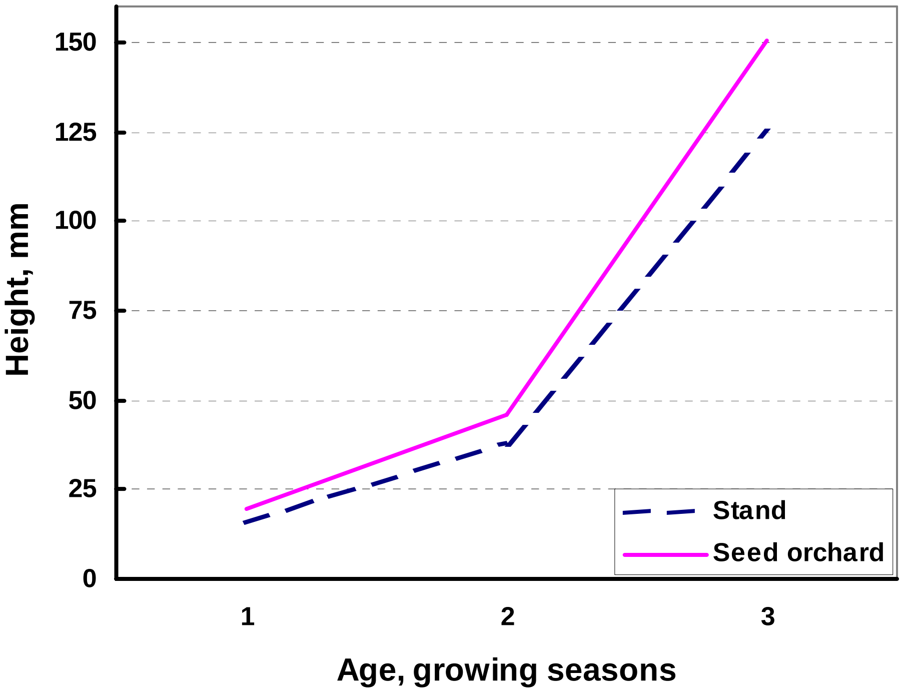


Figure 1. The average height of the tallest seedling in the sowing spot of seed orchard and stand seed lots in the first three growing seasons

Temporal and Spatial Change of the Mating System Parameters in a Seed Orchard of *Pinus tabulaeformis* Carr.*

X. H. Shen[1], D. M. Zhang[2], Y. Li[1], H. X. Zhang[3]
1 Beijing Forestry University, Beijing 100083. shenxh@bjfu.edu.cn
2 Institute of Shanghai Landscape Gardening Science，Shanghai 200232
3 Chinese Academy of Forestry, Beijing 100091

Abstract: It is more than 20 years, since we started to study flower characteristics and the mating system in the seed orchards of *Pinus tabulaeformis* Carr. A number of papers have been published in Chinese journals. Some results concerning variation of mating system parameters, pollen contamination and pollen dispersal with enzyme and SSR analysis are summarized in this paper.

Pinus tabulaeformis Carr. is an important tree species for Northern China due to its extensive distribution, tolerance in harsh sites, rather fast growth and high wood quality. Seed crops in seed orchards are stable, if insects are controlled. The high genetic quality and ample yield of seeds in the seed orchard is closely related with the mating pattern of seed orchards. In order to make a clear picture of the temporal and spatial change in the mating system parameters, namely outcrossing, inbreeding, selfing and contamination rates in the seed orchard, as well as pollen dispersal distance, field observation of flower characteristics with laboratory analysis was continuously carried out more than 20 years (Shen X. H. *et al.*, 1985; Wang X. R. *et al.*, 1991). Some results with enzyme and SSR analysis are shown in this paper.

Location of seed orchard and progeny plantation

The seed orchard of *Pinus tabulaeformis* Carr. for field observation and seed sample collection, is located in Xingcheng County, Liaoning province, China at NL 40°43’, EL 120°34’. It was built in 1974 on a mountain slope of 5°-15°, covering an area of 20 ha. The seed orchard comprises 49 clones, with systematical design, spacing 5m×5m. In 1993 roguing was conducted and about 1/3 trees were removed. A stand about 50 ha of the same species locates at 3km away from the seed orchard. The progeny testing plantation derived from the seed orchard locates more than 3 km away from the orchard.

Seed samples collection

Open-pollination seeds were collected from the seed orchard for 7 years, namely in 1984,

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* Financial support partially came from National Natural Science Foundation of China No 30371178

1987, 1993, 1996, 2000 and 2005, 2006. For study of three different populations seed samples were taken from the seed orchard, above-mentioned stand and progeny testing plantation. 5-8 cones were taken from each sample trees, while 8-10 seeds from them were used for enzyme analyses and 69 to 92 seeds for each of two clones for SSR analysis at 12 primer pairs.

Allozyme and Enzyme System

Horizontal starch-gel electrophoresis, including 10 enzyme loci was applied for both embryos and endosperm analysis with 10 loci as genetic markers. There were 8 kinds of enzyme.

- ACP（Acid phosphatase, E.C.3.1.3.2）,
- LAP（Leucine-amino-peptidase , E.C.3.4.11.1）
- GOT（Aspartate aminotransferase, E.C.2.6.1.1）
- PGM（Phosphoglucomutase, E.C.5.4.2.2）
- MDH（Malate dehydrogenase , E.C.1.1.1.37）
- ADH（Alcoholdehydrogenase, E.C.1.1.1.1）
- SKD（Shikimate dehydrogenase , E.C.1.1.1.25）
- MNR（Menadione reductse , E.C.1.6.99.2

Each of the first six enzyme possesses one locus, while the remaining two - two loci for every one (Zhan C. X. & Li Y., 1999).

Primers and SSR-PCR reaction system

Selected SSR primer pair sequences

Locus	Primer-sequences	Repeat motif	Bands
RPTest11	F)AGGATGCCTATGATATGCGC R)AACCATAACAAAAGCGGTCG	(CAT)7	5
PtTX2123	F)GAAGAACCCACAAACACAAG R)GGGCAAGAATTCAATGATAA	(AGC)8	7
PtTX4001	F)CTATTTGAGTTAAGAAGGGAGTC R)CTGTGGGTAGCATCATC	(GT)15	8
PtTX3116	F)GCTTCTCCATTAACTAATTCTA R)TCAAAATTGTTCGTAAAACCTC	(GTT)10	10
PtTX4011	F)GGTAACATTGGGAAAACACTCA R)TTAACCATCTATGCCAATCACTT	(GT)20	7
RPS160	F)ACTAAGAACTCTCCCTCTCACC R)TCATTGTTCCCCAAATCAT	(ACAG)3AGGC (ACAG)3	8
Cjgssr124	F)AAAATGGGTCATGTCATGT R)CATTCTCCATCTCACTACCTAT	(GT)36	8
PR203	F)TGGGACCCCATATTCTGATG R) CATTCCACTAGTTCTCTCGCAC	(GA)14	10
PR4.6	F)GAAAAAAAAGGCAAAAAAAAAGGAG R) ACCCAAGGCTACATAACTCG	(CA)21(TA)6	7
PR011	F)TGAGGAATCCATTGACATGC R)TGATCCGTGTGATCATCTTATG	(CT)21(CA)8	8
RPTest1	F)GATCGTTATTCCTCCTGCCA R)TTCGATATCCTCCCTGCTTG	(ATA)7	6
PtTX2146	F)CCTGGGGATTTGGATTGGGTATTTG R)ATATTTTCCTTGCCCCTTCCAGACA	(GCT)21	7

12 primer pairs with abundant polymorphism bands were selected and the SSR-PCR

reaction system was established. In 15μL PCR reaction: 30 ng DNA, 0.25 mmol / L Mg2+, 0.2 mmol/L dNTP, 250nmol/L Primer (F), 250 nmol/L Primer (R), 0.375U Taq polymerase are the best. Detected by 6% polyacrylamide gel, the length of amplification products was 100 - 250 bp, the number of alleles per locus was varied from 5 to 10 (Zhang D. M. *et al.*, 2007).

Data analysis and programs applied

Inbreeding, selfing rate of both single loci and multilocus are estimated with Ritland mix mating systematic model MLT (Ritland, 1990). Pollen contamination rates in the seed orchard were predicted with GENFLOW, written by Adams et al. (Adams, W. T. and J. Burczyk. 1993.). For paternity analysis CERVUS, written by Marshall was applied (Marshall, 1998; http://helios.bto.ed.ac.uk/evolgen).

Results

1 **Mating system parameters**

The variations of mating system parameters in the seed orchard over time, space and for different clones in the seed orchard were studied. Seed samples were taken in 1984, initial bearing age; 1993, fruitful bearing stage before rouging; 1996, after rouging; 2000, bearing age. Result indicates that the multilocus rates of outcrossing (t_m) has no much difference in 1984 and 1993, which is 0.975 and 0.962 respectively. There is a little difference in selfing coefficient, 0.025 and 0.038 respectively. In 1996 and 2000, outcrossing rates contain some difference, 0.795 and 0.801 correspondingly; the selfing rates are 0.205 and 0.119 respectively. Seed orchard was rouged in 1993, mating parameters differs markedly before and after the operation, the rates of the outcrossing decreases from 0.975 in 1993 to 0.795 in 1996, while the selfing coefficient increases from 0.038 in 1993 to 0.205 in 1996 (Zhang D. M. *et al.*,2001b; 2004).

Table 1 Mating system parameter in the seed orchard for different years

	1984	**1993**	**1996**	**2000**	**mean**
Multilocus rates of out crossing t_m	0.975(0.039)	0.962(0.019)	0.795(0.056)	0.801(0.046)	0.883
Difference of outcrossing rate t_m-t_s	0.076(0.028)	0.141(0.035)	0.341(0.052)	0.239(0.040)	0.199
Multilocus selfing rate 1-t_m	0.025(0.000)	0.038(0.019)	0.205(0.056)	0.199(0.046)	0.117

Note: Numbers in brackets are estimated standard errors, the same below.

Distribution of clones with different rates of outcrossing

The distribution of clones with different rates of outcrossing at different stages of the seed orchard is shown in Figure 1. There were 17 flowering clones in 1984, for 11.8% clones the outcrossing rate ranged 0.4 - 0.6, while the rates higher than 0.8 account for 82.4%. In 1993, there was no clone with the rates lower than 0.6 for total 34 clones in the seed orchard before rouging, while the rates higher than 0.8 accounted for 88.2%. Clones with the rates of 0.2 - 0.4 and 0.4 -0.6 respectively accounted for 8.3%, 6.7% and 20.8%, 13.3% in 1996 and 2000. Clones with the rates over 0.8 accounted for 54.2% and 40% in 1996 and 2000 as well. It shows that the thinning management apparently affects the mating parameters in the seed orchard (Zhang D. M. *et al.*, 2000).

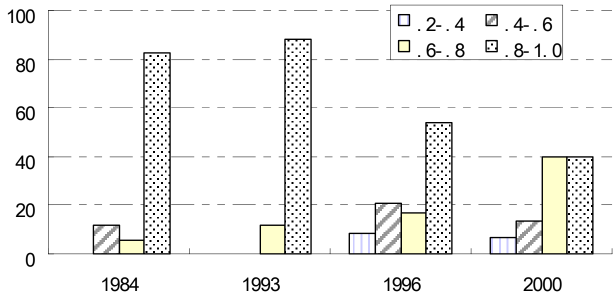

Figure 1 Distribution of clones with different outcrossing rates for four years

Outcrossing rates for three layers of crowns

Seed samples collected in 1987 were analyzed for this purpose. In general the outcrossing rates for seeds from upper crown are slightly higher than those from middle and lower positions (see Tables 2 and 3), although they are slightly various for different ramets-clones (Zhang D. M. *et al.*, 2004).

Table 2 Outcrossing & selfing rates for different layers of crown on an average

	Top	Middle	Low	Mean
Rates of multilocus outcrossing	0.910(.098)	0.909 (.098)	0.900 (.049)	0.906
Difference of the rates of outcrossing	0.160(.057)	0.044 (.057)	0.040 (.053)	0.081
Multilocus Selfing rate	0.090(.098)	0.091 (.098)	0.100 (.049)	0.094

Table 3 The outcrossing rates for three layers of five ramets

Clone	Upper			Middle			Lower		
4#	1.16*	(0.11)	n=46	2.00*	(0.00)	n=47	1.03*	(0.19)	n=71
5#	0.83	(0.06)	n=55	0.96	(0.09)	n=51	1.02*	(0.08)	n=48
19#				0.70	(0.09)	n=49	0.63	(0.18)	n=52
31#	0.96	(0.09)	n=53	1.27*	(0.13)	n=50	0.55	(0.12)	n=52
43#	1.12*	(0.06)	n=50	0.99	(0.07)	n=51	0.87	(0.09)	n=51

Note: According to the program stipulation, figures lager one are treated as one; n shows number of seeds analyzed.

2 Pollen contaminations

Based on comparing enzyme allozyme of clones with the seeds produced the seed orchard, foreign bands were discovered on locus *got2* allele *a, lap1 a, lap2 a* in 1984, 1983, 1996 and 2000; while *pgm1 e* - in 1993 and 1996 (See Table 4). It is obvious that the seed orchard was contaminated by the surrounding plantation (Zhang D. M. *et al.*, 2000).

Table 4 Foreign bands discovered in Seeds produced in the seed orchard

Locus	Allele	Clones	Gene frequency				Locus	Allele	Clones	Gene frequency			
			1984	1993	1996	2000				1984	1993	1996	2000
Got1	a	0.031	0.025	0.042	0.112	0.050	*Acp1*	a	0.255	0.055	0.381	0.191	0.128
	b	0.949	0.907	0.942	0.826	0.899		b	0.459	0.286	0.314	0.506	0.725
	c	0.020	0.068	0.017	0.062	0.050		c	0.286	0.658	0.217	0.237	0.147
Got2	a	0.000	0.014	0.017	0.158	0.050		d	0.000	0.000	0.053	0.000	0.000
	b	0.969	0.970	0.958	0.736	0.945		e	0.000	0.000	0.000	0.000	0.000
	c	0.031	0.016	0.025	0.106	0.005		f	0.000	0.000	0.036	0.000	0.000
Lap1	a	0.000	0.010	0.006	0.115	0.014	*Skd1*	a	0.020	0.161	0.107	0.248	0.115
	b	0.980	0.977	0.936	0.722	0.968		b	0.724	0.698	0.862	0.540	0.725
	c	0.020	0.013	0.036	0.152	0.018		c	0.255	0.141	0.030	0.211	0.161
	d	0.000	0.000	0.000	0.000	0.000	*Pgm1*	a	0.082	0.051	0.097	0.025	0.119
	e	0.000	0.000	0.000	0.000	0.000		b	0.357	0.327	0.300	0.220	0.468
	f	0.000	0.000	0.019	0.000	0.000		c	0.439	0.474	0.481	0.522	0.353
Lap2	a	0.000	0.018	0.045	0.152	0.037	*Adh1*	d	0.122	0.148	0.119	0.202	0.060
	b	0.980	0.980	0.877	0.761	0.927		e	0.000	0.000	0.003	0.031	0.000
	c	0.020	0.002	0.045	0.087	0.037		a	0.041	0.125	0.106	0.031	0.156
	d	0.020	0.002	0.045	0.087	0.037		b	0.673	0.770	0.883	0.866	0.784
	e	0.000	0.000	0.000	0.000	0.000		c	0.286	0.105	0.011	0.102	0.060
	f	0.000	0.000	0.034	0.000	0.000							

Based on the analysis of 8 enzyme loci for 49 clones and seeds collected from the ramets in the seed orchard in 1984、1993、1996 and 2000, the observed contamination rates are 0.326, 0.450, 0.532 and 0.385, while the estimated rates - 0.354、0.492、0.583 and 0.418 respectively. An average contamination rate is 0.462 (See Table 5). The contamination rate in 1996 is higher than that of 1993. It may be caused by rouging carried in 1993 (Zhang D. M. *et al.*, 2004).

Table 5　Estimates of pollen contamination in the seed orchard

Year	Seed analyzed	Observed rate	Correct coefficient	Estimated rate
1984	182	0.326	0.922	0.354
1993	149	0.450	0.914	0.492
1996	154	0.532	0.914	0.583
2000	109	0.385	0.922	0.418
Mean				0.462

To examine the contamination rates in different positions of crown, seed samples was taken from one ramet. The result is shown in Table 6. The rate in low layer is much higher than those in top and mid, but all the rates are much lower in comparison with the average rates in Table 5.

Table 6　Pollen contamination rate in different positions of crown in the seed orchard

Layer of Crown	Seed analyzed	Observed rate	Correct coefficient	Estimated rate
Top	186	0.108	0.891	0.121
Mid	255	0.123	0.891	0.138
Low	211	0.243	0.891	0.273
Mean		0.158	0.089	0.177

3 Pollen dispersal and pollination

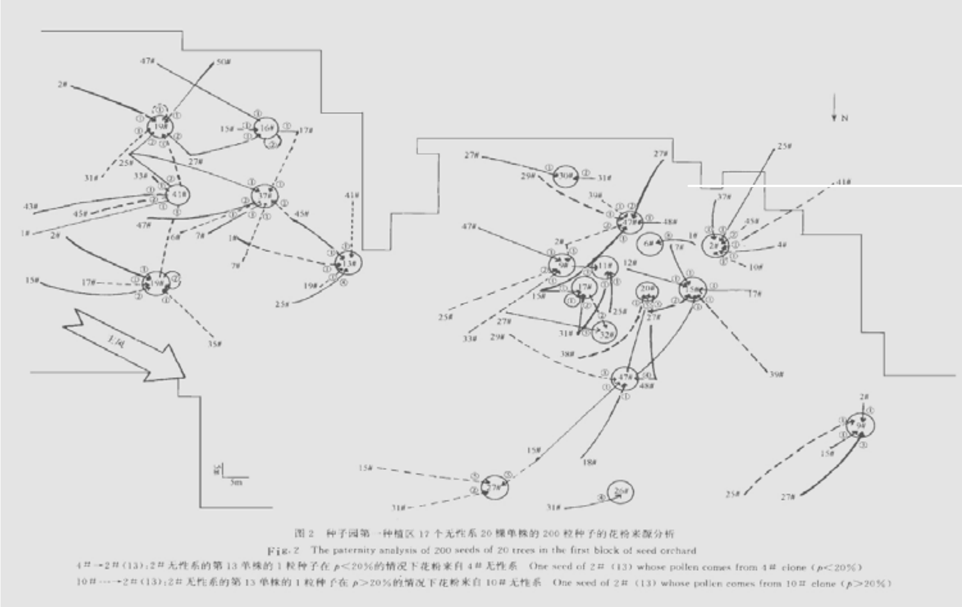

图 2　种子园第一种植区 17 个无性系 20 棵单株的 200 粒种子的花粉来源分析

Fig. 2　The paternity analysis of 200 seeds of 20 trees in the first block of seed orchard

4# →2# (13): 2# 无性系的第 13 单株的 1 粒种子在 $p<20\%$的情况下花粉来自 4# 无性系　One seed of 2# (13) whose pollen comes from 4# clone ($p<20\%$)

10# ---→2# (13): 2# 无性系的第 13 单株的 1 粒种子在 $p>20\%$的情况下花粉来自 10# 无性系　One seed of 2# (13) whose pollen comes from 10# clone ($p>20\%$)

Figure 2　The paternity analysis of 200 seeds in the seed orchard with enzyme

The effective dispersal distance of pollen was investigated using enzyme analysis. 89 seed samples from the seed orchard were examined for pollen-father source in detailed (Zhang D. M. *et al.*, 2001a). Figure 2 might give some ideas of the pollen dispersal and pollination incident in the seed orchard. 17.8% pollen-father comes within a radius of 7 m from the neighboring seed tree; 24.4%

approaches within 10 - 20 m; 55% pollen arrives within a radius of 20 - 30 m. The effective dispersal distance of pollen is less than 30 m. In addition, it demonstrates that pollen-father sources of 8 seeds for each ramet are quite diverse. For example, pollen sources for clone No 6 (ramet No 14) completely derived from clone No 1 at reliability > 0.95; 50% pollen for clone No 26 (25) came from clone No 31; 62.5% pollen for clone No 20 (1) – clone No 47, while for most of ramets, namely clones No 2 (13)、No 9 (6)、No13 (33)、No 16 (44)、No 17 (4)、No 37 (46) and No 41 (45) pollen-father derived diversely. In some cases pollen-father of 8 analyzed seeds originated from 6 clones.

The results with SSR analysis of open-pollinated seeds for two clones No 11 and 24 is shown in Figure 3. 11.1% -12.8% of pollen-father comes within a radius of 10 m from the seed tree; 37.0% - 40.4% within 10 - 20 m; while 17.2%- 22.2% comes within a radius of 20 -30 m from the seed tree. The outcome is all most the same as enzyme analysis.

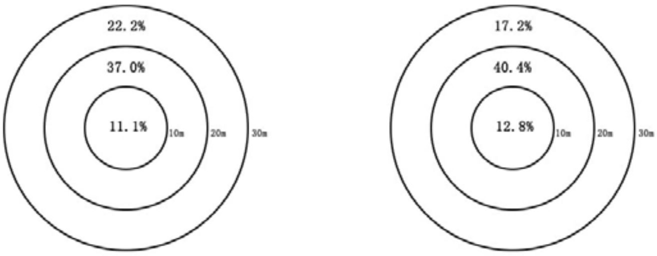

Figure 3 The paternity analysis for two clones (left – No 11; right – 24) with SSR

4 The mating system parameters for three different populations

Natural stand, seed orchard and Progeny testing plantation were examined. The estimated outcrossing rates of single-loci (ts) and multi-locus (tm), inbreeding and selfing rates are 0.638, 0.821, 0.742; 0.864, 0.962, 0.953; 0.226, 0.141, 0.211 and 0.136, 0.038, 0.047 correspondingly. There are considerable differences in ts and tm among the populations. It indicates that selfing rate is much high in the natural stand, while the inbreeding rate in the stand and progeny plantation populations is higher in comparison with the seed orchard (Zhang D. M. *et al.*, 2000).

Table 7 The mating system parameters for three populations

	Natural stands	Seed orchard	Progeny plantation	Mean
ts	0.638(0.049)	0.821(0.044)	0.742 (0.023)	0.734(0.039)
tm	0.864(0.048)	0.962(0.019)	0.953 (0.021)	0.926(0.029)

Tm-ts	0.226(0.042)	0.141(0.035)	0.211(0.019)	0.193(0.032)
1-tm	0.136(0.048)	0.038(0.019)	0.047(0.021)	0.740(0.029)
F	0.259(0.062)	0.211(0.083)	0.477 (0.029)	0.316(0.058)

Conclusion

Modern technology provides an opportunity to gain an insight into understanding essential, but invisible fact happened in seed orchards (El-Kassaby,Y. A. & K. Ritland, 1986; .El-Kassaby Y. A. et al., 1989; Harju A, Muona O. 1989; El-Kassaby Y. A. & S. Reynolds, 1990) and our investigation affords some idea about temporal and spatial variation on outcrossing, selfing, inbreeding and contamination rates as well as on pollen dispersal distance in a seed orchard of *Pinus tabulaeformis* carr.. All these data are theoretical importance for sustainable, healthy development of seed orchards, although they are not sufficient and not accurate enough as expected. To fully solve the facing problem we have long way to go. Inaccurate biology analysis and statistical methods used today should be improved and the study of mating system in combination with field observation is vital.

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Challenges and prospects for seed orchard development in South China

Run-Peng Wei
Sino-Forest Corporation
3815-29, 38/F Sun Hung Kai Centre
30 Harbour Road, Wan Chai, Hong Kong
E-mail: **runpeng-wei@sinoforest.com**

Abstract

This paper 1) briefly reviewed the history, development and status of seed orchard for different forest species, 2) discussed the difficulties or problems and the causes, and 3) proposed the solutions for further seed orchard development in south China. In the last four decades, many seed orchards were established, but most of them were the first generation with low realized genetic gain and many were not well maintained. Long-term seed orchard development should focus on major species for ecologically public-benefit forests and minor tree species. The idea of low-input seed orchard is particularly recommended. It is also proposed that newly growing private companies should greatly contribute to tree breeding and seed orchard through their involvement, input or cooperation.

Background

In South China, the first forest seed orchards were established in around 1964 after few years of preparation works such as plus tree selection, site selection, material collection, grafting etc. In the first decade, few species were considered, including Chinese fir (Cheng etc 1996; Qiu 2006) and slash pine (Zhu etc 1993). Chinese fir is one of the main wood production species in South China, while slash pine was introduced into Guangdong, China as early as in 1930s, and found well adaptive to the local environment (Zhu etc 1993). From 1975 to 1995, seed orchard programs were expanded or extended to more species but to the advanced generation only for few species (Cheng etc 1996; Chen etc 1999; DSOFF 2000; Yang 2001; Sun etc 2003; Qiu 2006). In the last decade, not really much progress was achieved even though forest plantation business has rapidly been booming up in the region.

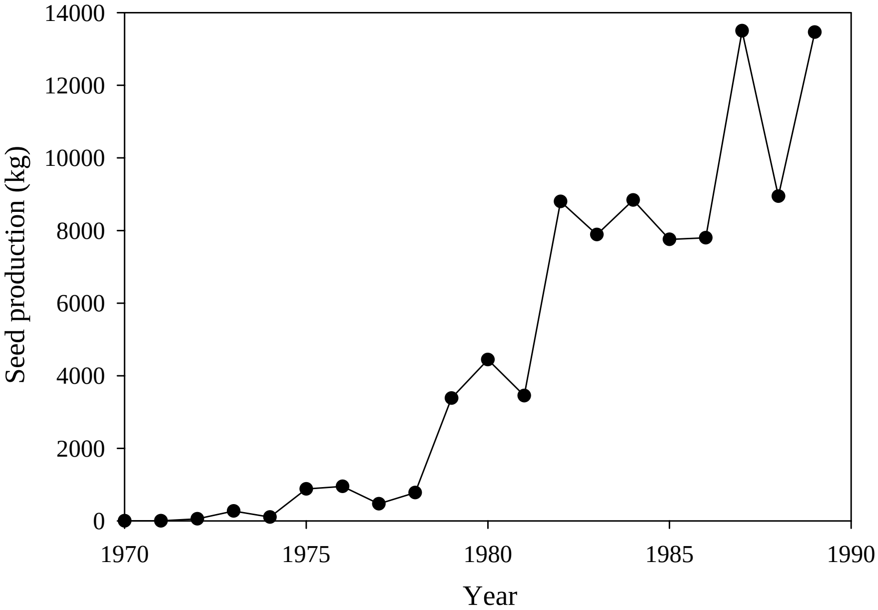
Up to date, most of the existing seed orchards are still in their first generation using plus trees as parent materials, and some in the advanced generation using genetic materials selected from progeny test trials (Shen 1992; Cheng etc 1996; DSOFF 2000; Sun etc 2003; Li etc 2005; Qiu 2006). Most of the seed orchards were/are located at, owned and managed by state-own forest farms and tree breeding bases that are under local forest bureaus or state-own forest companies. Technically, they are parts of the national & provincial tree breeding programs, and usually planned, designed and advised by forest geneticists from local universities or research institutes. On the other hand, these state-own forest farms or tree breeding bases are often managed as financially independent units.

More than 30 species were once put in seed orchards, including pines, Chinese fir, casuarinas, teak, eucalypts, acacias, *Castanopsis hystrix*, *Sassafras tzumu*, *Taxodium distichum*, *Taiwania flousian* etc (Shen 1992, 1994; DSOFF 2000; Bai 2004; Li etc 2005; Liang etc 2006). Some species might be included in more than one seed orchard, depending on the planting or replanting plan early made by national or local governments. These seed orchards naturally differed in area, management intensity and output.

Seed orchards were usually well designed and planted according to the standard textbooks (e.g. Faulkner 1975; Jiao etc 2004). It was planned that the parental materials covered relatively wide ranges of the natural distribution although actual plus-tree selection might not be so. Large amount of the selected genes were collected and exclusively conserved in the early established seed orchards. These collections were supposed to constitute the fundament not only for then seed orchard establishment but also long term breeding.

Some seed orchards flowered quite early and had good seed production as expected, providing huge amount of improved seeds for afforestation or reforestation. In a slash pine seed orchard located in south Guangdong, for instance, the grafts was planted out with a total

Fig 1. Seed production over ages for a slash pine seed orchard planted from 1965 to 1969 and expanded from 1974 to 1978 in South Guangdong



area of 60 ha from 1965 to 69, among which 6 and 20 ha were planted in 1965 and 1966, respectively; and an expansion of 50 ha with the same genetic materials was carried out from 1974 to 1978. The grafts started to flower at 4-year old and produce seed at 5-year old (Zhu etc 1993). The total seed production steadily increased from 1970 to 1989 (Fig 1). By the end of 1989, the accumulated seed production was 91,800 kg, which accounted for most of seed sources for slash pine planting before 1990 in south China. It was also observed that slash pine grafts had fast growth in cone production during the period from 8 to 14 years old and reached peak stage from 15 years. A caribaean pine orchard planted in 1974 started to flower at 3-year old and produced 300 kg cone or 15 kg seed per hectare at 8-year old (Zhu etc 1986).

Many seed orchards or even breeding bases were abandoned or converted to other uses due to poor management, deficient capital, less seed demand, low profit, high maintaining cost, land use change or economic development in the last decade. Seed orchards might not have enough budgets from national or provincial funds or income from seeds for maintaining their seed orchards (Wang etc 2006). Most of the early seed orchards aimed at improvement of growth. It was expected that some genetic gain could be obtained with use of seed orchard seed (Shen 1992, 1994). It seemed however that realized gain was generally lower than expected, if we look into the growth rate of plantations planted 10 or 20 years ago.

The definition of seed orchard is diverse. It is restricted in this paper to the sense of conventional or traditional mass-multiplication of genetically improved seed crops (Faulkner 1975). Almost all of early or existing seed orchards in south China were or are conventional. This paper discussed the issues and prospects of seed orchard development from seven perspectives, and particularly recommended low-input schemes for establishing and managing new seed orchards.

Ownership

Most of the existing seed orchards were/are owned and managed by state-own forest farms or breeding bases that do not have regular financial support from governmental budget or enough income from selling seeds (Li etc 2005; Wang etc 2006). This has been a key issue for maintaining or improving seed orchards since economic reform in the country. Few private companies may have their own seed orchards in the last several years, but they are technically still supported by the existing breeding programs. It could be expected that there will be a change in seed orchard ownership in the soon future.

Private companies become more and more important in managing commercial timber plantation forests. Desire of using improved materials including seeds in their planting is a strong drive for them to get involvement in seed orchard development. They may directly buy seed from the existing seed orchards for their short time purpose, or operate their own seed orchards for long run. A fast and efficient way is probably to change ownership through cooperation in maintenance and improvement of the existing seed orchards, as well as in establishment of new seed orchards. Private sector should play a major role in breeding programs and seed orchards, particularly for commercial plantation species. In the other hand, non-commercial planting with improved seeds is the country's long-term strategy to increase forest resources and protect environment (NFA 2006). The continuous investment of the public agencies is thus essential. In addition, there may appear professional breeding companies that operate seed orchards purely for commercial purpose.

Private sector or professional companies could focus on main commercial species such as eucalypts, pines, Chinese fir, and other fast-growing and high-value species, while state-own forest farms or breeding bases should pay more attention to species of both commercial and ecological values. As a most sustainable stakeholder, governmental support is also indispensable for commercial species. No matter what kind of ownership, a seed orchard may involve many different parties including shareholders, land owner, gene owners, technical contributors, seed users, local forest bureaus, local communities etc (stakeholders). Harmony cooperation among stakeholders is obviously a key for success of a breeding program as well as seed orchard management.

Investment and infrastructure

It has been a rule of thumb that good infrastructures with heavy investment are necessary for operating a seed orchard (Cheng etc 1996; Qiu 2006). These infrastructures include good land, transportation, irrigation, electricity etc. High-input is a psychological threshold to pass in planning and establishing new seed orchards for both public and private sectors. This has also been a main cause for instability of the early or existing seed orchards, particularly since market economic was adopted (Wang etc 2006). As the population is large and wide-distributed, and economic has been developing so fast in south China, many early seed orchards were converted for expansion of township, and construction of high-way, residence and factory. In addition, it seemed that the state or provincial policy support for funding tree breeding and research has gradually been switched from tradition breeding including seed orchard to molecular genetics and gene engineering in the last decade.

Is low-input seed orchard feasible? I give a very positive answer, at least in China and some developing countries. Ordinary forestland, usually hilly or mountainous lands in south

China, should be acceptable, even more suitable for producing improved seeds. Irrigation system is not necessary as rainfalls are usually high (>1200 mm) and relatively even in South China (e.g. Cheng etc 1996; CMA 1998). Simple forest road or access is perhaps enough. Other infrastructure investments may not be necessary or can just be low.

Technical operations and management can be simple and less costly (Lindgren and Wei 2006). Imagine a seed stand with mother trees designated and deployed as in a seed orchard. It may be a little bit more expensive to collect fruits or cones, but much cheaper in general. It may be less productive, but the production may be well enough for localizing or less overspreading the genetic materials. Such a low-input seed orchard has other advantages: 1) less likely to be changed for other purposes, 2) less human disturbance, 3) easy to maintain, and 4) flexible management. I will further discuss the potential low-input seed orchard management below.

Planting species

Main planting species varied much over time, although large scale planting has never been stopping in the last few decades (NFA 2006). Such change was more or less political, rather than scientific, technical or industrial-demanding. Before 1980s, the main planting species were Chinese fir and pines. In 1980s and before mid 1990s, the planting was switched to the southern pines including slash pine, loblolly pine and caribaean pine. Since late 1990s, eucalypts have been predominating over the whole region. A direct consequence was change in demand on specific species seed. This created great difficulties for maintaining high-input seed orchards.

Forest industry, particularly plantation business was opened up to private sector only in the last decade. Along with more involvement of private sector in forest resources, market demand will certainly regulate planting species to a more sensible and stable structure. This is very favorable for seed orchard management and development. It can also be predicted that more species would be considered for commercially planting as market economic develops.

In China, planting is not only adopted for commercial plantations but also for regenerating or rehabilitating other types of forests, which is a great drive for seed orchard development (Li etc 2005). It would always be a desire to use improved materials in planting. Sustainable harvesting with replanting is a development direction, which leads to a more stable demand on seed orchard seeds. Sexual propagation is still the main avenue for many species, which underlies the basis for developing seed orchards.

Gene resources

Plus tree selection work might not well cover the natural distribution of a species. Useful genes in nature might be missed in generating new planting materials. Gene stock deployed in a seed orchard might not well adapt to the planting region because proper breeding zones or seed use guidelines were usually not available. Lots of early selections were lost, not tested, or not deployed in seed orchards. Deployment of initially selected genes without testing could result in high genetic diversity that was probably unnecessary, since the number of deployed clones was usually large (Ai etc 2006; He 2006; Hong 2003). Pollen contamination was not much recorded but observable (Lai and Chen 1997), undesired for gain but a way to maintain high diversity. All of these issues should partly explain low realized gain in plantations.

Early used genes were exclusively kept in seed orchards, and many of them might be lost along with the disappearance of some seed orchards. In the other hand, the natural or secondary forests of many indigenous species become less and less, meaning a rapid loss of gene resources. It is an urgent task to conserve the plus trees early selected, and to collect valuable genes from the wilds. To conserve gene diversity for future improvement and seed orchard development, we need to consider an efficient cooperation that is different from a tradition one. Private sector should play a major role in strengthening gene conservation, seed orchard management and financial support. Through cooperation, an effective mechanism should be established so that the existing gene collections are best used, and the breeding and seed orchard programs best benefit from exchange of the gene resources.

Technically, it is of great potential to explore genetic gain through intensive and optimal use of genes in seed orchards. For exotic species, more gene resources are always positive for generating high gain, such as eucalypt improvement in China (DSOFF 2000). Early cooperation with foreign institutes in species and genes introduction was exclusively carried out by the state-own institutes such as forest farms, breeding bases, universities and research institutes. It becomes possible now for private sector to get involved in such cooperation, particularly as more and more foreign forest companies invest in China.

Breeding program

Well breeding strategy and/or plan were considered in the beginning for some species such as Chinese fir and loblolly pine. The corresponding seed orchards were established with sound design, technical input and management. However, such breeding and seed orchard operation usually could not continue as planned, because of less seed demand, deficient input and other undesired difficulties (Wang etc 2006). For many species, breeding work started with a simple breeding plan, or plus tree selection and seed orchard establishment. Technical input into seed orchards was usually not enough to ensure the expected genetic gain. In addition, almost all breeding programs were initiated and planned by universities or research institutes, but operated or managed by the financially and administratively independent units such as state-own forest farms and breeding bases, which was not favorable for effectively sustaining the breeding programs and improving gains.

A seed orchard is usually part of a breeding program. As market economic and private forest resources further grow, seed orchard development would mainly rely on the genetic quality of seeds produced. A successful breeding program with sound strategy and plan underlies the technical basis for a seed orchard with good output. However, most of breeding programs progressed slowly, or were not systematic or effective to support seed orchards with high gain, which was obviously inconsistent with the potential output. Private sector is young in this country, and it is difficult to say now how far they would like to go with their own tree breeding programs. A most quick, realistic and efficient way to better existing or work out new breeding and seed orchard programs is to effectively integrate the interests of all potential stakeholders. With improved strategy and plan, most existing breeding and seed orchard programs may greatly benefit in terms of sustainable operation and gain improvement.

Genetic testing of the breeding population materials is an indispensable part of tree breeding and seed orchard, which basically include 4 levels of trials: provenance, half-sib, full-sib and clonal. While seed-lots with provenance and half-sib structure were sometimes considered, most full-sibs with specific crossing design often came with the second and later generation breeding. Cloned materials may be used alone or combined with a high hierarchical structure in trials. Half-sib family trials that should be used to test the general performance of seed orchard seeds were often used for selection of parents for the next cycle of breeding.

Most breeding or seed orchard programs involved provenance or half-sib trials, even at late stage when crossing work could be easily achieved (DSOFF 2000). Some of them had good materials and experiment design. Full-sib family or hybrid trials were set up for several important species, such as eucalypts, pines, Chinese fir etc. However, only several advanced generation seed orchards were established. Clone testing becomes poplar in the last two decades, but mostly used for screening superior clones for plantations (DSOFF 2000). Common issues for genetic trials probably included too few genetic entries (20~30) or test sites (1~4) for improving the genetic quality of the existing or planned seed orchards.

Sound genetic testing should be emphasized to obtain high gain through improving the accuracy of genetic parameter estimates, seed orchard thinning or seed collection, selection of parents for the advanced generation breeding or seed orchard, and deployment of genetic materials in plantations. Large number of genetic entries in trials would definitely increase the sampling accuracy of genes, and intensity of selection. Properly increasing test sites would reduce the sampling skewness in representing normal planting lands.

Mating issue has long been neglected in terms of efficiency, necessity, operability, cost etc. Good crossing work was carried out for very few species such as some eucalypt species and Chinese fir (DSOFF 2000). Crossing is one of the most costly and difficult operations in practice. Systematic mating design with strong theoretical support is necessary for efficiently advancing breeding as well as seed orchard. This is particularly important when setting up completely controlled or two or few clones seed orchards. Open-pollination is still most realistic for most of species in terms of cost and operation. A wide genetic base with more parents or families is more effective for screening superior seed orchard materials (Lindgren etc 1997). A mating scheme should be planned by taking existing genetic knowledge, cost, operability, genetic testing and selection, sustainability etc into account. This is not easy but a direction for main commercial plantation species.

Selection of materials for seed orchards mostly aimed at growth traits such as height and DBH, but seldom at wood quality and other traits. It has been shown however that other traits such as wood density and fiber property would be as important as growth (e.g. DSOFF 2000). Genetic bases for selection were usually low, particularly those with a provenance or family structure because of low number of entries. Selection methods used such as family selection and between- and within-family truncation (He 2006; Hong 2003), were usually less efficient (Wei etc 1998). This was one of the main causes for the low realized genetic gain. Breeders should properly consider different traits of importance in selection for their final wood products. Overall gain could markedly be improved through properly widening genetic base or breeding population for intensive selection. In addition, selection or use of selections could be optimized to improve gain, diversity, and/or both in seed orchards.

When there was a demand, seeds from a seed orchard were often used in wide planting range, out of its targeting region. This has been an issue relating to breeding or seed zone. It was not considered when planning a seed orchard, neither when distributing the seeds (e.g. Xu and Liu 2002). For example, slash pine seeds from the earliest seed orchard in south Guangdong had once been used in more than 10 provinces (Zhu etc 1993). A direct lesson was that stands or trees did not perform well with slow growth and less resistance to insects or disease soon after planting. This work needs to seriously be considered in the future. Sound genetic trials that include rich materials and well cover the planting range are fundamental for delineating breeding or seed zones.

Vegetative propagation

Great progress has been achieved on mass vegetative propagation technology for many forest species. In China, cutting has been adopted in propagation of eucalypts, casuarinas, teak, Chinese fir, pines, poplars, Paulina, etc, and tissue culture for most eucalypts, teak, Paulina etc. Both cutting and tissue culture technology becomes more and more mature, and less and less costly over years for some eucalypts. It is particularly worthy to note that cutting materials have exclusively been used in planting of poplars and main eucalypt species for some years. Two years ago, some planters started to directly plant eucalypt tissue cultured materials in their plantations, and raised a high demand this year. It is predicted that tissue cultured material would replace cuttings in some years at least for some main eucalypt species, such as *E. urophylla*, *E. grandis* and their hybrids. This is probably one of the technological advantages that China has over other eucalypt planting countries. However, difficulties exist in using cutting or tissue culture technology to mass propagate some species for large scale planting, such as *E. dunnii*.

Somatic embryogenesis is an attracting direction in tree species vegetative propagation. Progress has been made for mass propagation of conifer species in some countries. Most of efforts were restricted to lab research in China (Wang etc 2007). However, people see a bright future in advancing this technology for some pine and other commercial species that are difficult to root. It is also believed that vegetation propagation would become predominant in propagation of planting materials for many tree species.

A seed orchard is needed or survives only when seed is needed for planting programs. Asexual material has many advantages over seed orchard seeds. Most commercial planters prefer cloning material. Will vegetative propagation become a competitor or terminator of traditional seed orchard? Success in vegetative propagation would no doubt marginalize seed orchard development in China. A good example is short-rotation eucalypt species such as *E. urophylla*, *E. grandis*, *E. tereticornis* and/or their hybrids, changing quickly from seedling (seed orchard), rooting to tissue-cultured cuttings in the last decade. As vegetative technology matures for many tree species, seed orchard existence may highly rely on non-technical legal regulations that are necessary for ecological, environmental or social concerns. Ecologically public-benefit forests are quite likely to become the first target of such legal regulation, using seeds or seedlings for replanting or rehabilitation. It would be difficult and unlikely to have similar regulations for commercial plantation species.

Management

In this part, I would mainly focus on management skills and technologies that are different from traditional ones, particularly the idea of low-input seed orchard or breeding (Lindgren and Wei 2006). First, breeders should probably consider sites that are stable and less likely to be converted to other uses like house or factory building. Ordinary plantation lands (hills or low maintains) should be acceptable. Forestland for ecologically public-benefit may be a good choice as this type of land is relatively stable and less disturbed by human according to the national or provincial regulations. Good infrastructure (transport and irrigation systems) is not necessary as discussed above.

Genetic quality of seeds from a low-input seed orchard should be the same or similar to those from traditional ones, resulting from breeding operation. Seed production is not a sole goal; timber production or ecological and environmental functions should also be considered. The multi-objectives may properly be adjusted according to the changing demand on seeds, through control of mother tree density. This is a cost-effective way to set up and secure a seed orchard. Seed orchard seed crop could be considered superior over asexual materials in conservation.

To guarantee genetic improvement, the same genetic materials as for traditional seed orchards should be used, such as selection grafts, cuttings, or seedlings. Conventional design and layout can still be valid (Faulkner 1975; Jiao etc 2004), but site topography needs to be considered. To keep the cost further low, half-sib family seedlings (no crossing) or cuttings of selections are probably good materials, at least for most of minor species. Optimal reselection or deployment of materials can be employed in such seed orchard to obtain high quality seeds.

Initial planting density could be relatively high, something between for a traditional seed orchard and a normal stand or seed stand. Thinning is considered for both seed crop and wood growth, and certain number of trees is remained to ensure the final wood production. However, it is flexible for late-stage adjustment of the objectives, depending on the need on seeds. Trees are more like timber production trees rather than fruit trees. Logs of large size are obtained at a rotation age deliberately considered. Density control through thinning as well as tending should also take ecological or environmental considerations into account.

Seed orchard site should be maintained at a status of more or less “nature”, with enough vegetation and less soil disturbance to control soil and nutrition erosion. However, it is also necessary to minimize the growth competition on space and nutrition of vegetation with mother trees. It is probably feasible to only keep low layer vegetation between tree rows. Weeding is only given within certain distance around trees, when trees are young or short, and when seed crop is harvested. Fertilizing is given together with weeding to ensure enough nutrition supply for tree growth or seed production. Hormone chemicals such as $GA_{4/7}$ may be used to stimulate flowering and seed yield.

Seed crop harvest is difficult not only for low-input seed orchards as proposed, but also for traditional ones, as manual collection is still popular. Mother trees in low-input seed orchards should be taller. It may be argued that operations such as grafting, seed crop harvest, field layout etc are expensive. In the labor-intensive countries like China, however, the costs for these operations can still be low. Seed yield per unit area may be low, but this issue can probably be solved by increasing the area or few more sites.

Conclusions

Seed orchard development has a more than 40-year history in south China. Most of the seed orchards were in the first, and some in their advanced generation. Many were discarded due to conversion of land use, less input, no seed demand, etc. The last decade was probably a relatively difficult period for seed orchard development. The ownership, concept of seed orchard including needs of high-input and good infrastructure, varying species, and vegetative propagation technology were the main factors that created difficulties in developing seed orchards. The problems existing with gene resources, breeding program and management were the main causes for the slow progress in seed orchard and low realized gain in plantations.

Private sector would play a role in improving the existing or future breeding program and seed production. Asexual materials may be just super and become predominant for commercial plantations. There exist rooms for seed orchard even in the future with better vegetative propagation technology. Seed orchard should be a good choice for major ecologically public-benefit forest species and many minor tree species. Low-input seed orchard and breeding is probably a realistic alternative with well theoretical support, at least for developing countries or regions.

A low-input seed orchard does not need good land, good infrastructure, heavy construction and intensive tending (weeding, fertilizing, thinning, topping, pruning etc). Besides seed production, it also serves as other functions such as timber production, ecological or environmental conservation, or cultural appreciations. Its parent materials and layout should be the same as or similar to those for a traditional seed orchard. The initial planting density may be high, and flexible for adjustment later if needed.

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Factors affecting effective population size estimation in a seed orchard: a case study of *Pinus sylvestris*

Dušan Gömöry$^{1)}$, Roman Longauer$^{2)}$, Ladislav Paule$^{1)}$, Rudolf Bruchánik$^{3)}$

1) Faculty of Forestry, Technical University in Zvolen, T.G. Masaryka 24, SK-960 53 Zvolen, Slovakia
2) National Forestry Centre, Forestry Research Institute, T.G. Masaryka 22, SK-960 92 Zvolen, Slovakia
3) Stredoslovenské lesy, SK-974 00 Banská Bystrica, Slovakia

ABSTRACT

Effective population size as a parameter closely correlating with the genetic and genotypic diversity of the seed orchard output is an important indicator of seed orchard functioning. It is determined by the variation of male and female gametic contributions of parental genotypes (including those outside the seed orchard), influenced by the variation in male and female gamete production, reproductive phenology, pollen dispersal within seed orchard and other factors. The assessment of fertility and phenological variation requires labor and finances. We tested empirically what is the relative importance of these factors for effective population size (status number) estimation by gradual adding one-by-one in the order of labor requirements (female contribution, male contribution, reproductive phenology, pollen dispersal) in three Scots pine (*Pinus sylvestris* L.) seed orchards in central Slovakia. The study has shown that in old, fully fructificating seed orchards, effective number of clones is a satisfactory estimator of the effective population size, but the inclusion of female and male fertility variation improves the estimate of status number. On the other hand, phenological variation and spatially-dependent pollen dispersal do not considerably affect N_S estimates and need not be assessed for practical purposes. In contrast, a young seed orchard proved to be unbalanced and phenologically not synchronized; consequently, effective number of clones was a poor estimator of the effective size and status number was affected by all factors (male + female fertility, phenology, spatial design)

Keywords: *Pinus sylvestris*, seed orchard, effective population size, status number, phenology, pollen dispersal

INTRODUCTION

In contrast to seed stands, clonal seed orchards generally contain a limited number of genotypes. However, the seeds they produce are used for reforestation of large areas. In Slovakia, forest reproductive material of European larch and partially Scots pine is almost completely limited to seed orchard seeds. Great attention must therefore be paid to the genetic composition of seed orchard crops.

Panmixis is frequently taken as a reference of functioning of seed orchards. An ideal seed orchard should function as a randomly mating Mendelian population, i.e. probability of mating for any pair of the population members should be independent of their genotypes (no mating preferences, equal fertility) and equal viability of all produced offspring zygotes. These conditions are known to be frequently violated in seed orchards, mainly in clonal seed orchards. Clones can be represented by different numbers of ramets with different fecundity, what produces variation in the numbers of produced male and female gametes. Differences in floral phenology may cause unequal probabilities of mating between particular parental pairs. Genetic incompatibilities (including self-incompatibility) result in different viability of embryos, e.g., in Scots pine, viability of selfed embryos was observed to be only approx. 30% as compared to non-inbred ones (Yazdani & Lindgren 1991).

Genetic marker studies performed during the past 30 years have considerably contributed to our understanding of mating processes in seed orchards. They generally showed only small deviations of genotypic frequencies from the Hardy-Weinberg expectations and low levels of self-fertilization in conifer seed orchards (citations). On the other hand, significant shifts in allele frequencies between seed orchard and its offspring, proving deviations from panmixis, have also been reported (Gömöry & Paule 1993). Nevertheless, marker studies are too laborious and too expensive to be operationally performed in every seed orchard to provide information on the genetic diversity in seed orchard outputs for management decisions. Various types of effective population sizes (based, however, on *a priori* assumptions about the mating process) are most frequently used for this purpose.

The concept of effective population size is characterized by two principal features: a concept of an ideal population, which generally is a panmictic population, and a characteristic variable, such as inbreeding coefficient, coancestry, or variance of gene frequencies (Gregorius 1991). Kjær & Wellendorf (1997) provided a good overview of effective population numbers and their specific meanings in case of clonal seed orchard crops. Lindgren *et al*. (1996) developed status (effective) number, which refers to the concept of group coancestry (Θ), i.e. the likelihood that two genes taken at random from the gene pool are identical by descent. Status number is defined as half the inverse of group coancestry ($N_S = 0.5/\Theta$). It measures the number of equally fertile clones in an ideal seed orchard that – following random mating in the seed orchard progeny – gives raise to the same amount of inbreeding as will be experienced following random mating in the present seed orchard progeny.

The simplest estimator of the effective population size of a clonal seed orchard is the effective number of clones, reflecting the variation in the numbers of ramets representing individual clones in the seed orchard. It is based on the (unspoken) assumption that all ramets contribute equally to the offspring, or, more exactly, that the contribution is not clone-dependent. As this assumption is not realistic, the potential parental contributions of individual clones have frequently estimated in seed orchard studies. The most easy-to-assess parameter is the female fertility, mostly estimated based on cone crop (number or weight of cones), eventually seed crop. Male fertility based on male strobili scores (what is, however, more labor-demanding) has also been frequently assessed (El-Kassaby & Cook 1994, Muona & Harju 1989, Kjær 1996, Kjær & Wellendorf 1997, Reynolds & El-Kassaby 1990). Less often, the role of reproductive phenology was recognized (Askew 1988, O'Reilly *et al.* 1982, Xie *et al.* 1994). Phenology scoring requires several visits of seed orchard suring the flowering period. The effects of the localization of individual clones on the genetic composition of seed orchard crop have been generally neglected. Their assessment requires previous studies on the shape and parameters of the pollen dispersal function. The effects of other factors, such as anisotropies in the pollen dispersal due to prevailing wind direction during the pollination season or genetic incompatibilities are difficult to assess and their incorporation in the modeling of the mating process is hardly possible.

The assessment of fertility and phenological variation requires labor and finances. From the point of view of practical management decision-making in seed orchards, it is necessary to know which factors affect considerably effective population size, in order to decide which are thus worth of being assessed. We tested empirically what is the relative importance of these factors for status number estimation by gradual adding one-by-one in the order of labor requirements (female contribution, male contribution, reproductive phenology, distance-dependent pollen dispersal) in three Scots pine (*Pinus sylvestris* L.) seed orchards in central Slovakia.

Table 1 Description of the investigated Scots pine seed orchards

Characteristic	Seed orchard		
	Kolkáreň	Háj	Sýkorová
Geographical coordinates	48E46'N 19E16'E	48E15'N 19E19'E	48E29'N 18E42'E
Altitude	450 m	350 m	475 m
Year of establishment	1982/1987	1979	1979
Census number of clones	43	30	35
Coefficient of variation of clone representation[1)]	40.8%	43.0%	59.4%
Number of ramets	589	574	372
Number of wrongly planted ramets	117	199	56
Number of alien genotypes	160	63	45
Number of alien ramets	160	81	45
Spacing	5 m × 7 m	8 m × 8 m	7 m × 8 m
Average cone yield per ramet 1995–1999 (fresh weights)	0.47 kg	2.38 kg	2.80 kg

[1)] Without alien genotypes

MATERIALS AND METHODS

A detailed description of the studied seed orchards is given in Table 1. Clone identity was verified using 7 polymorphic allozyme loci (for details on allozyme analysis, see Gömöry et al. 2003). In all seed orchards, a considerable proportion of alien material (overgrown rootstocks, non-registered ramets) was found and several ramets were placed on wrong positions. In all three seed orchards, cone crops of individual ramets were scored in five consecutive years (1995 to 1999). Numbers and sizes of male strobili were assessed in 1997 and 1998. Assessment of floral phenology essentially followed Jonsson *et al.* (1976). Phenological phases were scored on three to five days in each seed orchard in 1997 and 1998 (Table 2). A complete dataset (male strobili, cones, phenology) was available only for the year 1997, which was a year of full fructification.

Table 2 Description of the scored phenological phases and dates of phenological observations

Male strobili			Female strobili		
score	description of modal state	relative pollen shedding	score	description of modal state	relative receptivity
0	not visible	0	0	bud closed, completely covered by scales	0
1	developing, closed in integuments	0	1	ovuliferous scales visible at the apex	0
2	yellow, fully shedding	100	2	strobili developed, starting to open	25
3	brownish, weakly shedding	40	3	open, fully receptive	100
4	dry, brown, not shedding	0	4	open, scales sickle-shaped	30
			5	closed, starting to form cones	0

Seed orchard	Year	Observation dates
Kolkáreň	1996	May 13, 16, 18, 20, 22
	1997	May 14, 16, 19, 22, 26
	1998	May 12, 15, 18, 21, 25
Háj	1997	May 11, 15, 20
	1998	May 9, 12, 18
Sýkorová	1997	May 15, 20, 23
	1998	May 12, 15, 18, 21

To describe the dependence of mating success from the distance between mates, we used the model according to Adams & Birkes (1991):

$$\varphi_{ij} = e^{-\beta d_{ij}} \Big/ \sum_{k}^{N_{ram}} e^{-\beta d_{ik}}$$

where φ_{ij} is the mating success between female i and male j, d_{ij} is the simple aerial distance between female i and male j, N_{ram} is the number of ramets, and β is the distance parameter.

On the basis of the data published by Müller(-Starck) (1977), the value of β was set to 0.04. For the self-pollination within the crown of one ramet ($i = j$), we estimated d_{ii} as the average distance of two randomly chosen points on a globular surface, which is approximately equal to 1.3-multiple of the crown radius. The distance between ramets was estimated as the distance between crown centers.

For the assessment of the gametic contributions of individual clones, a modified method following Askew (1988) and Xie et al. (1994) was used. Under the consideration of all factors, relative gamete contribution of the pair of ith male ramet and jth female ramet was estimated as:

$$GC_{ij} = \Sigma_k\ \varphi_{ij} P_i p_{ik} O_j o_{jk} / \Sigma_i \Sigma_j \Sigma_k\ \varphi_{ij} P_i p_{ik} O_j o_{jk}$$

where P_i is the pollen gamete contribution of the ith ramet, O_j is the ovule contribution of the jth ramet, p_{ik} is the relative pollen shedding rate of the ith ramet at date k, o_{jk} is the relative receptivity of female flowers of the jth ramet at date k (p_{ik} and o_{jk} depend from the phenological phase of male and female strobili, respectively), φ_{ij} is the distance-dependent mating success of the ith male ramet with the jth female one. Gametic contributions were subsequently summed according to clones. When a particular factor was not considered (female fertility, male fertility, flowering phenology, distance), the respective parameter was set to 1.

It is only male (pollen) gametes, which compete in the fertilization. Unless pollen density is low (e.g., at the beginning or end of the pollination season), all available ovules have almost equal chance to be fertilized. Although this assumption is not absolutely realistic, the assessment of clonal gamete contribution was based on it, as it would be difficult to quantify the differences between clones in the proportions of fertilized ovules. Therefore, the total proportion of female gametes contributed by jth clone was estimated based on the produced ovules: $f_j = O_j/\Sigma_k\ O_k$, whereas the total proportion of male gametes contributed by the ith clone was estimated as as $m_i = \Sigma_j\ GC_{ij}$ (whereby $\Sigma_i\ m_i = \Sigma_j\ f_j = 1$).

Effective number of clones was calculated as:

$$N_e = 1 \Big/ \sum\nolimits_i p_i^2$$

where p_i is the relative number of ramets of the ith clone. Group coancestry and status number of seed crop were estimated according to Gömöry *et al.* (2000).

RESULTS AND DISCUSSION

A complete dataset (cone weights, male flower numbers, phenology) was available only for the year 1997 (i.e. cone collection in the spring 1999), which was a year of relatively rich flowering in all seed orchards under study.

In all three seed orchards, there is a considerable proportion of alien genotypes. Therefore, we considered three situations: (a) alien ramets were removed (census number of clones is equal to the planned number), (b) alien ramets were not removed, but no cones were collected from them (census number is equal to the total number of genotypes, i.e. planned clones plus alien genotypes, since alien ramets contribute to pollination), (c) cones were collected from all ramets (census number is equal to the total number of genotypes).

Male as well as female contributions of alien ramets exceeded 10% in all three seed orchards. Harvesting cones from alien ramets (c), and even leaving alien ramets in the seed orchard (b) lead thus to a substantial contamination of the gene pool of the seed crop by genes of parents of unknown phenotype. It is questionable, if this disadvantage is balanced by increased genetic diversity in the crops, since effective numbers increase negligibly as compared with the situation (a).

In both older and fully fructificating seed orchards (Háj and Sýkorová), effective number of clones seems to be a relatively satisfactory descriptor of the genetic diversity of seed orchard crops, especially when seed orchards do not contain alien ramets. Effective number of clones is generally substantially lower than the population census (table 3a, b). Status number considering the variation in female fertility substantially is not substantially lower. This, naturally, does not mean that there is no among-clone variation in female gamete production, but the differences among clones are not huge and actually compensate the differences in the ramet numbers per clone.

On the other hand, variation in male fertility (which is bigger in both seed orchards) decreased status number. Flowering phenology and distance-dependent pollen dispersal did not affect parental imbalance. In Sýkorová, the inclusion of the phenological variation into the estimation even increased slightly status number. Apparently, there is a kind of compensation effect, when some of the abundantly flowering clones are not sufficiently synchronized with the major part of seed orchard clones either in pollen shedding or in female strobile receptivity.

The situation is different in the younger seed orchard Kolkáreň. The estimated effective population sizes decrease steadily with the inclusion of each considered factor, whereby status number under each model is substantially lower than the effective number of clones.

Table 3 Effective number of clones and status number under consideration of different sources of parental imbalance in three Scots pine seed orchards

	Aliens removed		Aliens not harvested		All harvested	
	Θ	*N*	Θ	*N*	Θ	*N*
			Háj			
Census		30		93		93
N_e		25.5		34.1		34.1
N_S: F	0.0198	25.2	0.0171	29.2	0.0150	33.3
FM	0.0236	21.1	0.0207	24.1	0.0186	26.9
FMP	0.0241	20.8	0.0207	24.1	0.0186	26.9
FMPD	0.0248	20.1	0.0213	23.5	0.0190	26.3
			Sýkorová			
Census		35		80		80
N_e		26.1		33.5		33.5
N_S: F	0.0205	24.4	0.0181	27.6	0.0159	31.5
FM	0.0237	21.1	0.0199	25.1	0.0177	28.3
FMP	0.0234	21.4	0.0198	25.3	0.0176	28.4
FMPD	0.0236	21.4	0.0201	24.9	0.0178	28.0
			Kolkáreň			
Census		43		203		203
N_e		36.9		68.0		68.0
N_S: F	0.0159	31.4	0.0122	40.9	0.0095	52.4
FM	0.0214	23.4	0.0162	30.9	0.0123	40.7
FMP	0.0241	20.7	0.0177	28.2	0.0148	33.8
FMPD	0.0263	19.0	0.0197	25.4	0.0165	30.4

This means that the effective number of clones does not properly reflect parental imbalance in a yound seed orchard.

There is no unanimity about the significance of phenology for reproductive success of clones in a seed orchard: Askew (1988), El-Kassaby et al. (1984), Matziris (1994), Nikkanen (2001) or Codesido et al. (2005) underlined the effects of reproductive phenology on the genetic composition on seed orchard crops, but O'Reilly et al. (1982) consider these effects unimportant. Our results support rather the latter opinion, at least in old seed orchards. The effect of pollen dispersal seems to be more or less compensated by clone dislocation in the case of random seed orchard design. Only in Kolkáreň with more than one third of mislabelled or wrongly placed ramets, this effect was more pronounced. However, spatial pollen dispersal can be more important under clonal-row design, as indicated by the study of El-Kassaby et al. (2007).

The presented effective population number estimates (44%–85% of the seed orchard census) are to what has been reported from clonal seed orchards of conifers. In a large-scale study of 255 conifer seed orchards in Korea, Sweden and Finland, effective number of clones ranged between 20% and 100% of the census number with an average of 74% (Kang et al. 2001). This study considers only the variation in the ramet numbers per clone. Effective population size estimated considering fertility variation ranged in similar, even broader intervals: 60% to 93% in Scots pine (Muona & Harju 1989), 22% to 91% in East Asian pine species (Kang & Lindgren 1998, Choi et al. 2004), 54% to 73% in Norway spruce (Kjær 1996, Nikkanen & Ruotsalainen 2000) from Finland. In these studies, the effective number was estimated based on male and female gamete contributions of clones derived from the counts and/or weights of male strobiles and cones, without consideration of phenological variation and spatial pollen dispersal.

A different behaviour of the youngest seed orchard Kolkáreň is associated with age. Flowering is most irregular, therefore, relative status number much lower than in Háj and Sýkorová. Kjær & Wellendorf (1998) also observed a gradual change of the relative status number from 29% to 81% in a Danish Scots pine seed orchard within a period of 20-years.

The current model of mating process does not count with pollen contamination. Actually, no specific study of background pollination rates has been performed in either of the investigated seed orchards. However, all of them are situated in broadleaved areas, far from large complexes of Scots pine stands, which are situated approximately 150 km westwards in the Zahorie lowland and 80 km eastwards in the Spis region and are separated from southern Slovakia by mountain ridges. Scots pine is by far less represented in Slovak forests than in, e.g., Scandinavia, so that much lower pollen contamination rates are to be expected. Under this situation, genetic diversity of the seed orchard crop depends primarily on the pollination within the orchard. The study of Gömöry et al. (2000) has shown that in mature seed orchards, the among-clone variation in male gamete production is three to four times higher than for female gametes, and relatively stable over years (non-significant clone-by-year interaction). Our results indicate that male fertility is the key parameter determining effective population size in mature Scots pine seed orchards in Central Europe.

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Pollen contamination and after-effects in Scots pine

Jan-Erik Nilsson, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden E-mail: Jan-Erik.Nilsson@genfys.slu.se

Approximately half of the seeds produced in mature forest tree seed orchards in Scandinavia are from fertilization with the natural background pollen cloud. In young seed orchards with no male flowering pollen contamination is close to 100 %. Due to pollen contamination only between 50% and 75 % of the genes in young and mature seed orchards respectively, originate from the selected seed orchard clones and the genetic gain of the seed orchard progeny is reduced compared to all-internal pollination.

In northern latitudes pollen is usually found in the air one or more weeks before local pollen dispersal. The early pollen is often assumed to originate from more southern areas with higher temperatures in early summer and accordingly earlier pollen dispersal. For northern seed orchards southern pollen reduces the hardiness of the seed crop, mainly from delayed autumn cold acclimation. However fluctuations in wind directions, temperatures and rain fall in early summer can carry pollen from other directions, with other genetic composition, and with other effects on the seed orchard crops.

From artificial freeze testing comparing wind pollinated progenies with reference progenies from controlled pollination with pollen of known latitude origin, the hardiness and geographical origin of the natural pollen cloud can be estimated. However, evaluation of progeny tests at low age means that physiological after-effects from maternal environment etc. might affect the results and hide the genetic differences that are of main interest in such pollen cloud studies.

By producing both wind pollinated progenies and controlled crosses on the same trees , the maternal effects are largely eliminated from within tree comparisons, and the observed progeny differences will reflect genetic differences between pollen clouds (different days and/or different localities) and reference pollen.

As a basis for studies of genetic variation in the natural pollen cloud across central and northern Sweden, based o the described method of progeny testing, a series of small clone archives (with the same ten clones) has been established on 19 localities in Sweden between latitude 61 and 67^{o} N.

To make it possible to the study the pollen cloud on any localities, also a clone archive of mobile grafts was recently established. From this archive trees will be lifted from the soil and transported for pollination at selected localities just before female receptivity and the pollinated trees are returned to the clone archive immediately after pollination to allow all seeds to develop in the same locality and environment. Thus after-effects from maternal environment can probably be further reduced compared to the permanent clone archives, enhancing the precision in estimates of genetics pollen clouds differences.

Initial studies of lifting mother trees for pollination on different localities followed by seed development on the original locality show insignificant treatment effects on progeny cold acclimation in freeze tests of one year seedlings. This indicates that the method of utilizing clone archives as collectors of the natural pollen cloud followed by progeny freeze testing of young seedlings can be useful to understand more about the genetic variation and geographical origin of the natural pollen cloud of Scots pine over time and space. More knowledge about variations in the natural pollen cloud should also guide in establishing new seed orchards.

Mass Controlled Pollination at ArborGen in South Carolina, USA. Over 94 million control pollinated full sib seedlings have been planted operationally in the southeastern US since 2000. Photo: Dave Gerwig.

This group of symbolic rather well spaced trees (designed by Gun Lövdahl) is situated outside the main building of Faculty of Forest Sciences at Umeå, where the conference took place. With good will it could be interpreted as a symbol of “Perfect seed orchard trees”. Photo: Darius Danusevicius.

Pinus nigra male flowers. The picture is taken early morning, so the light is fresh and interesting bright water drops are visible. In Poland there are 23 seedling seed orchards of European Black Pine (110 ha). The purpose is to provide seeds for the forests located in polluted urban areas. Photo: Jan Kowalczyk.